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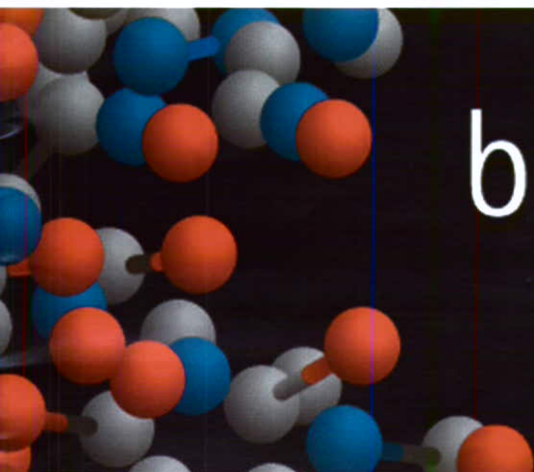

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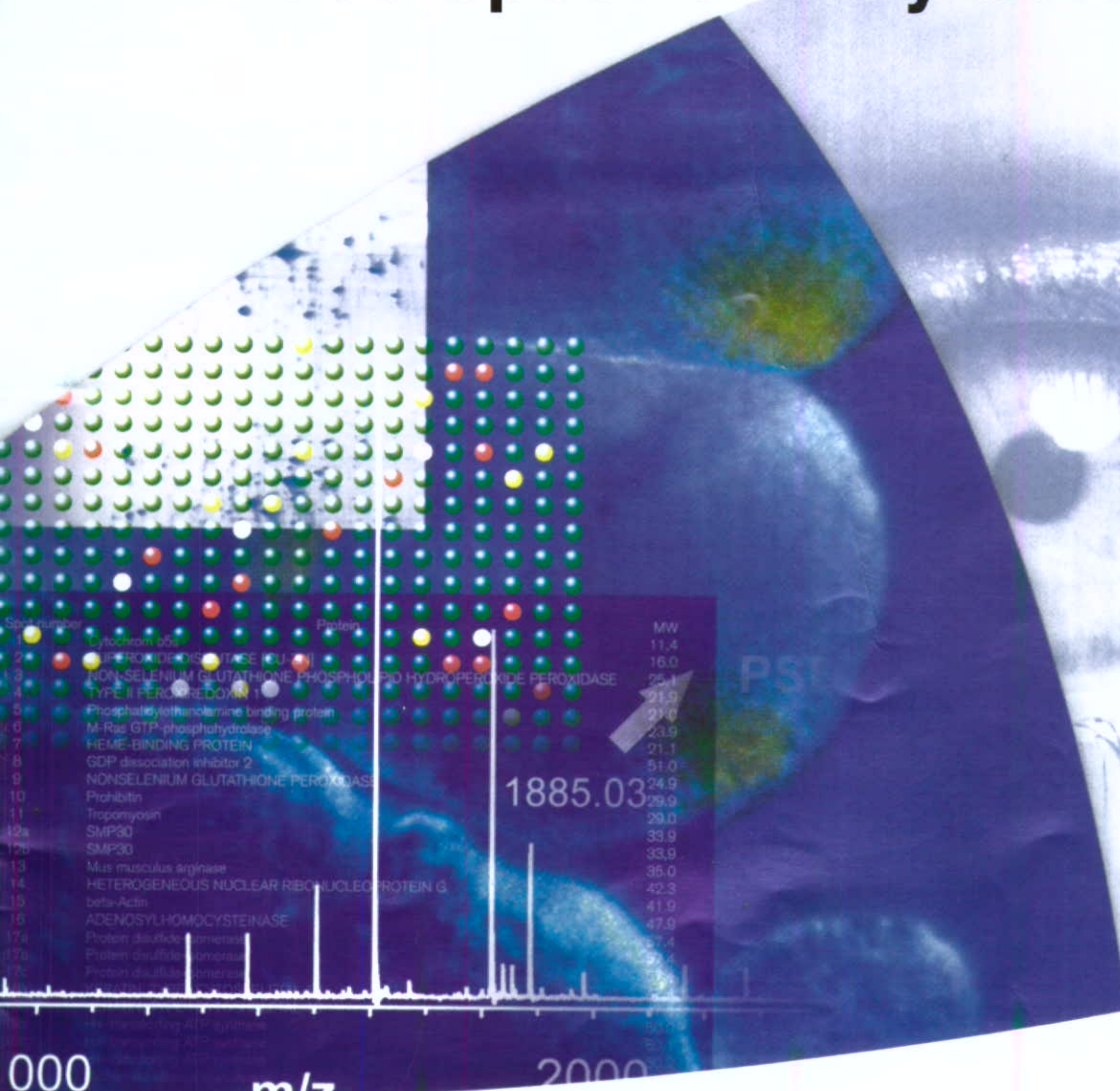

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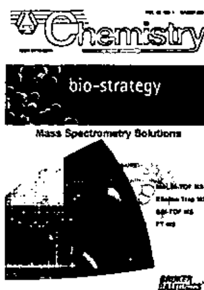

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NZ Science Scene

MACDIARMID YOUNG SCIENTISTS OF THE YEAR AWARDS ANNOUNCED

The Foundation for Research, Science and Technology is proud to announce, in association with Fisher & Paykel Appliances, the establishment of a new annual awards programme to honour New Zealand's best up-and-coming researchers and scientists.

The new Awards are named after one of New Zealand's greatest scientists – Professor Alan MacDiarmid, who won the Nobel Prize for Chemistry in 2000 for the discovery and development of conductive polymers. Professor MacDiarmid was born in the Wairarapa and educated in the Hutt Valley and at Victoria University. He is currently the Blanchard Professor of Chemistry at the University of Pennsylvania, USA and the James Von Ehr Professor of Chemistry and Physics at the University of Texas at Dallas, USA.

Key Details:

The 2004 Awards are open to all fellows and scholars who are current recipients of a scholarship administered by the Foundation for Research, Science and Technology.

Fellows and scholars are invited to enter a poster in one of eight new categories:

- Agriculture, Forestry and Fishing
- Manufacturing and Materials
- ICT and Creative Industries
- Biotechnology

Above: Professor Alan McDiarmid.

- Maori Innovation
- People and Society

The winner in each of these categories will receive a cash prize of $2,000 and the runner-up $1,000. From these eight categories there will an overall winner of the event who will be flown to Washington DC to join the winners of a similar competition organised by the prestigious American Association for the Advancement of Science (AAAS).

The overall runner-up will receive a travel grant to attend a science communication event in Australasia.

The new MacDiarmid Young Scientists of the Year Awards will be presented at a special gala dinner which will be held this year in Auckland on 22 June, which will be attended by Ministers of the Crown, business people, leading scientists and researchers, and science communicators – some of the most influential people from the New Zealand RS&T sector.

Finally, these new look Awards are due in no small part to the generosity of the Award's sponsors, including Fisher and Paykel Appliances who have naming rights sponsors and also the MacDiarmid Centre at Victoria University, The University of Auckland, Pulse Data International, Crop and Food Research, Landcare Research, Te Puni Kokiri, the *National Business Review*, the University of Waikato, AAAS, BTI Travel and ION Automotive.

SIR WILLIAM PICKERING AND THE PICKERING MEDAL

"Sir William Pickering was one of the outstanding scientific talents New Zealand has produced, Research, Science and Technology Minister Pete Hodgson said recently. "He stood alongside our Nobel Prize winners as an example of brilliance and achievement in the pursuit of knowledge."

Dr Pickering, the New Zealand-born giant of rocket science and space exploration, has died at his California home, aged 93. "I extend my sympathy to Sir William's family, friends and scientific colleagues for their loss," Mr Hodgson said. "His life was inspirational and his place in scientific history is assured."

William Pickering was born in Wellington in 1910. He was educated at Havelock Primary, in the Marlborough Sounds, Wellington College and Canterbury College, now the University of Canterbury, before moving to the United States in 1929 to study at the California Institute of Technology (Cal Tech), where he gained his PhD in physics in 1936. Dr Pickering became Director of the Jet Propulsion Laboratory in Pasadena and led the development of US unmanned space exploration, including the first US satellite, Explorer 1, the first successful American around-the-moon probe, Pioneer IV, the Mariner flights to Venus and Mars in the 1960s, the Ranger photographic missions to the moon and the Surveyor lunar landings of 1966-67. He became known as "Mr JPL".

"In 1941 Dr Pickering became an American citizen, but he never forgot New Zealand," Mr Hodgson said. "In a deliberate move to give something back to his country of birth, he established the WH Pickering Fellowship for New Zealand graduates to study engineering or science at Cal Tech." Dr Pickering returned to the University of Canterbury last year to be awarded an Honorary Doctorate of Engineering. More than 1500 people came to hear him speak, and gave him a standing ovation. Last year the Royal Society of New Zealand established the Pickering Medal to recognise excellence and innovation in the practical applications of technology. The Medal is to be awarded annually to a person who through design, development or invention has performed innovative work in New Zealand with results of national and international significance or significant commercial success. The first nominations are currently being sought. For further information:
<http://www.nasa.gov/home/hqnews/2004/mar/HQ_04094_pickering.html>
<http://www.jpl.nasa.gov/releases/2004/86.cfm>
<http://www.nexusresearchgroup.com/space_expl/pickering.htm>
<http://www.comsdev.canterbury.ac.nz/news/2003/030319O2.shtml>
<http://www.rsnz.govt.nz/awards/pickering/index.php>.

ANNOUNCEMENT OF THE BÜCHI NIR AWARD 2004

Since 2001 the Büchi NIR Award is presented every year. Previous awardees have been among others Dr. James Drennen, Duques University in Pittsburgh, USA; Professor Tony Moffat, University of London, School of Pharmacy, UK; Professor Yukihiro Ozaki, Kwansei-Gakuin University in Kobe, Japan and Professor Heinz W. Siesler, University of Duisburg-Essen, Germany.

Because in addition Büchi also sponsors the Tomas Hirschfeld Award, this year the BÜCHI NIR Award is dedicated for the first time to excellent diploma and doctoral theses in the field of NIR spectroscopy. Interested parties are encouraged to submit their NIR results and apply for the NIR Award. Deadline for applications is 30 June 2004. It's worthwhile: the prize money is US$2,000. Details and application forms can be requested at nir.award@buchi.com or via <www.nirsolutions.com.>.

NEW VICTORIA RESEARCH CENTRE TARGETS BIODISCOVERY

Exploring the new world of proteins to prevent, diagnose and cure human, animal and plant diseases is the goal of a new collaborative applied research centre to be established at Victoria University.

The Centre for Biodiscovery was launched by the Hon. Pete Hodgson, Minister for Research, Science and Technology, in the University's Hunter Council Chamber on March 18 .

Vice-Chancellor Professor Stuart McCutcheon said with the completion in 2000 of the mapping of the 30-40,000 genes that make up the human genome, the focus was turning to the more than two million proteins that are the products of genes. It is proteins that are the targets of most pharmaceutical drugs and vaccines.

"There is a growing realisation that to better diagnose, prevent and cure the array of maladies that afflict humans, animals and plants, we need to understand the nature of proteins. While proteomics – the study of study of proteins – is in its scientific infancy and experiencing explosive growth, at Victoria, our academics have been working in this field for the last 20 years.

"The new Centre for Biodiscovery brings together in one collaborative team, under the directorship of Dr Bill Jordan, researchers from the Schools of Biological Sciences, Chemical & Physical Sciences, Psychology and Mathematical & Computing Sciences to further enhance Victoria's research in this exciting new frontier of biological and chemical science.

"The Centre's researchers are working on at least seven major projects that involve about $7 million dollars of external funding from organisations as diverse as the Foundation for Research Science and Technology, Ovita, the United States' National Institutes of Health and the European Union."

Professor McCutcheon said Centre staff already had strong relationships with a host of other research organisations including Crown Research Institutes, such as AgResearch, Industrial Research and the Institute of Environmental Science & Research, as well as the dairy company Fonterra, the Wellington School of Medicine and Health Sciences, and the Malaghan Institute of Medical Research.

In a related development, the University and AgResearch have formed a new proteomics joint venture with an AgResearch laboratory established within the School of Biological Sciences so both organisations can work together in proteomics-based biology and biotechnology. This will assist in fulfilling the growing need for proteomics capability for the University and for AgResearch, as well as providing exciting long-term opportunities for new products from the discovery of novel proteins.

Research within the Centre includes: the development of a new anti-cancer drug from a marine sponge toxin; protein technology to help rapidly diagnose disease; understanding the activity of bacteria in a cow's rumen (one of four stomachs) to improve nutrition and reduce the greenhouse gas methane; and investigating proteins in cereals and wheat that cause adverse reactions in humans.

The Centre for Biodiscovery is the sixth applied research centre to be

launched following the establishment of the MacDiarmid Institute for New Materials and Nanotechnology (now a Government-funded Centre of Research Excellence), New Zealand Institute for Research on Ageing, the Crime and Justice Research Centre, the Roy McKenzie Centre for the Study of Families and the Centre for Applied Cross Cultural Psychology.

For more information, contact Centre Director, Dr Bill Jordan, on 04 463 6092.

PROFESSOR FIELD NAMED NEW LINCOLN VICE-CHANCELLOR

Professor Roger Field of Christchurch has been appointed Vice-Chancellor of Lincoln University and he will take up the position on 1 April 2004. Professor Field has been the University's Acting Vice-Chancellor since the retirement of Dr Frank Wood on health grounds in October last year, and he has also served in that capacity on two other occasions.

Announcing the appointment, Chancellor Margaret Austin says that Professor Field comes to the position with an outstanding combination of academic and administrative experience at a senior level and as a notable contributor to tertiary education in New Zealand, Australia and Europe.

The appointment was unanimously supported by the University Council and Mrs Austin said the selection process was robust and comprehensive and included consultation with groups drawn from all sectors within the University. "Professor Field is a respected as an educator, researcher, manager and leader and he has a reputation for decisiveness, clarity and excellence," said Mrs Austin. "He is a strategic thinker whose management strengths are recognised by his peers and by the University Council. "He has played a major role in the development and implementation of recent organisational and structural reviews at Lincoln University from which significant successes have already flowed, such as an increased student enrolment in this first semester of 2004."

A former Professor of Plant Science at Lincoln University and Head of the Plant Science Department, Professor Field moved into top university management in the mid-1990s, first as Pro Vice- Chancellor and since 1998 as full-time Deputy Vice-Chancellor. Professor Field's knowledge of Lincoln is extensive, says Mrs Austin, and dates from his appointment in 1970 as a Lecturer in Plant Science, with particular responsibility for plant physiology. He was promoted to Senior Lecturer in 1976, Associate Professor in 1984 and full Professor in 1986.

As an academic, Professor Field's undergraduate and postgraduate level teaching included applied plant and crop physiology, physiological aspects of genetics and plant breeding, weed science and agronomy and he utilised his extensive research programmes in these areas to inspire his lectures, laboratory classes and field work. In his time at Lincoln Professor Field has served on numerous administrative bodies including the Examinations Board, Research Committee, Professorial Board and Postgraduate Studies Committee. In the past he was active too in the Association of University Teachers (now Association of University Staff, AUS) serving as both a Lincoln branch chairman and member of the national executive.

Professor Field was a founder member of the New Zealand Society of Plant Physiologists and is a past president of that society. As Deputy Vice-Chancellor, Professor Field represented Lincoln University in numerous negotiations associated with recent Government reforms in tertiary education. For example he chaired the New Zealand Vice Chancellors' Committee's University Entrance group at the time of the introduction of the NCEA, and he is currently Chair of the Committee on University Academic Programmes.

Born in Britain, Roger Field completed a joint honours degree in botany and zoology at the University of Hull, followed by a PhD, also at Hull, studying the physiological mechanisms of herbicide and plant growth regulator translocation in plants.

He becomes Lincoln University's third Vice-Chancellor and 10th head since the institution was founded in 1878 as a school of agriculture. "Lincoln University has a history and culture of inclusion and camaraderie for which it is well known," said Mrs Austin. "The new Vice-Chancellor, Professor Field, inherits this legacy and can be expected to enhance and develop it in the years to come."

INTERNATIONAL SCIENCE AND TECHNOLOGY (ISAT) LINKAGES FUND

Applications are now being called for under the Bilateral Research Facilitation Programme of the 2004-2005 ISAT Linkages Fund. Guidelines and Application Forms will be available for downloading from the Royal Society's website at <http://www.rsnz.org/funding/isat> from 31 March 2004. Funding has been specifically dedicated to research collaborations involving Australia, France, Germany, Japan, Korea, Latin America, the USA, and the United Kingdom, but funding for collaborations involving all other economies is also available. Applications are for activities that commence before 30 June 2005. Applications close with the Manager, ISAT Linkages Fund, Royal Society of New Zealand, 4 Halswell Street, Thorndon, Wellington at 4.00 p.m. on Thursday 6 May 2004. No late applications will be accepted. Potential applicants should contact their institutional ISAT coordinators for further details.

INTERNATIONAL PRIZE FOR BIOLOGY

The Japan Society for the Promotion of Science seeks nominations for the 2004 prize, in the area of systematic biology and taxonomy. Nominations close on 20 May, 2004. See <http://www.jsps.go.jp/j-bionom.htm> for details and a nomination form. The international prize, which was instituted in 1985 in commemoration of the sixty-year reign of Emperor Showa, is for ten million yen. Recent recipients have come from the UK, USA, Japan and Australia.

SYNTHESIS OF PHARMACEUTICALS USING MOLECULAR CHESS AND AUTOMATED SYNTHESIS

Margaret A. Brimble
Department of Chemistry, University of Auckland, 23 Symonds St, Auckland

Introduction

The main theme of our research is focused on the chemical synthesis of complex natural products that have important biological activity. However, in the last two years we have built a parallel second strand of research focusing on the synthesis of peptidomimetics, glycopeptides and combinatorial libraries of bioactive natural molecules as lead compounds for development as pharmaceuticals. This latter area of research has been fuelled by commercial contracts with some of New Zealand's emergent biotechnology companies in the Auckland region. This area of research also coincides with the introduction of New Zealand's first BSc (Hons.) degree programme in medicinal chemistry that has already attracted 64 students at the end of its second year. This degree is interdisciplinary and comprises papers in organic and medicinal chemistry, analytical chemistry, genetics, molecular biology, physiology, pharmacology together with a new paper entitled "Current Issues in Drug Design and Development." This latter paper will include lectures on patent law, Good Manufacturing Practice and Good Laboratory Practice, ethical issues in the pharmaceutical industry, the current regulatory framework and several case studies on pharmaceutical development in New Zealand and overseas. The University of Auckland has also recognized the important role of chemistry by providing funds for the $NZ 8M refurbishment of the Department of Chemistry which located the synthetic organic and medicinal chemistry laboratories on the 7th floor of the building.

Natural Products Synthesis and Molecular Chess

Natural products have long been regarded as *Nature's medicine chest* providing a rich source of compounds that can be synthesized for possible pharmaceutical development. Natural products synthesis has also been described as an *'enabling science'* because it provides unlimited opportunity for discovery at the interface with biology and medicine.[1] Our research in synthetic organic and medicinal chemistry focuses on making and modifying naturally occurring bioactive compounds isolated from plants, animal tissue, microbes or marine and soil organisms, which are rare or difficult to isolate in abundance. The chemical synthesis of these complex bioactive molecules is a challenging endeavour because of the rich and diverse chemical structures that test the existing boundaries of organic synthesis. It demands academic and manipulative rigour, creativity, dedication, persistence, and hard work. The tactics and synthetic manoeuvring involved in executing a given synthesis have been compared to the logic and rationale behind a game of chess.[2]

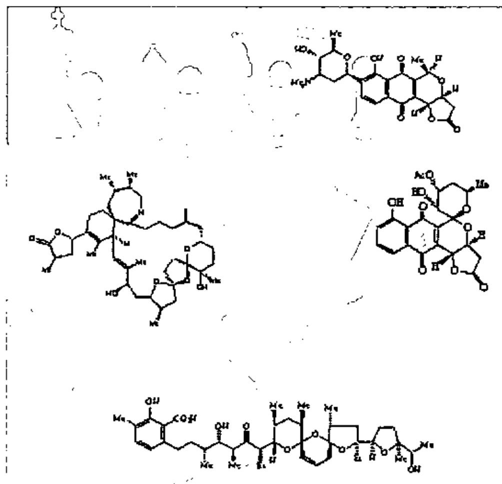


Figure 1. The concept of molecular chess with targets from the Brimble research group.

Current Natural Product Synthetic Targets

Over the last decade our research group in Australasia has focused on the development of flexible synthetic approaches to several natural products that have important biological activity. The synthesis of the molecules described in detail below has also allowed the preparation of synthetic analogues of the natural compounds which have led to improvements in biological activity and an understanding of the way the naturally occurring compounds act.

Synthesis of the Pyranonaphthoquinone Family of Antibiotics

The pyranonaphthoquinone family of antibiotics exhibit inhibitory activity against a variety of pathogenic fungi, yeast, and Gram-positive bacteria. Additionally, these compounds are able to act as bioreductive DNA alkylating agents via quinone methide intermediates thereby resulting in cross-linking of DNA strands. These alkylated DNA adducts then interfere with the cell replication process. Normal cells, which do not divide rapidly, are able to repair the damage. However, in rapidly dividing cancer cells, the DNA repair enzymes are unable to cope with the damage and the cells die. This concept of bioreductive alkylation offers an exciting mechanism of drug action for the development of new antineoplastic agents based on the pyranonaphthoquinone skeleton.

We have developed an efficient synthesis of several simpler members of pyranonaphthoquinone antibiotics[3] using a novel annulation of a 2-acetylnaphthoquinone using 2-

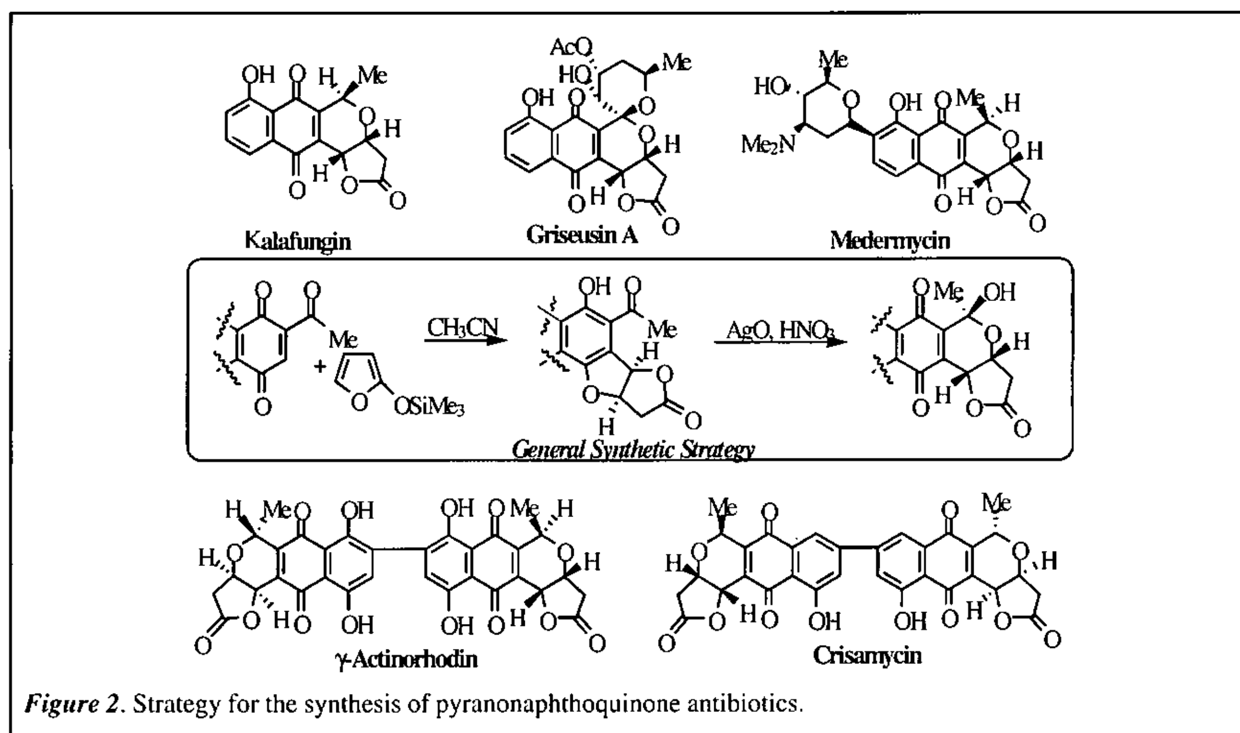


Figure 2. Strategy for the synthesis of pyranonaphthoquinone antibiotics.

(trimethylsilyloxy)furan to afford a furonaphthofuran ring system that undergoes oxidative rearrangement to the desired pyranonaphthoquinone ring system (Fig. 2). This methodology was then successfully applied to the synthesis of the spiroacetal-containing pyranonaphthoquinone, griseusin A, and the *C*-glycoside pyranonaphthoquinone, medermycin (which is effective against neoplastic cells *in vitro*, antibiotic resistant cell lines of L5178Y lymphoblastoma and inhibits human leukaemia K 562 cells as well as platelet aggregation). The first efficient synthesis of a dimeric pyranonaphthoquinone, as present in the antiviral agent crisamycin A, has also been successfully effected using an efficient double furofuran-oxidative rearrangement strategy starting from a bis(2-acetyl-1,4-naphthoquinone).

Synthesis of Polyether Ionophores

The polyether antibiotics salinomycin and CP44,161 (Fig. 3) exhibit antimicrobial activity against Gram-positive bacteria, mycobacteria, and fungi. They also have important roles in veterinary medicine as growth promoters in ruminants. The characteristic property of these polyether antibiotics is their ability to act as ionophores and conduct ions across membranes.

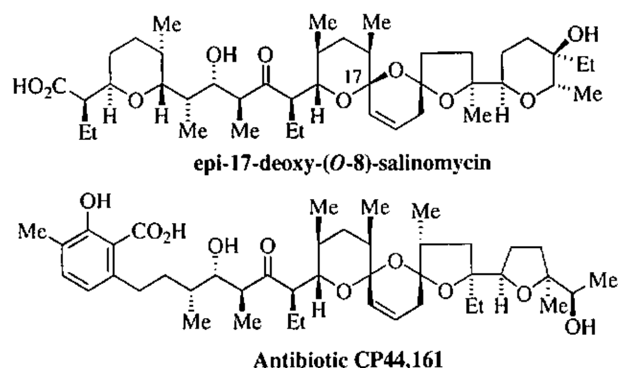


Figure 3. Structures of polyether ionophores.

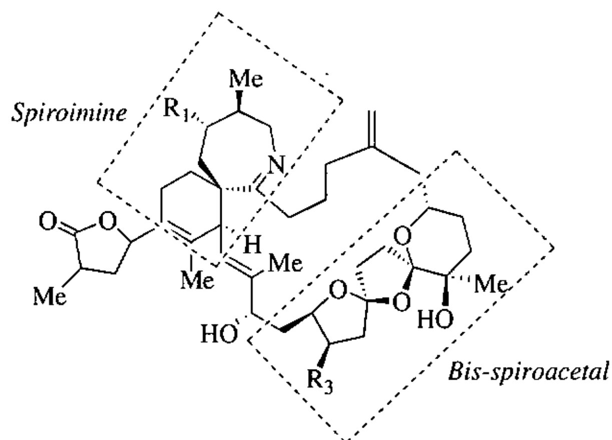
Their main structural feature is the presence of the bis-spiroacetal ring system. This complex functional group is thought to contribute to the binding and transport of metal ions across membranes. Our synthetic approach to the bis-spiroacetal ring systems of *epi*-17-deoxy-(*O*-8)-salinomycin and antibiotic CP44,161[4] hinged on the use of a key oxidative cyclization of a hydroxyspiroacetal to a bis-spiroacetal.

Synthesis of Shellfish Toxins

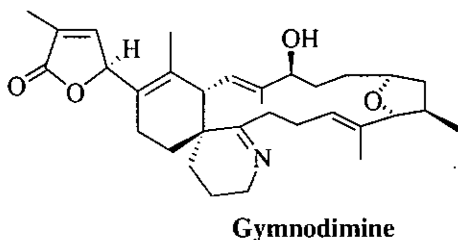
The production of shellfish toxins (also known as phycotoxins) is associated with dinoflagellate blooms (the *red tide* phenomenon) that can result in massive kills of fish and marine animals in temperate waters in both the Atlantic and Pacific Oceans. The shellfish feed upon the micro algae and concentrate the toxins thereby acting as a vector transferring these toxins further up the food chain when carnivores such as fish and crabs eat shellfish. During algal blooms the level of toxins within healthy shellfish can reach a level that is harmful to humans and causes symptoms ranging from diarrhoea to extreme cardiovascular and neurotoxic effects even at concentrations as low as a few parts per billion. These shellfish toxins have rich and diverse chemical structures and their complex molecular architecture poses a significant challenge to synthetic organic chemists engaged in their total synthesis. Many of these toxins have unique biological activity and can act as therapeutic agents and pharmacological probes.

One of our research programmes is directed towards the synthesis of these complex shellfish toxins. The spirolides A-D (Fig. 4) comprise a novel family of pharmacologically active macrocyclic imines found in the polar lipid fraction obtained from the digestive glands of contaminated mussels (*Mytilus edulis*), scallops (*Placopecten magellanicus*) and toxic plankton from the eastern coast off Nova Scotia, Canada. The spirolides A-D cause potent and characteristic symptoms in the mouse bioassay (spirolide A – LD_{50} 250

µg kg⁻¹) and are activators of type L calcium channels. These macrocycles contain a novel bis-spiroacetal ring system as well as an unusual spiroimine moiety. Commercial shellfish beds in New Zealand are often closed due to the high levels of the marine biotoxin gymnodimine found in the shellfish. Gymnodimine contains a spiroimine ring system analogous to that present in the spirolides A-D that is thought to be the active pharmacophore in these toxins. The chemical synthesis of these toxins and partial structures of the molecules provides compounds for the development of pharmacological probes for the activation of L-type calcium channels. Blockage of such channels by drugs is exploited as a therapeutic principle to treat cardiovascular disorders such as hypertension and angina pectoris. We have recently synthesized the bis-spiroacetal moiety of the spirolides and spiroimine ring systems.[5,6]



Spirolide A: $\Delta^{2,3}$, R_1=H, R_2=Me
Spirolide B: R_1=H, R_2=Me
Spirolide C: $\Delta^{2,3}$, R_1=Me, R_2=Me
Spirolide D: R_1=Me, R_2=Me



Gymnodimine

Figure 4. Structures of the shellfish toxins spirolides and gymnodimine.

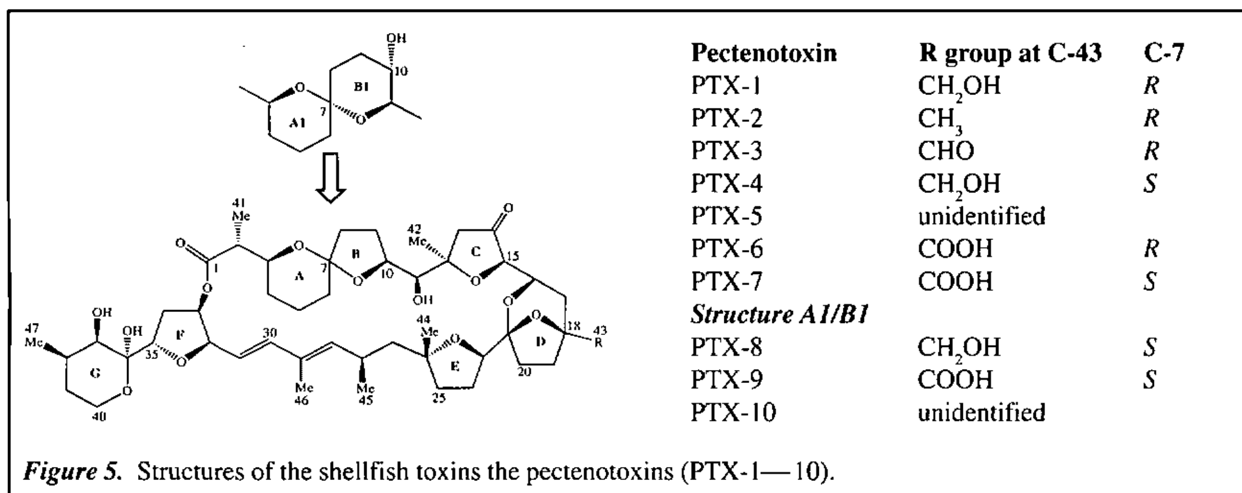
The pectenotoxins (PTX) (Fig. 5) are the agents chiefly responsible for the onset of severe diarrhoea and liver damage (diarrhetic shellfish poisoning - DSP) following the ingestion of contaminated mussels (*Mytilus edulis*) and scallops (*Patinopecten yessoensis*) in Japan and Europe, and are produced by the micro algae *Dinophysis acuta, D. fortii, D. accuminata,* and *D. caudate.* Physiological studies on pectenotoxin-2 (PTX-2, C43 = Me) revealed selective cytotoxicity against several cell lines representing ovarian, renal, lung, colon, CNS, melanoma, and breast cancers, with LC_{50} values in the nanomolar range and with differences in LC_{50} values between sensitive and resistant cell lines of 100-fold or more. Pectenotoxin-2 also interacts with the actin cytoskeleton at a unique site rendering it an important research tool in the study of basic cellular processes.

PTX-2 is an architecturally complex natural product that represents a formidable synthetic challenge in that the structure contains two spiroketals, three tertiary ethers, three substituted tetrahydrofurans, and 19 stereocentres embedded within a 40-carbon chain. This exquisitely complex structure, together with the intense demand for supply of the toxins by organic synthesis for further exploration of clinical utility and for establishment of methodologies to prevent the intoxications, renders pectenotoxin-2 a valuable synthetic target that we are is currently addressing.

Synthesis of the Human Telomerase Inhibitors, the Rubromycins, as Anticancer Agents

An important goal in devising novel treatments for cancer is the identification of targets that are uniquely and vitally important to cancer cells, but not essential or even present in normal cells. Attacking this critical target would afford the desired selectivity by killing the cancer cells while leaving normal cells unaffected. Existing cancer therapies generally have not fulfilled this goal because the targets of most therapeutic agents are not unique to cancer cells.

Telomeres are the natural ends of linear chromosomes, essential for maintenance of stable chromosomes, and are important for maintenance of the cell cycle clock. Tumour cells typically have shortened telomeres that are maintained by the highly specialized telomerase enzyme. Elevated telomerase levels are found in almost all human cancers



Pectenotoxin	R group at C-43	C-7
PTX-1	CH_2OH	*R*
PTX-2	CH_3	*R*
PTX-3	CHO	*R*
PTX-4	CH_2OH	*S*
PTX-5	unidentified	
PTX-6	COOH	*R*
PTX-7	COOH	*S*
Structure A1/B1		
PTX-8	CH_2OH	*S*
PTX-9	COOH	*S*
PTX-10	unidentified	

Figure 5. Structures of the shellfish toxins the pectenotoxins (PTX-1—10).

and levels frequently correlate with disease progression. The rubromycins (Fig. 6) are a unique class of antibiotics produced from a strain of *Streptomyces* that inhibit human telomerase. The novel aryl-fused spiroketal ring system present in the rubromycins has been suggested as the main structural feature responsible for the observed telomerase inhibition hence the synthesis of this structural motif has been undertaken in our laboratory.[7]

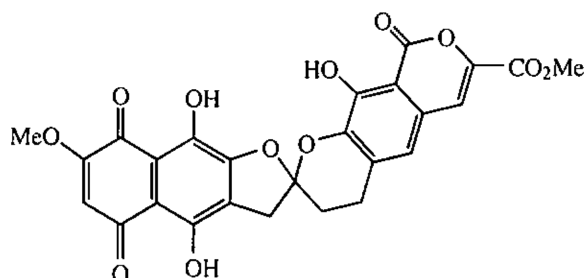


Figure 6. Structure of γ-rubromycin, a human telomerase inhibitor.

Synthesis of the Anti-*Helicobacter pylori* agent CJ-12,954

Recent studies have shown a relationship between gastric and duodenal ulcers and the presence of the microaerophilic spiral-shaped Gram-negative bacterium *Helicobacter pylori*, which is present in the mucus layer of the stomach. The natural product CJ-12,954 (Fig. 7), produced by *Phanerochaete velutina*, exhibits potent activity against *H. pylori* and is therefore a lead compound for the treatment of ulcers in humans. Recent research has been directed towards the first synthesis of CJ-12,954 and analogues in order to provide novel anti-ulcer agents.

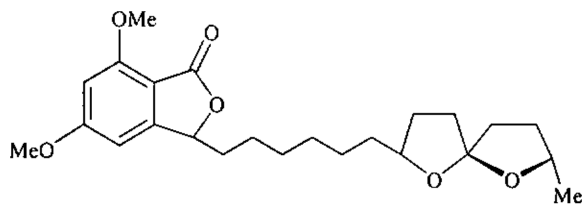


Figure 7. Structure of anti-*Helicobacter pylori* agent CJ-12,954.

Synthesis of Ligands for Nicotinic Acetylcholine Receptors Based Upon Methyllycaconitine

Work on the toxic components of *Delphinium brownii*, a cattle-stock poison in Western Canada, led to the identification of methyllycaconitine (Fig. 8) as the principal toxin. Both its toxicity and insecticidal activity have been attributed to its ability to act as a potent inhibitor that prevents binding of α-bungarotoxin to the nicotinic acetylcholine receptor (nAChR) in mammalian and insect neural membranes. At this subset of the nAChR, methyllycaconitine is the most potent small molecule antagonist yet reported. It is, therefore, a valuable neurobiological tool for the study of the comparative pharmacology of nicotinic acetylcholine receptors and is a lead compound for the treatment of Alzheimer's disease.

The synthesis of tricyclic ABE analogues of methyllycaconitine (Fig. 8) has been undertaken by us to

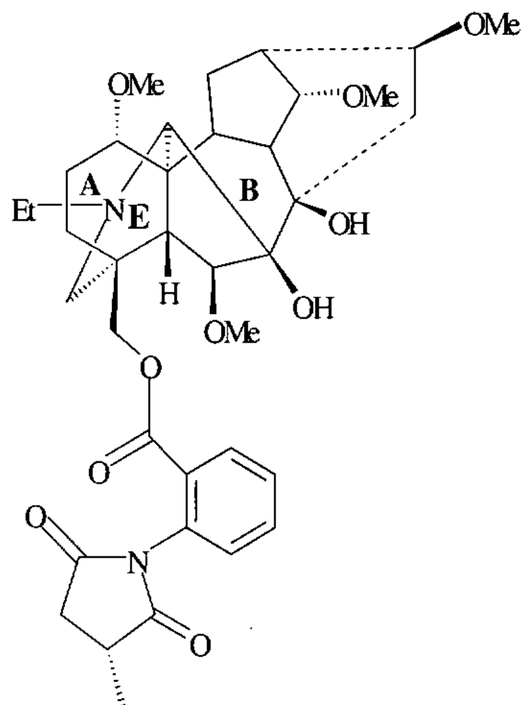


Figure 8. Structure of the alkaloid methyllycaconitine.

evaluate the pharmacological properties of these simpler analogues. To date our work has focused on assembly of the ABE 3-azabicyclo[3.3.1]nonane ring system using a double Mannich reaction. Appendage of a 7-membered B ring to the AE bicyclic framework has been accomplished using Grubbs' ring-closing metathesis. Use of a solid phase combinatorial approach to these molecules is also being undertaken on the recently purchased Advanced Chemtech 390 automated synthesizer (Fig. 9).

Commercial Synthesis of Peptidomimetics For the Treatment of Neurodegenerative Diseases

Professor Brimble also works at the interface of academia and the commercial world in that, since May 2001, she is a consultant and Head of Medicinal Chemistry for one of New Zealand's emerging pharmaceutical companies, NeuronZ Ltd. This commercial research is directed towards the synthesis of novel peptide mimics with potential for commercial development as therapeutic agents in the treatment of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. The NeuronZ chemists work directly with their in-house team of biologists who carry out the *in vivo* and *in vitro* screening of the synthetic compounds.

Synthesis of Glycopeptides as Components for Cancer Vaccines

The recent purchase of the Advanced Chemtech 390 automated synthesizer has also opened up a new field of research by facilitating the preparation of large peptides and glycopeptides. This instrument rapidly generates libraries of peptides and small organic molecules for drug discovery and development and is the only instrument of its kind in New Zealand. The immune system often recognizes tumour cells and infectious agents from the unique peptides on their surfaces, so synthetic peptides of similar structure can be used as vaccines to stimulate the

immune system. A collaborative project with Rod Dunbar (Biological Sciences) is based on the design and synthesis of peptides and glycopeptides for their ability to stimulate human T cells whilst being resistant to proteolytic degradation by common human proteases. Promising compounds will also be tested in an *in vitro* assay using human skin to model responses to vaccines for human injection, allowing the best compounds to proceed to clinical trials. This project represents a paradigm shift in our research from synthetic organic chemistry to the exciting field of chemical biology.

Figure 9. The Advanced Chemtech 390 automated peptide and small molecule synthesizer.

Acknowledgements

The author acknowledges the enormous contribution that her research team members, both past and present, have made to the synthetic work briefly described above. Several current members of the research group are pictured below.

Above: Some current members of the Brimble research group: Caryn Burgess, Janice Choi, Prabhakar Bachu, Kit Tsang, Dr. Michael Trzoss, Isabell Haym, Dr. Mark Levi, James Robinson, Darryl Crimmins, Dr. Paul Harris (NeuronZ), Scott Houghton.

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Author Profile

Professor Margaret Brimble was awarded the 2003-2004 Novartis Chemistry Award in recognition of outstanding contributions to natural products synthesis and the development of new synthetic methodology. She will give lectures on her academic research at five Novartis research sites in USA, UK, Japan, Switzerland, and Austria in May 2004. She is also one of the first recipients of the Rosalind Franklin Lectureship sponsored by The Royal Society to expose young scientists in the UK to a greater number of internationally recognized and successful women scientists; she will give several lectures throughout the UK in April 2004. She was also recently elected as President of the International Society of Heterocyclic Chemistry.

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POLYMER ELECTRONICS FOR THE 21st CENTURY

Jadranka Travas-Sejdic, Graham A. Bowmaker, and Ralph P. Cooney
Polymer Electronics Research Centre, The University of Auckland, Private Bag 92019, Auckland

Forrest L. Carter of the Washington DC Naval Research Laboratory first advanced the notion of computers based on molecular devices in 1982.[1] Nowadays, not many in the field of electronics have serious doubts about his statement that the electronics of the 21st century will be based on molecular chemistry/physics instead on semiconductor physics. As semiconductor devices approach their physical size limits, there is a drive to devise new ways to miniaturise components of electronic circuits, and the realization that single organic molecules can be employed as components in nano-scale molecular circuits has led, in recent years, to the establishment of the new interdisciplinary field of molecular electronics.[2,3] Thus we have been fortunate to witness significant discoveries in the field, such as molecular wires[4] and molecular switches,[5] but there are many more to come. The overlap of the field of molecular electronics with numerous areas of nanotechnology and biotechnology presents unprecedented opportunities for technological advancements in electronics, biology and medicine.

Polymer electronics, at the centre of the molecular nanotechnology revolution, holds the promise of an exceptional range of future research opportunities.[6] The award of the 2000 Nobel Prize in Chemistry to New Zealand-born Professor Alan MacDiarmid for his work on conducting polymers has given a high impetus to the field.

The Polymer Electronics Research Centre at The University of Auckland

The Polymer Electronics Research Centre (PERC) is a newly established Centre at The University of Auckland with the aim of promoting interdisciplinary activities in the broad area of polymer electronics, and facilitating appropriate collaborations and long-term alliances between researchers across the University. The Centre brings together for the first time in the University some fourteen academic researchers from Science, Medicine, and Engineering to develop new nanotechnologies based on polymer electronics. Many of these staff researchers have been active in the field for some time.

The work of the Centre focuses around the key themes listed below that range from fundamental research on cutting-edge projects, which may have commercial outcomes in the longer term, to shorter term projects aligned with well-defined industry needs.

- Health and Bioelectronics, *i.e.* the application of polymer materials to bioelectronic devices suitable for use in medical research and human health care. This area also includes the utilization of polymer materials in bioelectronic devices specifically designed for veterinary applications.
- Polymer Electronics in Devices, *i.e.* the development of devices based on molecular electronics, such as, sensors, photovoltaics, improved light-emitting diodes, and electrochromic windows.
- Molecular Circuitry, *i.e.* the development of molecular wires and molecular electronic switches based on conducting polymers and other organic materials.
- Corrosion, i.e. applications of conducting polymers in corrosion protection.
- Sustainable Energy, *i.e.* the development of conducting polymer-based materials for sustainable energy applications.

The Centre is striving to establish close working relationships with a number of leading international research groups. Thus, strong cross-institutional links with the *Interfaces with Molecular Materials and Macromolecules Unit* (SI3M Unit) at CEA (The French Atomic Energy Commission) in France are expected to follow the recent visit of Dr. Jadranka Travas-Sejdic (Director, PERC) to the CEA. Currently, the major collaborative effort with CEA is in the development of biosensor technology based on conducting polymers (see

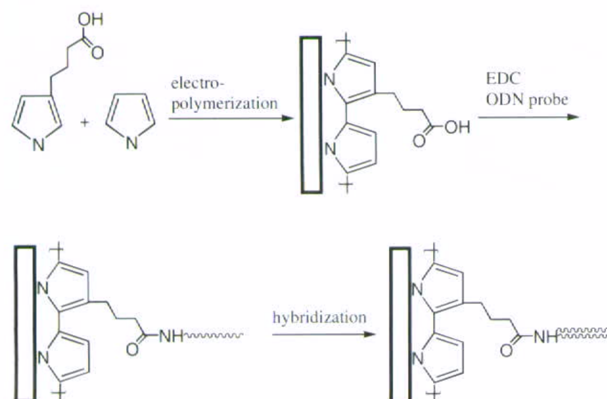

Figure 1. Schematic of the preparation of the electrochemical DNA sensor based on the functionalized polypyrrole.

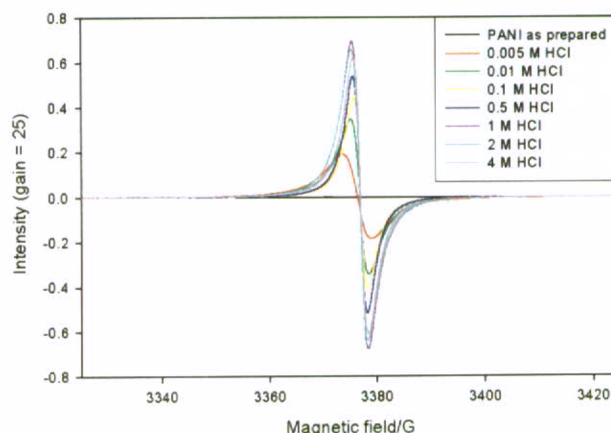

Figure 2. ESR spectra of HCl-doped polyaniline.

below). Other international linkages include the research groups at the Department of Chemical and Environmental Engineering at the National University of Singapore, with staff at Massachusetts Institute of Technology, the Intelligent Polymer Research Group at the University of Wollongong, and CNAM in France.

The Auckland Centre is also committed to the exchange of information and skills with national partners and collaborators that include Industrial Research Limited, the MacDiarmid Institute for Advanced Materials and Nanotechnology at VUW, and the Nanomaterials Research Centre at Massey University.

Current Research

Conducting polymer-based gene sensors

Analysis of gene expression using gene sensors is rapidly becoming the most powerful way to accurately diagnose diseases, discover mutations and monitor biotechnology processes in the post genomic era. Application of conducting polymers in medical sensors is a new and promising area of research and requires cross-disciplinary expertise. The research in this field is an intersection of several revolutionary developments of modern science: materials, molecular-level electronics and molecular biology. The current project on the development of gene sensors based on conducting polymers is a collaborative effort of scientists from Chemistry, Physiology and Electrical and Electronic Engineering.

Conducting polymer substrates functionalized with DNA and oligonucleotides (ODN) can provide sensors for gene fragments that allow the quantitative detection of unlabeled fragments with high specificity and sensitivity. The detection of complementary DNA sequences is based on a change in the opto-electronic properties of conducting polymers caused by a hybridization event that is based on the complementary principle of the base pairing between probe ODN sequence and target DNA sequence. Such DNA sensors are expected to have a number of advantages over the conventional detection methods. Conducting polymers can be easily electrodeposited on the electrode surfaces thereby allowing miniaturization. Versatility of monomer/polymer functionalization provides a suitable interface for immobilization of biological sensing elements. Moreover, intrinsically conducting polymers are active substrates that also act as a *molecular wire* and therefore provide a direct connection between the sensing element and the electronic transducer.

In this project, we have been investigating suitable conducting polymers for DNA sensors and have synthesized a new conducting copolymer, poly[pyrrole-co-4-(3-pyrrolyl)]butanoic acid, and a DNA sensor based on this polymer has been constructed.[7] Figure 1 shows a schematic of the preparation of the electrochemical DNA sensor based on the functionalized polypyrrole.

Upon hybridisation with complementary ODNs, changes in the electrochemical properties of the sensor films have been observed. In particular, the viability of the sensor is illustrated from significant changes in cyclic voltammograms and AC impedance spectra after hybridisation with complementary ODNs. The development of a DNA sensor based on the changes in optical properties of conducting polymers is underway.

Spectroscopic studies of variously doped polyanilines and their radical savaging properties

Polyaniline (PANI) has attracted considerable attention over the last 10 years due to its straightforward polymerization, chemical and environmental stability and high electrical conductivity. Doping and oxidation levels are two of the most important factors affecting the electrical conductivity and other properties of PANI. Doping with protic acids and various oxidants can be followed by infrared, electron paramagnetic resonance (EPR) and nuclear magnetic resonance (NMR) spectroscopy. Figure 2 shows EPR spectra obtained for PANI doped with HCl. The plots illustrate an increase in polaron level in PANI as the doping level is increased by exposure to increased concentrations of HCl.

The potential antioxidant activity of conducting polymers has been also studied by determining their radical scavenging properties using the free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH) that is stable and an ideal model. The antioxidant property of conducting polymers is potentially important in biomedical applications, such as drug delivery systems and tissue engineering. UV, FTIR, NMR, and EPR spectroscopy are used to monitor the electron-transfer processes involved[8] and cross-polarisation ^{13}C magic angle spinning (CP MAS) NMR studies before and after the reaction of PANI with DPPH[9] serve to illustrates the changes induced (Fig. 3).

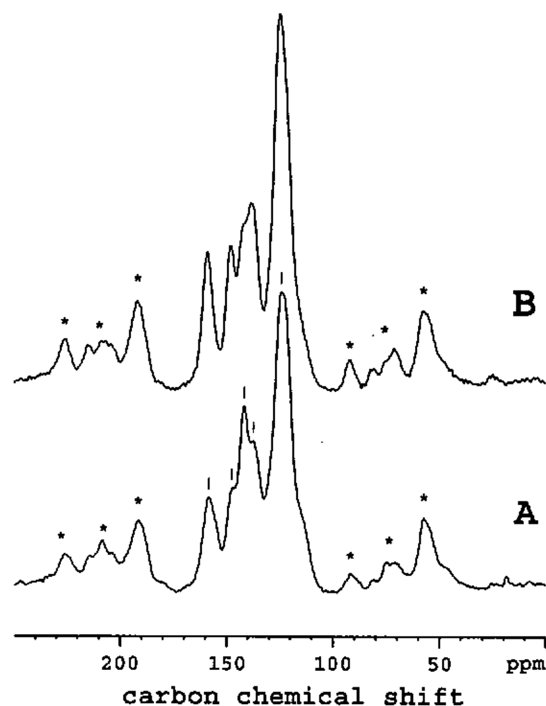

Figure 3. CP MAS spectra of **A**) PANI and **B**) PANI after reaction with DPPH.

In-situ ***spectroscopy studies of the polypyrrole doping-undoping cycle***

The brief of this project is to investigate the mechanism of action of ICP gas sensors. Specifically, we are interested in whether the presence of redox-active transition metals incorporated in the ICP matrix alters their response characteristics. With these goals in mind, we are attempting to

use molecular spectroscopic techniques that have the potential to provide *in-situ* evidence of changes to the ICP structure and oxidation state in the presence of gaseous analytes.

Thus far, we have predominantly focussed on the Raman spectroscopy of polypyrrole (PPy). Specifically, we have undertaken *in-situ* experiments under electrochemical control of PPy with various dopants. In such experiments there are, indeed, spectroscopic parameters that give reliable indications of the change in oxidation state of the ICP. In addition, it appears possible to monitor the oxidation state of incorporated transition metal dopants such as hexacyanoferrate, although difficulties exist for such *in-situ* electrochemical experiments because of a tendency for the dopant to be lost from the PPy matrix during redox cycling. We are now attempting to positively correlate various Raman peaks with cationic and dicationic charge carrying species of PPy using *in-situ* EPR. We believe that the results from such experiments may be extrapolated to the events taking place in ICP films that act as gas sensors. PPy films exposed to analytes such as ammonia do indeed show changes in the Raman spectrum that can be interpreted in terms of doping and undoping, and changes to the length of the polymer conjugation.

Studies of poly(3,4-ethylenedioxythiophene) doping

A study of the effect of different dopants and doping levels in chemically prepared poly(3,4-ethenedioxythiophene (PEDOT) dispersions, the way the dopants are incorporated in the polymers, and the resulting electrical conductivity properties is being made. In addition to conductivity studies, EPR, FTIR and Raman spectroscopy are employed for that purpose. It has been shown[10] that PEDOT pre-doped with sulfate can undergo both secondary doping and dopant-exchange processes when treated with iodine/triiodide solutions. Raman spectra of such samples reveal a correlation between the shifts in the position of the symmetric C=C stretching band and the overall doping level in the polymer.

Multilayer composite films basedup on CPs

Multilayer thin films comprised of sequentially adsorbed layers of polyaniline and polyanions (sulfonated polystyrene), a functionalized C_{60} derivative, and an oligonucleotide were fabricated via the use of a simple layer-by-layer technique. The electrostatic attraction between the oppositely charged polymer species is used as a driving force for the formation of the multilayers. The method is very simple: a substrate (pre-cleaned glass substrates, ITO slides), having some surface charges, is immersed in an aqueous solution of a species carrying the opposite charge and this generates an adsorbed monolayer on the surface. After rinsing, the sample is again dipped into an aqueous solution of a compound with the opposite charge. This leads to adsorption of a second monolayer. The repetition of this deposition process then results in multilayer formation. The building up of the multilayer films was characterized by measuring the increment of the adsorbed amount by UV-visible spectroscopy. The electrochemistry of the bilayer films deposited on the ITO slides has been investigated using cyclic voltammetry. The potential application of such thin films is in photovoltaic cells where fast charge separation between electrons and holes is required.

AC Impedance studies on conducting polymers

The AC impedance spectroscopy of conductive polymers gives a good indication of the conduction mechanisms of these materials. Various attempts have been made to model the observed impedance spectra through the construction of equivalent circuits representing the various processes thought to affect the conductivity of the conductive polymers. However, none of the available models have proved to be very accurate. The aim of this collaborative project between Chemistry and Electrical and Electronic engineering, is to develop a procedure for the impedance modelling of conductive polymers.

Polyaniline anti-corrosion coatings on aluminium

The nature of the interaction of polyaniline with aluminium surfaces and its potential to improve the corrosion resistance of aluminium is being studied. We have been characterising (by X-ray photoelectron spectroscopy) the chemical interaction between PANI and the surface oxide on aluminium, and the influence of the metal surface pre-treatment on this interaction.

Bright Future

It seems certain that advances in modern electronics will be strongly dependent on the development of molecular electronics. However, the benefits will be not limited to the area of electronics alone, but will apply equally to developments in areas of nano/biotechnology and health, such as intelligent miniaturized sensors or molecular machines for cellular-level reaper. At the present time, it is hard to predict the precise nature of such future developments, but it is certain that polymer electronics (and more broadly molecular electronics) is an exciting and highly promising new field of research that will change our everyday life in the future.

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Patent Proze

By John Landells and Helen Palmer

"Reach-Through" Patent Claims and Research Tool Patents

Wouldn't it be useful for the owner of a patent covering a biological research tool if he or she could also obtain patent protection for future "downstream" research products based on that biological research tool?

For example, as part of your research, you may have discovered and isolated a novel enzyme that has a particular function. You can patent the isolated enzyme. If your enzyme can be used in a particular assay and you can describe the assay method, you may also be able to patent the assay. But your scope of protection could be increased even more if you could also claim in your patent a series of compounds discovered using that assay.

Such a claim is known as a "reach-through" claim, and relates to products or compounds identified using a screening assay. This type of claim "reaches through" the scope of the other patent claims, and endeavours to obtain protection for something that the inventor has not yet shown how to make.

A reach-through claim could strengthen the patentee's rights over future discoveries, and in view of the crucial role that patents play in the burgeoning field of biotechnology, obtaining such protection might be desirable for patent owners. However, there is fine line between obtaining the maximum protection possible, and crossing over into areas that the inventor has not yet fully investigated.

The courts have endeavoured to strike a balance so that the patentee can obtain the protection to which he or she is entitled, without allowing the scope of this protection to be so broad as to encroach into areas that the patentee has not touched upon.

In the US case of *University of Rochester v G D Searle Inc.* (US patent 6,048,850) the inventors had discovered the gene encoding the mammalian prostaglandin H synthase-2 (PGHS-2). The patent disclosed methods of assaying for PGHS-2 inhibitors and tests of some well-known compounds such as aspirin. However, no particular inhibitors were identified. The claims of the patent were reach-through claims directed to methods of inhibiting PGHS-2, and encompassing methods of treatment employing any PGHS-2 inhibitor.

The court in *University of Rochester* took the view that the patentee was attempting to obtain protection for something that had not been fully investigated. Nowhere did the patent specify which compounds were inhibitors of PGHS-2 and although the patent disclosed an assay for identifying a compound as a PGHS-2 inhibitor, it merely identified categories of compounds that might work. The patent specification did not provide fair basis for the reach-through claims. The court said "what the inventors did not do, however, is succeed in taking the last, critical step of actually isolating such a compound" and held that the patent was invalid.

As the *University of Rochester* case shows, reach-through claims can raise a number of patentability issues. Where then does this leave a patentee in terms of the scope of protection that he or she can obtain for a research tool?

The recent US case of *Integra v Merck* has provided some comfort for owners of patents relating to research tools. In that case, researchers at Scripps Research Institute (under an agreement with Merck) conducted pre-clinical tests for the identification of potential drug candidates to inhibit angiogenesis. The researchers used particular peptides that had been patented by Integra. Integra sued Merck for patent infringement.

The issue that the court had to resolve in *Integra* was whether the pre-clinical tests were exempt from liability for patent infringement under the US Hatch-Waxman Act (1984). This Act permits competitors to conduct experiments in advance of patent expiration, as long as those experiments are reasonably related to securing regulatory approval. This is similar to the recent amendment made to the New Zealand Patents Act (see Patent Proze in *Chemistry in New Zealand*, **67**:2 (June 2003). The Court of Appeals for the Federal Circuit narrowly construed the Hatch-Waxman Act, and recognised that patented research tools are valuable. It held that the activities by the researchers did amount to patent infringement.

It seems that the courts, at least in the US, have limited the scope of protection available for research tool patents so that reach-through claims are likely to be held invalid. However, the decision in *Integra* does strengthen the position of patentees in that pre-clinical testing using a patented research tool will be patent infringement, rather than falling within one of the infringement exceptions.

Whilst *Integra* is good news for owners of research tool patents in the US, the situation in New Zealand remains unclear. We have the recent amendment to the Patents Act which allows experimentation for the purpose of developing information required for regulation of the manufacture or sale of products. However, it is unclear how the New Zealand courts would interpret this provision in relation to research tool patents.

A reminder: if you have any queries regarding patents, or indeed any form of intellectual property, please direct them to:

Patent Proze
Baldwin Shelston Waters
PO Box 852, Wellington
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John Landells

Helen Palmer and John Landells of Baldwin Shelston Waters specialise in chemistry and biotechnology patents. Helen joined BSW in 2000. She has a PhD in chemistry from The University of Auckland and postdoctoral research experience. John joined BSW in 2003. He has a PhD in chemistry from the University of Otago and is in the final stages of completing an LLB at Victoria University of Wellington.

Helen Palmer

WASTE TYRE DISPOSAL ISSUES FOR NEW ZEALAND

Alistair G. Bingham
JCL Air & Environment Ltd, PO Box 27-251, Mount Roskill, Auckland

The late John Asher, Professor of German at The University of Auckland, recounted his experiences as a student in Switzerland just after the end of the Second World War at a German Academic Exchange reunion in Auckland in 1985. One of his more poignant memories involved a trip to the German side of Basel where the rusting hulk of a bombed out Reichstrain bore the slogan Räder Müssen Rollen! (Wheels must roll!).

Nowadays most wheels do not roll on rails but on roads, thanks largely to three inventions: John Loudon MacAdam's revolutionary improvements to road construction (1816), Charles Goodyear's vulcanisation of rubber (1839), and John Dunlop's pneumatic bicycle tyre (1880). Modern Society's subsequent love affair with the car sees an annual global production of between 700 million and 1 billion tyres[1] most of which do not last for the lifetime of the vehicle and almost all of which become a waste for disposal when the car is scrapped. Used tyres present a significant waste-handling problem for nearly every developed country. New Zealand is fast waking up to the fact that it too must face quite serious waste tyre disposal issues.

The Scale of the Waste Tyre Problem

In the US two to three billion scrap tyres are in landfills and stockpiles and approximately one scrap tyre per person is generated every year.[2] Australian waste tyre production in 2000 was estimated to be at a similar per capita rate to that of the US.[3] New Zealand's relatively high rate of vehicle ownership, coupled with the importation of a significant proportion of its vehicle fleet as second hand units, suggests that here too waste tyre production is probably at the rate of one tyre per person per year. A recent report to the Inter Regional Committee of New Zealand's Regional Councils estimated that there were currently 3–3.3 million waste tyres generated for disposal in this country.[4] However, vehicle numbers in New Zealand continue to increase significantly on an annual basis as shown in Figure 1. From 1990 to 2000 passenger car and van numbers increased from 920,000 to 2,180,000,[5] and cars continue to be imported in ever increasing numbers. Car registrations are 14% higher at the time of writing (December 2003) than they were a year ago and are estimated to be 230,000 for the year – about 140,000 of which are used cars.[6] However, vehicle numbers are only one part of the indicators for the increasing tyre waste equation. The other important parts are the weight of the tyre and the number of kilometres it has been driven.

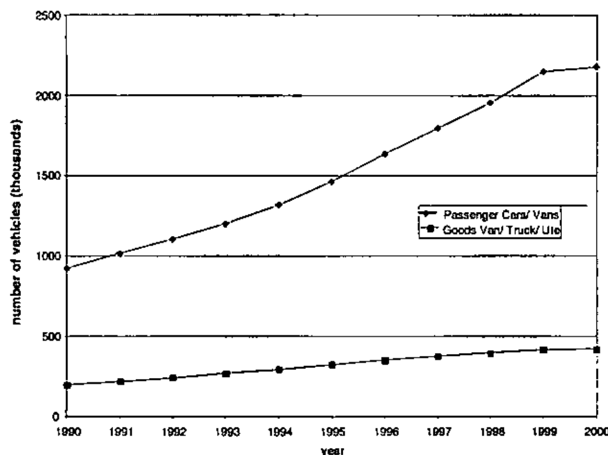

Figure 1. Changes in New Zealand Vehicle Numbers 1990-2000.

Tyres come in a variety of shapes and sizes and vary considerably in weight. In Great Britain, although 87 per cent of tyres in use are on cars, they only comprise about 70% of tyres by weight. On the other hand, tyres on heavy goods vehicles, which account for only three per cent of tyre numbers, comprise 20% of tyres by weight.[1] Tyre weight data is not available for New Zealand, but the goods van/truck/utility class of vehicle monitored by the Ministry of Transport has retained a constant 14% share of the total vehicle fleet with numbers increasing 8.8% from 1990 to 2000.[5]

The Ministry for the Environment has commented that the kilometres travelled by heavy commercial vehicles has increased by about 64% over the last two decades, attributing the cause to the deregulation of the rail freight industry in the early 1980s and the removal of the 100 km limit for transporting goods by means other than rail. Over the same period private cars travelled nearly twice as many kilometres – attributed to people using their vehicles for longer and/or more frequent journeys.[7]

Even within different vehicle fleet types significant changes are occurring. For instance, the types of vehicle that comprise New Zealand's passenger car category have changed markedly over the last five years. One vehicle in six is now of the Sports Utility or 4-wheel drive class. These vehicles typically have greater kerb-side weights and tend to have large, wide profile, all terrain tyres. In general, it is also fair to say that the average motorist is driving a larger car with concomitantly larger sized tyres than they would have been ten years ago. We need look no further than the new generation Mini and Volkswagen Beetle that have significantly larger tyres than their predecessors. Consequently, the combined effects of increased vehicle numbers across all major classes, increased aggregate tyre weight within classes, and increased number of kilometres driven will ultimately lead to increases in waste tyre production.

Current Disposal Practices for Waste Tyres

OECD countries

The fate of waste tyres in a number of OECD countries as at 1996 is shown in Table 1.[3] The *Recycling* option in the table refers predominantly to the conversion of the tyre material into crumbed rubber for use as a roading material additive and in safety surfaces. Even amongst OECD countries there are significant differences in the uses to which waste tyres are put. The US is now in the position of either recycling or recovering the energy from all the waste tyres it produces and has started to consume the vast quantities of tyres held in its stockpiles. On the other hand, France still sends 45% of its waste tyres to landfills.

From July this year EEC Directive 1999/31/EC prohibited the disposal of whole tyres to any landfill in European Community countries. Disposal of shredded tyres will be prohibited from July 2006.[1]

Australasia

An Australian study estimated that its waste tyres were disposed of in the following proportions:[3]

- landfill 57%,
- energy recovery 22%,
- inappropriate disposal 13%,
- recycling 5% and
- other uses 3%.

There are no firm data on the utilisation and fate of New Zealand's waste tyres but tyre recyclers based in Auckland and Wanganui use a small proportion. Anyone travelling through rural Waikato will see that many are still used for holding down the covers on silage pits. A recent *New Zealand Herald* article indicated that the growth of imports of second-hand tyres had stifled local re-treading activity. The article also reported that New Zealand and Russia are now the world's largest importers of used tyres with New Zealand importing 500,000 used tyres p.a.[8] It is probable that land filling of whole and shredded tyres and inappropriate disposal in the form of open tyre dumps are the predominant fates of waste tyres in this country. Over the last few years there have been several well-reported instances of *illegal* disposal and large stockpiles of tyres being abandoned. A number of these problems have arisen in the Waikato, possibly because of its proximity to Auckland – the largest single source of tyres – and comparatively lower land prices.[4]

New Zealand authorities are aware of the difficulties that inappropriate management and disposal of waste tyres pose. The 2002 New Zealand Waste Strategy, developed in partnership between the Ministry for the Environment and Local Government New Zealand, described the concept of extended producer responsibility (EPR) and proposed its application to special wastes including tyres. Used tyres are a priority for the Ministry's Sustainable Industry Group for the year 2003-04, and the Ministry is working with industry, council, motor trades and end-user groups to identify sustainable management practices. At present the Ministry's preference for excess waste tyres is disposal to landfill or storage in appropriately constructed facilities.[9,10]

Tyre Chemistry

Understanding the environmental issues surrounding waste tyres and their disposal requires some knowledge of the chemistry associated with tyre production. The major raw materials for modern tyre production are rubber compound, steel, and textile. Charles Goodyear's serendipitous discovery of the vulcanisation process in 1839, whereby heating rubber and sulfur together at high temperature resulted in a product that was no longer brittle at high temperatures, nor sticky at low temperatures, paved the way for the modern rubber industry.

Prior to the 1930s, tyres were made solely from natural rubber, a polymer of isoprene. Natural rubber is obtained from the latex discharged on cutting the bark of the Hevea tree (*Hevea brasiliensis*), originally native to South America, but since the 1880s grown in extensive plantations in Thailand, Indonesia, and Malaysia. The natural rubber plantation industry still produces some 6.4 million tons annually, which represents between 30 to 40% of total rubber usage.[1,3,11] The occupation of the Malay peninsula by the Japanese in World War II forced the United States to rapidly develop its synthetic rubber industry (which before had been restricted to Germany and Russia). Today, a wide variety of different rubber types are produced from the polymerisation of a number of petrochemical feedstocks. More than half the world's synthetic rubber is styrene-butadiene rubber (SBR) made from styrene and butadiene monomers, and three quarters of this goes into tyre production.[11]

Although tyres are commonly, and mistakenly, perceived solely as a product of modern petrochemical industrial processes, a significant proportion of certain tyre types still employ natural rubber in their composition either wholly or at least in part. This is because natural rubber often has superior performance to synthetic rubber types in applications that demand elasticity, resilience, tackiness, and low heat build-up. For instance natural rubber is indispensable for the treads of tyres for racing cars, bus and truck tyres, aeroplane tyres, and any other form of transport where conditions are particularly severe and a build up of heat inside the tyre could cause a failure.[11]

Table 1. **Fate of Waste Tyres in Selected OECD Countries.**[3]

	France	Germany	Italy	UK	Belgium	Netherlands	Sweden	USA
Retreading	20	17.5	22	31	20	60	5	-
Recycled	16	11.5	12	16	10	12	12.5	28
Energy	15	46.5	23	27	30	28	64	72
Landfill	45	4	40	23	5	0	5	-
Export	4	16	2	2.5	25	NA	7	-

In addition to synthetic and natural rubber, rubber compound is made from the following chemicals:[1]

- carbon black (typically 26%) for use as a filler to strengthen the rubber compound,
- oil, (typically 9%) to act as a processing aid, plasticizer, and extender,
- zinc oxide (typically 2%) to enhance and control the vulcanisation process,
- sulfur (typically 1%) to provide the cross linking during vulcanisation,
- antioxidants – either amines, such as dimethylamine, or substituted phenols and dibenzyl disulfide,
- vulcanisation accelerators such as 2-(morpholino)thiobenzothiazolesulfenamide (MBS), ziram, 1,3-diphenylguanidine, 2,2´-dithiobis(benzothiazole),
- stain protectors such as 2,5-di-*t*-pentylhydroquinone,
- dessicants such as calcium oxide,
- colouring pigments such as iron oxide, and
- flame retardants such as zinc metaborate dihydrate and sodium borate.

The other main constituents of a tyre are steel and textile. Steel wire is used to make the tyre's beads and to reinforce the rubber. Bead wire is the enclosed circular ring of wire on the inner rim of the tyre, which assists the tyre in forming an airtight seal on the wheel hub. Radial wire strands are used to provide strength and rigidity to the main portion of the tyre. The diameter of bead wire is significantly greater than that of the radial wire. In addition, nylon and polyester and rayon plies (belts) can comprise up to 3-4% of a tyre in both car and truck tyre types.[12]

During tyre manufacture the component rubber parts that comprise the tyre are made separately. The principal parts are the sidewalls, beads, and tread. The rubber compound and other chemicals listed above are mixed together, the fabric and steel wire are coated and the parts shaped. The final step involves linking the beads together then adding the sidewalls and the tread. The structure is then put in a press containing a mould of the desired tread and a rubber bladder is inflated to give the right shape. Heating to 140 °C and applying pressure forms the tyre. The heat activates the vulcanisation process and the pressure causes impression of the tread.[1]

Problems Associated With the Current New Zealand Practice of Land Filling and Storing Waste Tyres

The disposal of whole tyres in landfills can lead to a number of waste handling and engineering problems. For instance, whole tyres reportedly *float* in the compacted waste and may destabilise a landfill and impact on its suitability for future use.[3] Whole tyres have large *voids* (approximately 75% of the volume of a whole tyre is void) and these consume the available space. Landfill space is a resource and is limited in many areas. Waste tyres are generated at greater rates where the population is highest and these are precisely the areas where landfill space is limited. In Australia on a worst-case whole tyre basis, the volume occupied by tyres disposed to landfill represents 8% of the volume of solid waste.[3]

There is, therefore, a trend away from accepting unshredded tyres for landfill worldwide. Tyres in the Auckland region are shredded prior to landfill, and an increasing number of landfills in the lower North Island are also using shredding services.[13] Tyre shreddings are often being used for landfill drainage covers, and it is only excess material that requires disposal.[13]

Accidental fires associated with waste tyre storage

Accidental fires in tyre dumps can be prolonged because of the difficulty fire fighters often have in extinguishing the fire. The poor combustion conditions prevailing result in the discharge of thick palls of smoke-laden particulate matter. Additionally, these fires can discharge significant quantities of hazardous air pollutants including polynuclear aromatic hydrocarbons (PAHs), polychlorinated dibenzo-*p*-dioxins and dibenzofurans (dioxins and furans), hydrogen chloride, benzene, polychlorinated biphenyls (PCBs), and metals such as arsenic, cadmium, nickel, zinc, mercury, chromium and vanadium. These emissions can represent significant acute (short term) and chronic (long term) health hazards to fire fighters and nearby residents. Depending upon the length and degree of exposure, the health effects could involve irritation of the skin, eyes, and mucous membranes, respiratory effects, central nervous system depression, and cancer. Open tyre fire emissions are also estimated to be 16 times more mutagenic than residential wood combustion in a fireplace and 13,000 times more mutagenic than the emissions from a modern coal-fired power station.[2]

Smoke discharges to air are not the only environmental effect caused by tyre fires. Equally serious are potential contamination of land and surface and groundwater by the liquid breakdown products. In February 2002 a fire in a tyre stockpile at an unauthorised cleanfill site near Rotowaro affected with smoke nearby residents including a child who was hospitalised. However, the fire also produced a liquid oil that was difficult to contain. In total 3,000 litres of this material was collected from the fire.[4] Globally, many tyre fires have proved just too tenacious to extinguish. For instance a fire started in 1989 in a tip containing 10 million buried tyres at Knighton Powys in Wales, is still burning underground.[1]

Another potential problem may exist in New Zealand rural areas. Under the Forest and Rural Fires Act (1977) responsibility for fire fighting rests with the Rural Fire Authority, which is usually the District Council. They operate under the mandate: *"if...the fire constitutes a hazard to life or property, endeavour by all practical means to extinguish the fire and prevent the spread thereof and to save lives and property in danger"*. It is not certain whether the National Rural Fire Authority would fund fire fighting for a tyre fire in a rural area that is not a threat to adjacent property or life.[4]

Waste tyres as breeding grounds for mosquitoes, vermin, and weeds

Tyres trap water and this in turn provides a breeding ground for mosquitoes.[14] This has the potential to become a serious health risk if cavity breeding, disease bearing mosquitoes become established in New Zealand. Outdoor tyre stockpiles can also provide a habitat for weeds, and a breeding ground and habitat for vermin such as rats if appropriate food sources are in or near the stockpiles.[3]

Alternatives to Disposal – Reduce, Reuse, Recycle

The mantra for waste minimisation formerly known as *The Three R's* (Reduce, Reuse or Recycle), has been updated to *The Five R's* (Reduce, Reuse, Recycle (material), Recover (energy), and Residual disposal).

Waste tyre reduction

Reducing the number of waste tyres produced has a great deal to do with using existing tyres more efficiently. The Environment Agency of England and Wales has suggested a number of options and practices for achieving more efficient tyre use – some readily palatable to the average motorist and some less so:[1]

- Reduce the number of road vehicles,
- Reduce the distance travelled by road vehicles,
- Improve the maintenance of tyres with frequent checks on tyre pressure,
- Improve car maintenance to prevent unnecessary wear on tyres,
- Increase the use of re-treaded tyres,
- Ensure that all new tyres are manufactured to be re-treadable,
- Increase the durability of tyres without compromising safety,
- Replace tyres as soon as they reach the minimum legal tread depth,
- Improve driving standards – reducing speeds on bends puts less sideways force on tyres.

New Zealand's current practice of importing large volumes of second hand tyres, which are sold at a discounted price, has a compounding negative effect on any likelihood of a reduction in waste tyre numbers. Firstly, the projected life of these tyres will be shorter than that of a comparative new tyre and, secondly, the availability of cheap tyres directly attacks the market niche occupied by retreaded tyres.

Re-use/Recycling of Waste Tyres

Retreading

Retreading is the only method that re-uses tyres for their original purpose. With the exception of some suitable truck tyres that may have the tread physically re-grooved, retreading involves the physical removal of remaining tread rubber and its replacement with a new tread. As shown in Table 1, a significant proportion of waste tyres are retreaded in many OECD countries. About 6.7 million retreaded car tyres were produced in the UK in 1995 compared with about 29 million new car tyres. However, the proportion of retreaded truck tyres was significantly higher - 1.5 million vs. 3.3 million new truck tyres.[1]

Granulated rubber

Used tyres can be broken down into granulated or crumbed rubber particles either by mechanically grinding whole tyres or by cooling fragments in liquid nitrogen. The granulate has a variety of uses including road and sports surfaces, impact absorption areas in children's playgrounds, porous hosepipe, rubber boots, and carpet backing. A small percentage of granulate (<5%) is used in new tyre manufacturing where it confers benefits to the production process.[1] In the US, legislation enacted in 1991 requires that at least 20% of all federally funded highways be built with asphalt containing 1–3% crumbed rubber. Research into the use of rubber in roads is also underway in New Zealand,[13] but currently it is used here largely for children's playgrounds, horse arenas, and for industrial matting. A new tyre crumbing operation being established in Auckland is understood to be looking at markets for product within New Zealand and abroad.[13]

Physical reuse of tyres

Partly due to their abundance and durability, used tyres are used in a number of secondary applications. These include silage-sheeting clamps, use as fenders to protect the sides of boats, and in various engineering applications such as artificial reefs and flood defence. Ultimately, tyres used in this way must still be disposed of. For instance tyres used as silage clamps will last for 10 to 15 years after which the rubber compound will begin to degrade resulting in the tyre crumbling and exposing steel reinforcing. This can then present a hazard to livestock.[1]

Canadian researchers have found that tyres submerged in fresh water initially produce leachate which is toxic to rainbow trout.[15]

Energy recovery

In the US tyre derived fuel (TDF) is generally defined as a scrap tyre that has been shredded and processed into a rubber chip with a range in size of <50 mm to 100 mm. TDF may also be processed to remove bead wire and radial wire, which can comprise up to 10% of a tyre's weight.

TDF represents an ideal fuel source because its moisture content is low (1-3%) thereby reducing the amount of energy required for moisture vaporisation.[16] Its energy value is high with a volatile content of roughly 66%, indicating rapid heat release, and a relatively low ash content (3-5%). In many respects TDF is a superior solid fuel to coal. It has a higher heating value and lower moisture content. TDF contains more carbon, about as much sulfur as medium-sulfur coal but much less fuel-bound nitrogen which can result in a significant reduction in NO_x emissions.[16] TDF does contain, however, significantly higher levels of zinc than coal. Zinc is added to control vulcanisation, assist blending, and provide UV resistance. Zinc oxide makes up about 1.2% of a passenger tyre.[3] In modern, well designed combustion facilities zinc emissions from TDF combustion tend to have their highest concentrations in the fly ash with little or no zinc in the bottom ash. Both electrostatic precipitator and bag-house type air pollution control technologies are very effective in capturing the fine particulate matter with which zinc emissions are associated.[3] Tables 2 and 3 show a comparison of gross composition and elemental ash composition for typical TDF and coal analyses.[2,16]

Of the 192 million tyres used for energy recovery in the United States in 1998, 30% were used in cement kilns, 20% in boilers in the pulp and paper industry and a further 20% in other utility boilers.[3] In such cases TDF is used as a supplementary fuel, typically comprising about 10% of the total fuel feed. Only 8% of tyres were combusted in

Table 2. **A comparison of gross composition parameters for TDF and coal.**

Fuel Type	Composition (%)							Heating value (kJ/kg)
	Carbon	Hydrogen	Oxygen	Nitrogen	Sulfur	Ash	Moisture	
TDF	83.9	7.09	2.17	0.24	1.23	4.78	0.62	36,023
Coal	73.9	4.85	6.41	1.76	1.59	6.23	5.24	31,017

Table 3. **A comparison of elemental ash composition for TDF and coal.**

Fuel Type	Elemental Ash Analysis (% oxide form)							
	Aluminium	Calcium	Iron	Sulfur	Phosphorous	Titanium	Silicon	Zinc
TDF	13.11	3.80	2.37	6.72	0.68	0.95	35.05	34.81
Coal	20.70	3.30	18.89	4.33	0.62	0.82	47.98	0.02

dedicated tyres to energy facilities. Ironically tyres are not produced in sufficient quantities in the areas surrounding commercial combustion facilities to sustain demand and the economics of waste tyre transport then come into play. A pulp mill in Tasmania investigating the use of tyres as fuel in its boilers estimated that the total annual Tasmanian supply of waste tyres could be consumed in only 2- 3 months of operation.[3]

Greenhouse gas issues associated with waste tyre combustion

The type of tyres used, and to some extent their age, will determine to what extent TDF can be regarded as a renewable resource and thus not a net greenhouse gas contributor. In Australia, when total tyre consumption is taken into consideration, natural rubber is seen to comprise about 30% of total rubber usage. Consequently, TDF is seen in Australia as contributing about 75 kg greenhouse CO_2/GJ compared to a value of 90 kg greenhouse CO_2/GJ for coal.[3]

Summary

New Zealanders are producing waste tyres at an ever increasing rate. The currently preferred New Zealand option of disposal to landfill uses valuable landfill space especially in highly populated areas where it is at a premium. In addition the tyre's material is lost forever as a resource for new products or energy recovery. Unauthorised tyre storage facilities present the risk of potentially significant environmental effects, particularly from fires. The numbers of waste tyres sent to landfill or storage can be reduced by:

- Strategies designed to extend a tyre's lifetime including re-treading and driver education.
- Re-using the tyre in new products such as supplementing asphalt with crumbed tyre rubber.
- Recovering the energy of the tyre through its use as a supplementary fuel in modern well-designed boilers in a range of industries.

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HYPOXIA-ACTIVATED PRODRUGS: A NEW APPROACH TO CANCER CHEMOTHERAPY

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Introduction

Following a large research effort over the last 20 years, there is some evidence that improvements in cancer chemotherapy are assisting in improving survival rates in some solid tumours in adults. However, the gains have not been sufficient to reverse the trend of an increasing incidence and mortality as the population ages. A major cause of this limited success is the narrow therapeutic index of typical systemic cytotoxic (DNA-targeted) agents. While these have advantages as anticancer drugs, especially their ability to kill large numbers of cancer cells, they are not truly selective, and their therapeutic efficacy is limited by the damage they also cause to proliferating normal cells such as those in the bone marrow and gut epithelia. This is particularly true in the treatment of solid tumours, where the majority of the tumour cells themselves are not dividing rapidly. With this in mind, there has been a sustained recent research effort to develop non-DNA targeted drugs, particularly inhibitors of the signalling and checkpoint control pathways, where there are differences between tumour and normal cells. This is beginning to bear fruit, with the introduction of successful signalling inhibitors such as Herceptin (a monoclonal antibody to the erbB2 growth factor receptor) and Glivec (a small-molecule inhibitor of the ATP site of c-abl kinase).

The Problem of Hypoxia in Solid Tumours

The major cause of treatment failure in the common solid tumours to both radiotherapy,[1] and much chemotherapy, is because of the presence of hypoxic (oxygen-starved) cells. Solid tumours must recruit a blood vessel network in order to grow to macroscopic size, but these networks are limited and relatively chaotic,[2] with the result that a proportion of cells are too far from a viable blood vessel (above *ca.* 200 μm) for oxygen to diffuse. Hypoxic cells are resistant to radiotherapy because oxygen is required to oxidise the initial radiation-generated DNA radicals to species that result in lethal DNA strand breaks. They also rapidly go out of cycle into a quiescent but still viable state, and are thus become more resistant to classical cytotoxic drugs that primarily kill cycling cells. Finally, they tend to be the farthest from blood vessels, making drug access difficult.[3] There is also extensive evidence that hypoxia turns on a variety of genes that code for cell survival, resistance, and invasiveness.[4] Tumour hypoxia is widespread, with the best estimate that 65% of all human solid tumours possess a significant proportion (>35%) of severely hypoxic cells.

The Concept of Hypoxia-Activated Prodrugs

While severe hypoxia is a major block to successful therapy, it also represents an opportunity, since this physiological property is essentially unique to solid tumours. Thus there is a clear biochemical rationale for the development of compounds (hypoxia-activated prodrugs) that can distribute widely in the body, but become toxic only in the absence of oxygen, since such prodrugs would directly target a major treatment-limiting cell population. To be effective, hypoxia-activated prodrugs must fulfil a number of exacting criteria. They must be stable and distribute efficiently, to reach the remote hypoxic regions in tumours, and in these cells must undergo selective and efficient chemical transformation to generate an active drug. This should be a potent cytotoxin, able to kill tumour cells at all stages of the cell cycle, and capable of limited back-diffusion, in order to kill surrounding non-hypoxic tumour cells, the *bystander effect* (Fig. 1).[5] To achieve this, the activated drug must also have sufficient stability and rate of diffusion, and not bind tightly to common macromolecules such as DNA and proteins. Despite the compelling rationale, these exacting criteria have meant that only a few compounds have been brought to clinical trial, and only one (tirapazamine, see later) has proved useful to date.

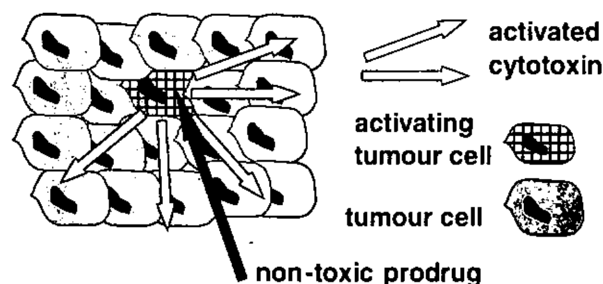


Figure 1. The bystander effect.

Design Principles for Hypoxia-Activated Prodrugs

We have proposed a modular design strategy for hypoxia-activated prodrugs, dividing them into three separate domains; trigger, linker and effector (Fig. 2).[6] Conceptually, this allows the individual domains to be optimised for their specific roles:

- The *trigger* is designed to undergo rapid and selective metabolism in hypoxic cells, by one of two major mechanisms (see below).
- The role of the *linker* domain or mechanism is to greatly suppress the latent activity of the effector domain, then

ensure its rapid activation following metabolism of the trigger.

- The *effector* is the actual cytotoxin, designed to kill cells rapidly and under all conditions, and to have a substantial bystander effect.
- Finally, the initial *prodrug* must have appropriate overall stability, and physicochemical properties, to ensure its rapid extravascular diffusion to reach the hypoxic cells farthest from blood vessels.

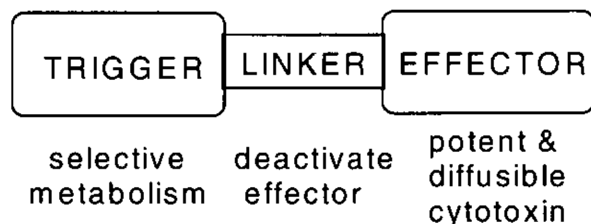


Figure 2. Modular concept for prodrug design.

Mechanisms of Prodrug Activation

There are two major mechanisms for the hypoxia-selective activation of prodrugs:

- Metabolism by ubiquitous one-electron reductases (such as cytochrome P450 reductase) that are present in all cells, to form a transient one-electron adduct (Fig. 3). In normal (well-oxygenated) cells, these transient adducts are rapidly re-oxidised by molecular oxygen to regenerate the non-toxic prodrug. The efficiency of this step depends less on the specific structure of the trigger than its one-electron reduction potential, which is suggested should be in the range –300 to –450 mV.[7] In hypoxic cells, the initial one-electron adduct is further reduced or undergoes spontaneous rearrangement, to generate the cytotoxic effector. Prodrugs activated by this broad mechanism we term *Bioreductive Enzyme Prodrugs (BEP)*; these represent the vast majority of hypoxia-activated prodrugs to date.

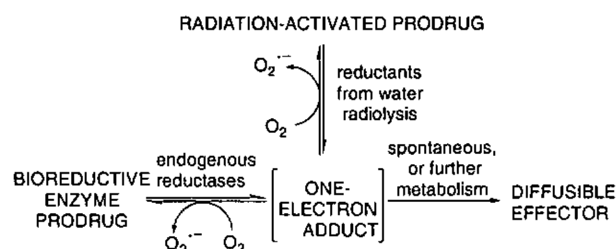


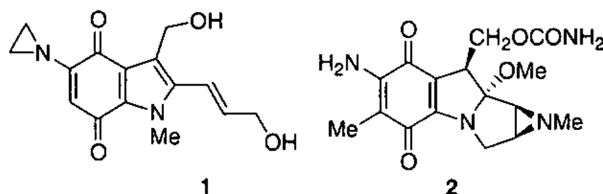
Figure 3. Mechanisms of hypoxia-selective activation of prodrugs.

- The reactive radicals formed by the radiolysis of water when cells are subject to ionising radiation during radiotherapy (Fig. 3) can be used to activate a second class of prodrugs, designated *Radiation-Activated Prodrugs (RAP)*. This activation is doubly restricted, to hypoxic cells within the radiation field, and exploits the targeting ability of radiation therapy. Such prodrugs have the potential advantage of not relying on enzymes (whose levels can vary) to release the cytotoxic agents, and offer a potential adjunct treatment for use in conjunction with radiotherapy, which is the treatment modality for approximately 50% of all cancer patients with solid tumours.

Classes of Bioreductive Enzyme Prodrugs

Quinones

The first clinically-tested agents were quinones such as EO9 **1** and Promycin **2**, which are activated by one-electron reductases to semiquinone radical anions that can be re-oxidised by oxygen in normal cells.[6,8] Following activation they undergo complex but well-understood fragmentation to species that cross-link DNA.[9] However, both of these prodrugs failed in early clinical trials, largely because the quinones are also substrates for two-electron reductases, particularly DT diaphorase. This reduces them directly to the hydroquinones, bypassing the oxygen-sensitive step and leading to toxicity in oxygenated cells.[10]

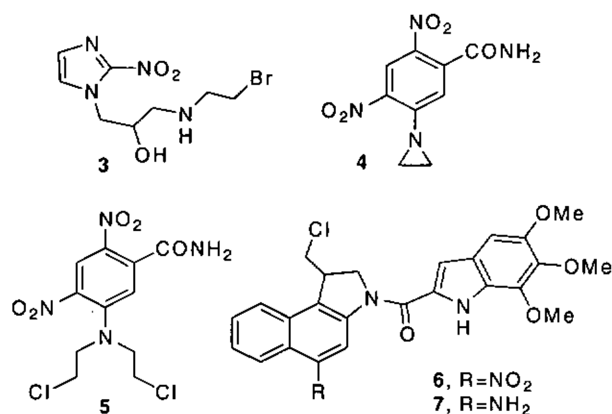


Nitroaromatics

These are perhaps the most obvious design for bioreductive prodrugs, being reduced in cells by a number of flavoprotein enzymes, via a first oxygen-sensitive step to form a nitro anion radical that can be scavenged efficiently by molecular oxygen, followed by further reduction to powerful electron-donating hydroxylamines or amines. Reduction of nitro groups is efficient only under severely hypoxic conditions (<1 μM O_2), and nitroaromatics are rarely substrates for oxygen-insensitive two-electron reductases. Nitroaromatics have been used to define and characterise many of the design concepts for bioreductive prodrugs,[6] but no nitroaromatics have yet been evaluated clinically as BEP. The (bromoethyl)nitroimidazole CI-1010, (*R*)-**3**, was designed to convert (by fragmentation of the initially-formed hydroxylamine) to a DNA cross-linking agent. It did undergo preclinical evaluation but was shown to cause irreversible retinal damage in animals.[11]

Nitrobenzenoid compounds are potentially attractive as BEPs since they form relatively more stable hydroxylamines on reduction, allowing the accompanying large electronic change as a molecular switch to activate a pre-positioned effector such as a nitrogen mustard.[7] The aziridine, CB 1954, **4** is being evaluated as a prodrug in suicide gene therapy, following bioreduction by an introduced nitroreductase.[12] We have shown[13] that dinitrobenzamide mustards, *e.g.* **5**, are hypoxia-selective, being reduced by endogenous reductases, and generate relatively stable effectors with significant bystander effects.[14] This is important in addressing the problem of killing hypoxic cells that are at intermediate oxygen concentrations (from 1-10 μM O_2). These desirable properties make them worthy of continued development.

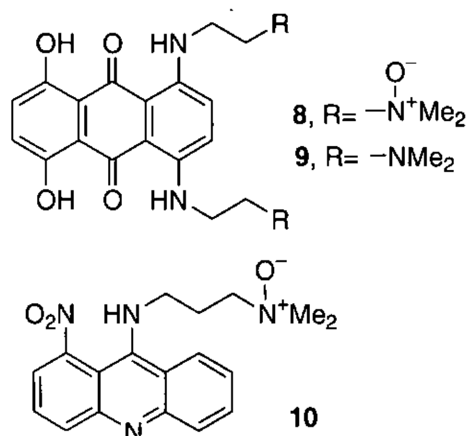
Nitroindolines are also potential nitroaromatic BEPs. We have reported the synthesis and evaluation of several classes of nitroindolines, particularly nitroCBIs, *e.g.* **6**.[15] Despite very low reduction potentials (below –550 mV), these nitroindolines are effective as BEPs both in cell culture and *in vivo*. On reduction they generate extremely potent



hydroxylamino- or amino-indolines, *e.g.* **7**, related to the well-known hydroxyindoline antitumor antibiotics,[16] and with IC_{50}s in the sub-nM range (unpublished work from this laboratory).

Aliphatic amine N-oxides

This class is represented in the clinic by the di-*N*-oxide AQ4N, **8**, of the anthraquinone-based DNA intercalator AQ4, **9**, which is a topo II inhibitor related to the clinical agent mitoxantrone. We have reported on related compounds, *e.g.* **10**.[17] Reduction of the aliphatic *N*-oxides in these compounds to the corresponding tertiary amines raises their pKa by about 5 units, ensuring their protonation. This confers tight binding of the generated tertiary amine effector to DNA, activating its topoisomerase II poisoning effects. The reduction is carried out largely by the cytochrome P450 3A family and is not a one-electron process, but is inhibited by oxygen probably because of direct competition between oxygen and the drug at the enzyme site.[18] AQ4N was developed initially at the University of Leicester, and Phase I clinical trials are currently being conducted by KuDos. The large-scale synthesis of AQ4N was developed in the Auckland Cancer Society Research Centre (ACSRC).[19] It remains to be seen whether a prodrug that generates such a tightly DNA binding effector will be useful.



Aromatic di-N-oxides

The benzotriazine di-*N*-oxide tirapazamine, TPZ, **11** (Fig. 4), was shown in a random screen to have selective toxicity towards hypoxic cells in cell culture and in preclinical tumour models,[20] and has become the most advanced BEP in clinical trials. Phase III trials of TPZ are in progress with Sanofi-Synthelabo, and it is expected to become the first hypoxia-activated prodrug to be registered for use. It is reduced initially to reducing radical **11a**, and we have shown recently that this initial product is then converted, by spontaneous loss of water with a rate constant of 0.84 sec^{-1}, to the oxidising benzotriazinyl radical **11b** that abstracts protons from the ribose unit of DNA, causing strand breaks (Fig. 4).[21] Despite working via the generation of DNA radicals, TPZ effectively kills hypoxic cells because both it and its two-electron reduction product **11c** can act as oxygen mimetics, converting the initial DNA radicals to strand breaks.[22] The short-lived nature of these intermediate radicals means that TPZ has no bystander effect, but this drawback is countered by the fact that it is activated at much higher oxygen concentrations (up to 3 μM).

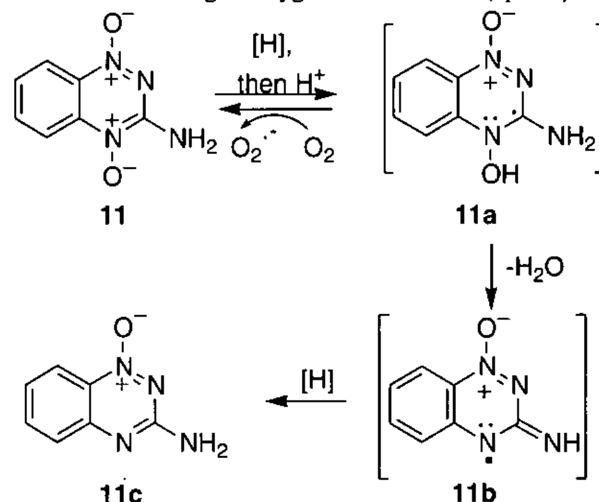
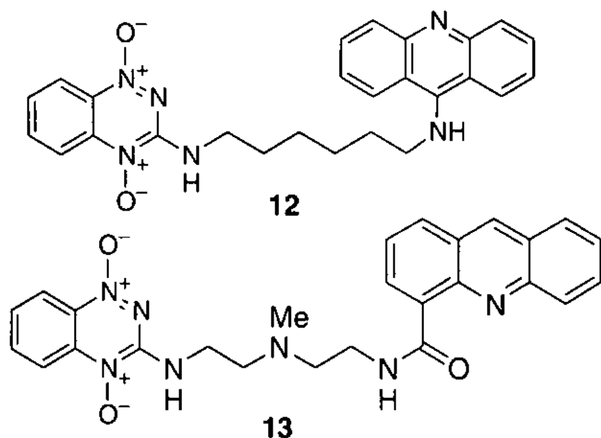


Figure 4. Mechanism of activation of tirapazamine.

Although TPZ was the first bioreductive drug to show clinical proof of principle, it has limitations, with modest hypoxic selectivity *in vivo*,[23] and a relatively low rate of extravascular diffusion.[24] In addition, its activation at higher oxygen concentrations, while overcoming to some extent its lack of bystander effect, also increases its toxicity towards normal oxygenated cells. A manifestation of this is an irreversible retinal toxicity in mice due to moderate levels of hypoxia in the retina.[24,25] While this is not an issue clinically, it is one that has to be kept in mind in analogue development. Using a series of ring A substituted derivatives, we have shown that the one-electron reduction potential of these compounds can be precisely controlled by substituents on the benzo ring.[26] This is a critical property that largely determines rates of formation, back-oxidation and dehydration of the initial reducing radicals.[21] Analogues with reduction potentials in the range –450 to –510 mV retain high potency and hypoxic selectivity in cellular assays.

Although the hypoxic toxicity of TPZ is due to its metabolism in the cell nucleus, there is evidence that some reduction also occurs in the cytoplasm. This is unproductive, and likely counter-productive, by contributing to some of the toxic side effects,[27] and also by compromising efficient diffusion of the prodrug into hypoxic zones.[28] We considered targeting the drug to DNA by attachment to a DNA-binding carrier, to minimize this unproductive metabolism, and showed that the acridine analogue **12** retains hypoxic cell selectivity, but was up to 200-fold more potent than TPZ in cell culture.[29] However, this strong DNA binder (K_a = 1.43 x 10^5 M^{-1} at 10 mM



ionic strength) was not active in potentiating tumor cell kill by irradiation *in vivo*, likely due to its excessively tight DNA binding limiting extravascular transport. For a series of analogues with a range of lower levels of DNA binding, there was a strong positive correlation between binding strength and high potency, but no correlation between binding strength and hypoxic selectivity.[30] A number of the analogues, *e.g.* **13**, showed increased potency and excellent hypoxic selectivity, confirming the initial hypothesis and making further development of this class of compound of interest.

Classes of Radiation-Activated Prodrugs (RAP)

There has been much less work done in this field even though the concept has some added attractions. These include:[31]

- Additional tumour specificity because of activation only by hypoxic cells *in the radiation field.*
- Potentially enhanced selectivity for hypoxic cells because of the addition of a second mechanism of inhibition of activation by oxygen (competition for reductants).
- The use of prodrugs of much lower reduction potential, minimising adventitious metabolic consumption.
- Exploitation of what is commonly the largest hypoxic compartment in many tumours, the necrotic regions (not available for activating BEP due to loss of enzyme activity).

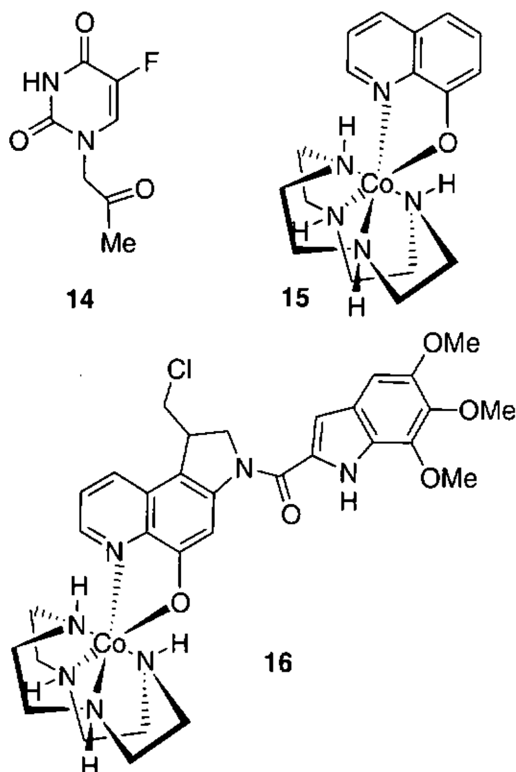
The main design difficulty with RAP is that a typical clinical dose of radiation (2 Gy) generates only a small yield of reducing radicals (0.6 μmol/kg). This requires the use of prodrugs with very efficient triggers that release very potent effectors.

Uracil dimers

The above point is illustrated by some nice work on a series of uracil derivatives, *e.g.* **14**, that were shown to undergo one-electron reduction by hydrated electrons on irradiation in hypoxic aqueous solution, followed by cleavage to release 5-fluorouracil in high yield.[32] However, even at 30 Gy, insufficient effector was generated to observe any therapeutic effects.[33]

Cobalt(III) complexes

We have explored Co(III) complexes of 8-hydroxyquinoline ligands, and shown that, in conjunction with azamacrocyclic amine auxiliary ligands, these complexes, *e.g.* **15**, undergo hypoxia-dependent reduction under radiation to the much less stable Co(II) species, which then rapidly release the 8-hydroxyquinoline ligand.[34] Use of the much more cytotoxic azahydroxyCBI ligand, an 8-hydroxyindoline derivative, provided Co(III) complexes such as **16**, which shows efficient radiolytic activation in hypoxic (but not aerobic) human plasma, at therapeutically relevant radiation doses of about 2 Gy.[35] This preliminary work shows that, with the right choice of trigger and effector, radiation-activated prodrugs are a viable concept for cancer therapy.



Conclusions

Hypoxia is a major reason for the limited effectiveness of current treatment for the common solid tumours, but at the same time (as a tumour-specific phenomenon) offers a potential opportunity for tumour-specific therapy. The major general design issues for hypoxia prodrugs have now been identified as diffusion of the prodrug to the (remote) hypoxic regions, selective activation under hypoxia, and back-diffusion of the active effector to surrounding tumour cells, the *bystander effect*. The likely registration of tirapazamine as the first hypoxia-activated prodrug will give an important boost to this field of drug design, which remains a major research programme in the ACSRC. The commercial arm of The University of Auckland (Auckland UniServices) has formed the start-up company Proacta Therapeutics to further develop this research programme. The aim of the company is to take lead compounds into scale-up, toxicology and Phase I/II clinical trials, and to conduct this work within New Zealand wherever possible.

Acknowledgements

Many people have carried out the ACSRC work summarised briefly here. Particular acknowledgements are made to Bill Wilson, Michael Hay, Swarna Gamage, Graham Atwell, Shangjin Yang, Moana Tercel, Ralph Stevenson, David Ware, Bob Anderson, and Sujata Shinde.

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Obituary: David Haden MNZIC (1944-2003)

Environmental Affairs Advisor for New Plymouth's Methanex New Zealand, David Haden, passed away suddenly on 8 November 2003. He was a much respected and loved man both within Methanex and across all of the different organisations that he worked with.

David touched us all and his premature passing leaves us feeling a great loss. His strength was his positive outlook on what life brought his way, a smile that never seemed to leave him and an amazing patience with people. He had 19 years experience, first with New Zealand Synthetic Fuels Corporation and then with Methanex. As a highly qualified chemist, David built a personal reputation of accuracy and integrity. After his early years in the laboratory, David turned his talents to environmental matters and quickly established himself as the Methanex *guru*. His thoughts and opinions influenced the direction that Methanex has taken in the area of environmental science. He will be greatly missed by the teams that he led and participated in and by the many external agencies he worked with.

David leaves a wife, Robin, three children and four grandchildren.

Geraldine Kennedy, Methanex NZ Ltd.

CORONATINE: A PLANT HORMONE IMPOSTOR?

Robin E. Mitchell
Horticulture and Food Research Institute, Private Bag 92169, Auckland

Historical Perspective

Numerous species of bacteria from the *Pseudomonas* genus have been reported in earlier literature to be pathogenic to plants, each with a specific set of host plants. The infection process occurs with an exponential multiplication of the bacterial cells after entry into the plant through leaf stomata or injury sites. The invading bacteria source their required nutrients from the plant, and secrete their products within the plant. In fact, they have evolved to be masters of the process. The infected plants suffer in numerous ways, and the external indication of an infection has a range of forms, such as wilting, chlorotic spots on leaves, necrotic or dead areas of tissue, changes in pigmentation; the plants may live on in an impaired way or die off. Substances secreted by the bacteria, known as phytotoxins, often cause these effects. Coronatine is one of several such phytotoxins that cause a leaf chlorosis symptom in bacterial plant diseases.

Thirty years ago two different laboratories, one in Japan and one in New Zealand, were independently investigating the cause of leaf chlorosis in plant diseases from the bacteria *P. coronafaciens* subsp. *atropurpurea* (now *P. syringae* pv atropurpurea) on Italian ryegrass and *P. syringae* pv glycinea on soybean, respectively. In 1977 a structure for the phytotoxin from the former was published[1] and named coronatine. Following on from this the principal phytotoxin from the latter, *P. syringae* pv glycinea, was established to be coronatine.[2] From 1976 to the present day this compound has attracted considerable international scientific interest and research, and it is not surprising that an enormous volume of knowledge has accumulated around the molecule and its production and function. Even so, there remains today a desire to unravel the mystique that is still associated with it, and to completely harness its potential usefulness in biology. This article presents the coronatine story.

Structure

Coronatine is an amide of the bicyclic coronafacic acid (cfa) and the unusual cyclopropane amino acid, coronamic acid (cma) (Fig. 1).[1] Coronafacic acid was identified as a co-metabolite with coronatine in liquid cultures,[2] while more recently cma has been shown also to be a secreted metabolite,[3] albeit at low concentrations. An asymmetric total synthesis of cfa and coronatine has been reported.[4]

A range of biologically active structural analogues of coronatine have been discovered as shown in Chart 1. Thus, norcoronatine,[5] coronafacoyl-L-valine,[5,6] coronafacoyl-L-*allo*isoleucine and coronafacoyl-L-isoleucine,[7] coronafacoyl-L-serine, and coronafacoyl-L-

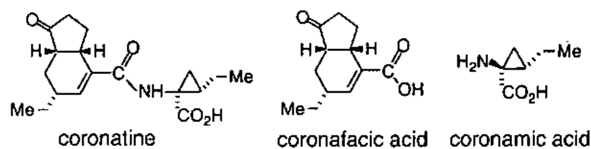
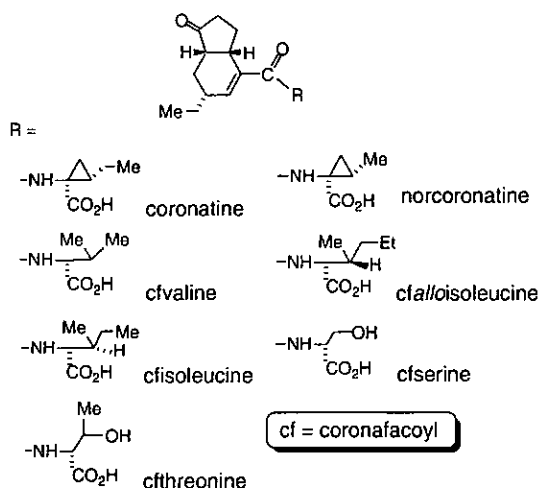


Figure 1. The structures of coronatine and its components.

Chart 1. Naturally occurring analogues of coronatine.



threonine[8] are all amino acid amides of cfa and together they emphasise a significance of the coronafacoyl (cf) moiety to the bioactivity when bound as an amide, since cfa itself lacks chlorosis-inducing biological activity. Their occurrence also demonstrates a degree of flexibility in the bioassembly of these compounds.

Properties and Detection

Coronatine is water soluble and extractable into organic solvents as a carboxylic acid when protonated at pH < 5.0. It can be obtained in crystalline form from polar organic solvents, *e.g.* propan-2-ol or ethyl acetate. The best analytical methods for the coronatine family of compounds are HPLC[8-10] or GC[6] of their methyl ester derivatives formed from reaction with diazomethane. There is scope for enhancing the sensitivity of these methods.

Genetic Basis For Coronatine Synthesis

The coronatine biosynthetic genes are often located on large (80-110 kb) plasmids.[11,12] One such plasmid from *P. syringae* pv tomato was transformed into coronatine non-producing *P. syringae* strains, which then became producers of coronatine.[11] It has been noted that the genes can also be chromosomal.[13] Plasmid DNA sequences in several

coronatine-producing *P. syringae* pathovars were found to be highly conserved, and the coronatine genes resided on a 30 kb region of the plasmid DNA.[12] In a detailed analysis of the coronatine gene cluster in *P. syringae* pv glycinea #4180 (p4180A), coronatine-defective mutants were obtained and subjected to genetic and chemical analysis and their phenotype established by cross-feeding experiments and chemical analysis.[10] This study gave a clear picture of the genetic make-up of p4180A and the various steps involved in the biosynthetic pathway to coronatine. The biosynthetic cluster encoded by p4180A has now been fully characterised by a variety of approaches that have been reviewed.[14] In summary, the significant features are:

- a 32-kb contiguous region is essential for coronatine biosynthesis
- a 6.9-kb region contains the genes for cma synthesis
- a single 19-kb transcriptional unit contains the cfa genes which have characteristics of a polyketide synthase
- a region required for coupling of cfa to cma contains a 1.4-kb gene designated *cfl* which encodes the enzyme coronafacate ligase.

The genetic analysis therefore gives the schematic course to coronatine formation shown in Figure 2.

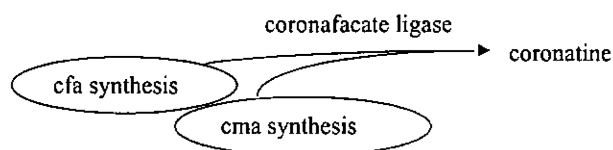


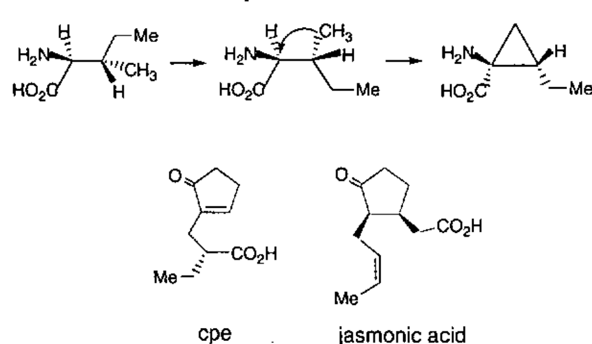
Figure 2. Schematic of coronatine formation.

By implication, coronafacate ligase is able to accommodate a range of amino acid substrates while, in contrast, the specificity for cfa is yet to be ascertained. Actual biochemical studies of coronafacate ligase itself have not been undertaken to date because the enzyme activity has proven elusive.

Biosynthesis of cma

The amino acid L-isoleucine is incorporated into the cma of coronatine.[15] The route (Scheme 1) involves epimerisation at C-3 to L-*allo*isoleucine and then direct C-C bond formation between the C-3 methyl carbon and C-2, without prior functionalisation of the methyl group, which results in the cyclopropane ring.[16] This last step is unprecedented and a feature unique to coronatine biosynthesis.

Scheme 1. Possible biosynthetic route to cma from L-isoleucine.



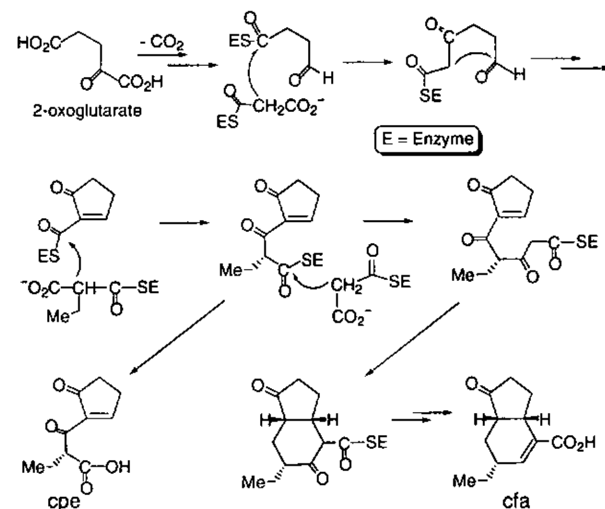
Norcoronatine consists of cfa coupled to the cyclopropane amino acid with a 2-methyl substituent in place of the 2-ethyl of cma. The absolute stereochemistry of norcoronatine was established by synthesis to be the same as for coronatine.[17] By analogy with cma biosynthesis, norcoronatine is likely to be an independent metabolite that is derived from L-valine with ring closure between a C-3 methyl and C-2 of valine.

Biosynthesis of cfa

The polyketide origin of cfa was demonstrated by labelling experiments with ^{13}C-acetate,[18] which established the incorporation of pyruvate, three acetate units, and butyrate. Further experimentation showed that the pyruvate was converted to 2-oxoglutarate, which is the starter for the polyketide chain,[19] with C-1 of this being lost at an early stage. The ethyl substituent of cfa was derived from the incorporated C-4 unit (ethyl malonate). Convincing insight to a probable mechanism of cyclisation of the ketide chain came from the discovery of a co-metabolite in culture broths that was determined to be 2-[1-oxocyclopent-2-en-2-ylmethyl]butanoic acid (cpe).[20] The absolute configuration of cpe, confirmed and established by chemical synthesis[21], matches the configuration of cfa.

It is not known yet whether cpe is an intermediate that lies directly on the pathway to coronatine, or whether it is a product shunted from the pathway. However, it is easy to envisage a 2-carbon extension at the carboxyl moiety of cpe, with the resulting product then mechanistically set up for ring closure via a Michael addition to the cyclopentenone. This looks to be the likely route operative in cfa biosynthesis. Scheme 2 depicts possible pathway processes for the biosynthesis of cfa.[22-24]

Scheme 2. Projected biosynthetic route to cfa.



Biological Properties and Mode of Action

Coronatine has a wide range of effects on plants, in addition to the visible effect in leaves that give a yellowing, or chlorosis response, or in *Arabidopsis* (and perhaps other crucifers) increased pigmentation ascribed to anthocyanin accumulation.[25] A swelling (hypertrophic) response to coronatine in potato tubers has been used as a bioassay.[26] Its effects on application to tomato plant leaves are a

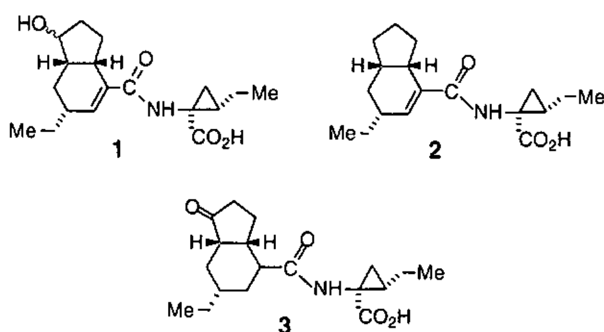
thickening of the epidermal cell wall, shrinkage of chloroplasts, and induction and accumulation of proteinase inhibitors.[27]

The structural analogy between cma and ACC, the ethene precursor *in planta*, has been considered as a basis for the bioactivity of coronatine. However, although coronatine was found to stimulate ethylene production,[28] this was considered to be a secondary effect.

Several reports have noted a structural similarity between cfa and jasmonic acid,[29-31] a signalling/hormonal molecule operative in plants. Activities of coronatine and cfa have been found to be similar to those of jasmonic acid (see above) – all three compounds are tuber inducing, cell expansion-inducing, cell division inhibiting, and senescence-promoting.[32] They promote the production of volatile compounds in leaves, and they promote the production of phytoalexins.[33] It is currently believed that coronatine functions *in situ* as a jasmonate mimic and disrupts the normal operative signalling processes. However, a specific site of action for coronatine has not been identified to date.

Structure and Activity

Modifications to the coronatine structure were undertaken to gain an understanding of what structural aspects are important for the chlorosis-inducing activity of the molecule.[34] The bicyclic coronafacoyl ring is a key component, but it also requires an amide linkage to an amino acid with a free α-carboxylic acid as the methyl esters of these compounds are inactive. When the carbonyl group of the cyclopentanone ring was reduced with $NaBH_4$ to hydroxyl, product **1** retained activity, and when the oxygen substituent was completely reduced to the cyclopentane-containing **2** the activity was still retained. These results demonstrate that the cyclopentane oxygen substituent is not an essential factor. However, when the cyclohexene moiety was hydrogenated, product **3** was inactive, demonstrating the importance of the α,β-unsaturated amide functionality. An analogue of cfa has been synthesised where the cyclohexene ring has been fully aromaticised,[35] and this product then coupled to various amino acids; these products had similar activity to coronatine.[36]



Coronatine Antibodies

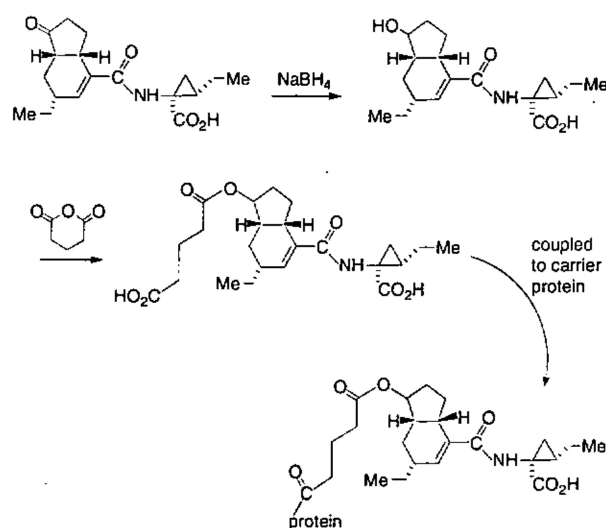
Preparations of monoclonal antibodies to coronatine have been undertaken to further the studies of coronatine by making available tools for sensitive coronatine assays and for the identification of receptors in plants. In the first instance, coronatine was conjugated through its free carboxyl group to various carrier proteins, and these conjugates injected into mice for antibody preparation. This was followed by the preparation of selected monoclonal antibodies which had high specificity for coronatine and coronafacoylvaline.[37] In order to obtain different specificities to the two different structural components of coronatine, suitable haptens were also prepared[38] to facilitate the presentation of these different structural aspects of the coronatine molecule to immunoglobulins, as outlined in Schemes 3A and 3B. Linkages to carrier protein were achieved through:

A. The cyclopentanone ring by reduction to the cyclopentanol, and reaction with glutaric anhydride, resulting in a 5-carbon extension terminating in a carboxylic acid group that was then available for coupling to carrier protein, in order to present the cma moiety (Scheme 3A).

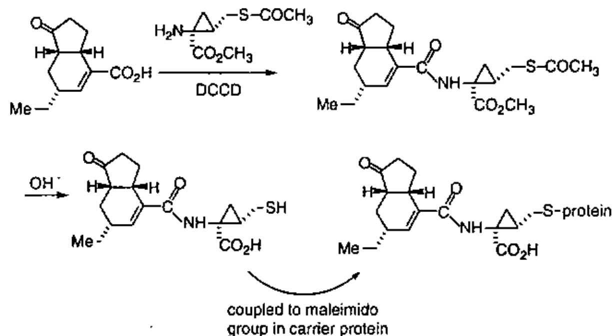
B. The cyclopropane ring. To achieve this we obtained the cyclopropane amino acid, 1-amino-2-methylthioacetyl-1-carbomethoxylcyclopropane from a commercial synthesis and coupled it to cfa. Base hydrolysis gave the required product with a free carboxylic acid and a thiol group available for subsequent coupling to a carrier protein in order to present the cfa moiety (Scheme 3B).

Scheme 3. Synthesis of haptens for antibody production.

A. Hapten presenting cma moiety.



B. Hapten presenting cfa moiety. The cyclopropane amino acid was procured by commercial synthesis as the N-Boc/S-acetate/methyl ester derivative, and de-Boc'd immediately prior to use.



Tests demonstrated that the monoclonal antibody product in the first case predominantly recognised the coronamic acid aspects of coronatine, while in the second case

predominantly recognised the coronafacoyl amide part structure of coronatine.[38] A recent study demonstrates the utility of these antibodies in the detection and localisation of low levels of coronatine in infected plant tissue.[39]

What Does All This Prove?

In summary, the discovery of coronatine arose from investigations to understand a phytotoxic chemical response in a plant caused by an invading bacterium. The results from that early work have lead to the generation of a significant volume of knowledge and an understanding of interplays between chemistry and biology. This has come from work accomplished in many different laboratories internationally thereby vindicating the original *blue sky* research.

To answer the question – does coronatine have plant hormone character? The probable answer is yes, but definitive proof is still to come!

The topic of chemical communication in biological systems receives much scientific attention.[40,41] The human equivalent of dialogue could be referred to in plants as a *cross-talk* which occurs between different hormone-dependent signalling pathways. It is feasible that the chemical relatedness of coronatine to plant hormone structures gives it the capacity to interfere with normal plant cross-talk, and thereby deliver its "desired" impact.

It is not certain where the coronatine story will finish, but clearly there is more to come. For example, a recent report[42] depicts a potential agricultural use of coronatine in the Florida citrus industry, whereby spray applications of dilute solutions can bring about abscission of fruit that could eventually lead to a reduction in picking costs. On the fundamental side undoubtedly there will be more depth added to the science underlying this intriguing topic.

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Book Review

The Third Man Speaks

The Third Man of the Double Helix: The Autobiography of Maurice Wilkins, Oxford University Press, Oxford, United Kingdom, 2003, pp. 273, $40.

Wilkins initiated work that led to the determination of the double helix structure of DNA when he was appointed to King's College, London under Professor John Randall. In 1962 he shared the Nobel Prize for Physiology or Medicine with James Watson and Francis Crick. The discovery of the double helix in 1953 rates as one of the most publicised scientific discoveries in history, begun by Watson's controversial book *Double Helix*. Rosalind Franklin, who unfortunately died in 1958, has been the subject of books by Ann Sayre and Brenda Maddox while Francis Crick has contributed a short memoir. Now we hear from Maurice Wilkins, the *Third Man*.

As there have been differing opinions about the actual role of Maurice Wilkins, this book is particularly welcome since he was criticised for his inability to collaborate successfully with Rosalind Franklin, and accused of providing her results to the Cambridge team without her knowledge or permission. He resolves the first question fairly successfully by blaming John Randall (the Professor at King's) for his misleading instructions. A letter from Randall to Franklin, found amongst her belongings after her death, had the following passage: *as far as the experimental X-ray effort is concerned there will be at the moment only yourself and Gosling*. Franklin evidently interpreted this to mean that Wilkins was no longer working on X-Rays. When it became obvious that this was not so, she became unfriendly.

It is easy to place too much emphasis on this unfortunate misunderstanding. Both of them seemed to have got bogged down in false leads but, despite their apparent hostility, Wilkins describes an occasion in December 1951 when they had a cordial conversation coming very close to deducing the correct structure. As for Rosalind Franklin's famous X-Ray photograph showing the helical structure of DNA, he admits that *in retrospect, I had been rather foolish to show it to Jim [Watson] during our hurried conversation in the corridor* but it is clear that this incident has been over emphasised.

The details of the final publication in *Nature* in 1953 are fascinating. Watson and Crick offered joint publication to Wilkins but he turned it down as he felt that he had not contributed sufficiently to their structure. So he wrote the paper with Stokes and Wilson which emphasised his earlier photograph. The reaction of Franklin was surprise, as it turned out that she had already written a short paper (with Gosling) that summarised her contribution at a time when Wilkins was under the impression that she no longer supported a helical structure. The three papers were published simultaneously on 25th April 1953.

Wilkins can claim to have been lucky. He recounts that he would probably never have obtained the job at King's if Allan Nunn May, the prime candidate, had been appointed Professor. As it turned out, Nunn May was arrested for spying and John Randall took the chair. Wilkins heard of Avery's proof that DNA was the hereditary substance before Avery's paper was published. He obtained a beautiful DNA sample from Signer and was able to start the quest for a structure before others realised its importance. He might also never have become a Nobel Prize winner as the most influential member of the Nobel Committee, Lawrence Bragg, apparently wanted the prize to go to Watson, Crick and Dorothy Hodgkin, not Wilkins.

Instead of expressing gratitude for his great good luck, Wilkins seems content with the role of *Third Man* to Watson and Crick. He is excessively modest, with (as was said of Clement Attlee) plenty to be modest about, but he shares his common humanity with us all, and we can still be proud of his New Zealand origins and achievements to which, whatever he tries to say, he made crucial contributions.

The account of his political beliefs and activities parallel those of my own. He was a member of the Communist Party for several years and he campaigned for nuclear disarmament and social responsibility in science with people I knew. The account of his psychological states, his toying with suicide, and his diffident approach to women is, on occasion, a bit embarrassing. I suppose the public likes to hear these things. Finally, it might be said that although this book gives valuable information on the actual discovery, it is rather defective as an autobiography. The text has a large font and double spacing, otherwise the book would be much shorter. There is an irritating lack of detail and dates throughout. There is no bibliography of Wilkins's publications although many of these can be found in the works of Olby, Judson, and Gribbin.

Vincent Gray
22 February 2004

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FROM GENES TO PROTEINS TO DRUGS VIA CRYSTALLOGRAPHY: NEW APPROACHES TO A DEADLY PATHOGEN, *MYCOBACTERIUM TUBERCULOSIS*

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In the popular media, commentaries on the so-called *genomic revolution* have mostly highlighted the sequencing of the human genome. This is not surprising, and there is no doubt that this one event will profoundly affect our future health and welfare in ways that we cannot yet foresee. Behind the headlines, however, the sequencing of large numbers of other genomes, of plants, microbes, parasites, and other living species has continued apace. These have equally dramatic implications and offer unprecedented opportunities to do science in different ways and address problems we simply could not begin to before.

Many of these opportunities cannot be realised, however, until we know more about the proteins that are the ultimate products of the genome sequence data. As an illustration, consider the bacterium *Mycobacterium tuberculosis*, which is the focus of a large part of our research. The *M. tuberculosis* genome[1] includes nearly 4000 genes that are assumed to carry the necessary information to make 4000 different proteins. The problem is that most of these, perhaps as many as 65%, are of unknown or uncertain function. Hardly any have been studied directly before (most functional inferences are made by sequence comparisons with proteins in other organisms) and there are clearly many important proteins, metabolic pathways and other cell processes that we cannot yet recognise.

Here we describe a research programme that was begun in Auckland about four years ago and aims to address these fundamental and important questions. Our slant on this is to try to use protein 3-D structure as a means of interpreting and exploiting genomic sequence data. The term given to this approach is *structural genomics*.[2-4] It is based on the fact that protein structures are much more strongly conserved during evolution than are sequences, meaning that relationships can be seen that are not apparent at the sequence level. Further, the structures can lead to the discovery of bound ligands or recognisable active site motifs or binding sites, leading to functional hypotheses that can be tested, and providing a basis for future drug development.[4,5]

Our focus is primarily on the bacterium *Mycobacterium tuberculosis*, the cause of TB, and we believe that both the way the project is organised, and the goals and results, could be pointers to new ways of conducting large-scale coordinated research.

Mycobacterium tuberculosis and TB

The disease of tuberculosis, known in the past as consumption or the white plague, is a devastating infectious disease. Current estimates by the World Health Organisation are that one-third of the world's population is infected, and 2-3 million people die annually – about 7000 per day, and one every minute in India alone.[6,7] The incidence is increased by urbanisation, population growth and migration, and by a deadly synergy with HIV. The one vaccine in use, the Bacille Calmette-Guerin (BCG) vaccine, is of only limited efficacy and although several effective anti-TB drugs (isoniazid, ethambutol, pyrazinamide, rifampicin) are available,[8] their usefulness is limited both by the development of multi-drug resistance and by the strange nature and lifestyle of the organism.

M. tuberculosis is a very slow-growing organism, making it difficult to study directly. It has a thick, waxy, cell wall that is rich in unusual lipids, glycolipids, and polysaccharides, such as the mycolic acids.[9] These present a fairly impenetrable barrier to exogenous small molecules, provoke host reactions, and help to maintain mycobacterial longevity. Most remarkable is the phenomenon of persistence. Usually, specialised immune cells called macrophages destroy inhaled bacteria. When *M. tuberculosis* is taken up by macrophages, however, it is not killed but remains in a semi-dormant state within structures called granulomas or tubercules. Here it switches its metabolism, living off its own lipids as a source of carbon, and up-regulating its biosynthesis of amino acids and other essential compounds.[10] This semi-dormant state can last for many years, re-emerging as an active infection when immunity wanes due to ageing or immune suppression.[11] These problems necessitate long treatment regimes, typically a combination of up to four antibiotics, taken for 6-9 months – this is *short-course therapy*!

The TB Structural Genomics Consortium

Worldwide discussion of the concepts of structural genomics began in 1998.[12] Realising we could not participate effectively on our own, we joined with colleagues at the Los Alamos National Laboratory, UCLA, and UC-Berkeley to form a consortium of laboratoriess whose intention was to coordinate our efforts. In the first instance our focus was on a hyperthermophilic organism,

Pyrobaculum aerophilum, whose genome sequence was being determined at UCLA. With the completion of the genome sequence of *M. tuberculosis* in 1998,[1] however, we switched our efforts to this organism because of its enormous health importance. This collaboration has now become the International TB Structural Genomics Consortium that consists of a group of more than 50 collaborating laboratories from 10 countries, see: <http://www.doe-mbi.ucla.edu/TB/>.

The goal of the Consortium is to use protein structure both to understand TB biology and to provide a foundation for the development of new anti-TB drugs.[12] Five *core* United States groups are funded by NIH to develop new *high throughput* methods for protein production, crystallization and structure determination, and maintain central protein production and crystallization facilities, synchrotron beamlines, and a gene knockout unit. These facilities, and the technologies developed, are immediately available to consortium members. Many of the consortium partners are structural biology laboratories, like ours, and coordinate their efforts *via* a common website where progress is logged. Not all the consortium members are involved in protein structure determination, however. Some are chemists, with an interest in collaboration in structure-based drug development. Others are biologists seeking structural collaborations on their favourite TB proteins, or with interesting technologies, *e.g.* for screening possible ligands, that they wish to test.

The University of Auckland Effort

Our focus is on protein targets of two types. First, we select proteins that are clear potential drug targets. These are mostly proteins of known function, but unknown structure, that are involved in processes essential to the viability of the bacterium, such as the biosynthesis of cell wall components, or of essential amino acids, cofactors or other metabolites. Second, we target proteins that are of unknown function but which are implicated in important aspects of TB biology that need to be understood better, *e.g.* the ability of the bacterium to enter its dormant or persistent state, its response to antibiotics, or its virulence. Some of these have been identified by gene knockouts or in microarray experiments that test which genes are upregulated in response to different environmental conditions.[13] Currently we are working on the following:

- Enzymes involved in biosynthesis of aromatic and branched chain amino acids.
- Enzymes involved in biosynthesis of cofactors and vitamins (thiamine, menaquinone).
- Enzymes involved in biosynthesis of mycothiol, a mycobacterial antioxidant.
- Proteins implicated in the phenomenon of persistence.
- Proteins implicated in the response to common antibiotics.
- Secreted proteins, which are likely to mediate host-pathogen interactions.

Practical Issues

To make a structural genomics enterprise even remotely viable requires that many aspects of the structure determination process need to be scaled up. Most critical is the ability to produce any protein of choice quickly and easily, at a level of purity suitable for crystallization. The required new technologies in molecular biology and protein chemistry will, however, be of huge benefit to all protein-based research, relevant to chemists and biologists alike. Our approach to the various practical steps is as described below:

Gene cloning

Because the DNA sequence of every gene is known, from the genome sequence, genes can be very quickly and easily amplified, *viz.* isolated in good amount, from a small sample of genomic DNA, using the Polymerase Chain Reaction (PCR).

Protein production

The gene of interest must be inserted into an *expression vector* which is then introduced to a suitable expression *host*. Usually this is done using a plasmid, a small piece of DNA into which the gene can be inserted, and which is itself designed to be integrated easily into the host. Many expression vectors are also designed such that they add another piece of DNA, coding for an affinity tag, to one end of the gene. A commonly-used tag comprises a short sequence of amino acids that contains six consecutive histidine residues (a His-tag) which can then be used for protein purification because it binds with high affinity to a column carrying immobilised Ni^{2+} or Co^{3+} ions. After insertion of the expression vector that carries the target gene into the host (often the laboratory standard organism, *E. coli*), the host cells are grown up in the laboratory, broken open, and the target protein isolated. Provided the protein is expressed in soluble form, and in reasonably good yield, passage down two columns, an affinity column and a size-exclusion column, usually gives a sample of sufficient purity.

Protein crystallization

Protein crystallization has become much more systematic in recent years, although it can still be inhibited by any inherent heterogeneity or flexibility in the target protein. Most methods use vapour diffusion, in which small drops of protein are equilibrated against crystallization "screens" comprising several hundred potential precipitating solutions that vary in pH, buffer, and concentration and nature of precipitant. By using small drops, typically 1–2 μL, very small amounts of protein go a long way.

Structure determination

X-ray crystallography remains the structure determination method of choice, because it is not limited by the size of the protein, being able to cope equally well with large assemblies such as viruses, it can give very high resolution, and it frequently leads to the discovery of bound ligands, ions, etc. that give important clues to function.[4,5] Many new and innovative approaches have greatly accelerated the structure determination process[14] such that this is no longer the rate-limiting step – crystals can be frozen at liquid nitrogen temperatures, taken or shipped to a synchrotron, data collected in a few hours, and in favourable cases automated electron density interpretation and model-building methods can result in a structural model

in a few days or weeks. NMR is a very important complement to X-ray crystallography for those proteins that cannot be crystallized, and the impending installation of high-field NMR facilities in New Zealand (Auckland and Massey Universities) will be a great advance.

Experience to date

In the course of this programme we have gained sufficient experience to recognise where the main bottlenecks occur. From a total of over 100 genes targeted, there is attrition at each step. Cloning by PCR is almost 100% successful, and most proteins, close to 90%, are successfully expressed. The principal bottleneck is solubility. For reasons we do not fully understand, many of the expressed proteins, particularly those from *M. tuberculosis*, prove to be insoluble, meaning that they are aggregated or incorrectly folded. Some can be rescued by refolding, or by slow expression at low temperatures, but the success rate is still such that only 30-40% of expressed proteins are soluble. The second bottleneck is protein crystallization. In our hands, about 50% of soluble proteins can be crystallized, but often not with sufficient crystal quality for X-ray analysis; optimisation of the conditions, often with the addition of seeding methods, can take some months.

The solutions to these bottlenecks will mostly come from advances in protein expression methods, perhaps incorporating new expression vectors, and from streamlining the processes so that alternative vectors and hosts are tried in parallel. We have recently received funding from FRST with just such a goal – which is critical to fully exploit the new opportunities from genomics. Robotics will play a part, as it already does for protein crystallization.[15] Recently, within the Centre for Molecular Biodiscovery we have installed a fully robotic system that allows potential crystallization conditions to be screened using protein drops as small as 50-100 nL. This means that not only is much less protein needed, but also that experiments are performed much more quickly and reproducibly, while protein samples are fresh.

A Pot-Pourri of New Protein Structures

We have determined a total of 11 new protein structures in the past 3 years in the course of this structural genomics programme. Six are from *M. tuberculosis* and five from *P. aerophilum*. A few examples serve to illustrate the diversity of outcomes.

MshB – a deacetylase involved in mycothiol biosynthesis

Most living organisms maintain a highly reducing environment inside cells to protect against uncontrolled oxidation. Mycobacteria, including *M. tuberculosis*, do not use glutathione as most other species do, but instead synthesise a novel compound called mycothiol that is built from the sugars inositol and *N*-acetylglucosamine and the amino acid cysteine.[16] The protein MshB was known to participate in mycothiol biosynthesis by removal of the acetyl group from *N*-acetylglucosamine (NAG), but was otherwise uncharacterised.[17] The crystal structure (Fig. 1A) identified the active site, deep in a pocket formed by polypeptide loops. Two unexpected bonuses were our discovery of a metal binding site at the bottom of this pocket, and a detergent molecule, β-octylglucoside (BOG), bound adjacent to it. Since both BOG and the NAG moiety of the natural substrate are substituted glucose residues, we were able to infer how the substrate binds and to propose the probable mechanism of action of the enzyme.[18] This is a good example of how protein crystal structures can reveal binding sites, sometimes quite fortuitously.

TrpD – anthranilate phosphoribosyltransferase

The pathways by which aromatic compounds, such as the aromatic amino acids, are made in plants and bacteria are attractive targets for the development of new herbicides or new antibiotics. This is because they are essential to the plants or bacteria but they are not present in humans (we have to take such compounds up in our diet). The enzyme known as TrpD is a good example, as it has been shown that if its gene is knocked out, *M. tuberculosis* cannot survive in the lungs.[19] Our TrpD structure (Fig. 1B) is therefore a good candidate for the development of inhibitors that might serve as anti-TB drugs. Binding studies are under way with this goal. The TrpD structure illustrates how protein crystallography has changed. The protein was expressed, purified and crystallized. A second sample was then prepared in which selenomethionine (SeMet) was substituted for the sulfur-containing amino acid methionine (done simply by growing the bacteria in a minimal medium that contained SeMet but no Met). This was crystallized, seeding with the native crystals, and the SeMet-containing crystals were frozen at liquid nitrogen temperatures and shipped in a dewar to a synchrotron. There the data collection took less than a day, the structure was solved (by finding the Se atom positions) in less than 24 hours, and the model was completed by a combination of automated model-building and manual building by computer graphics, in a few days.

MenG – a supposed enzyme involved in menaquinone biosynthesis

Menaquinone, Vitamin K_2, is the sole quinone used by *M. tuberculosis* and is essential for processes such as electron transfer. Because it is synthesised by the bacterium, but not by humans, the biosynthetic enzymes that produce menaquinone are again good drug targets. In this case, however, the structural analysis of MenG, which was believed to act as a methyltransferase in the final biosynthetic step, brought a very unexpected result. The protein structure (Fig. 1C) was completely different from that of any other known methyltransferase and strongly suggested that the protein had been wrongly identified in the original analysis of the genome sequence.[20] This is a well-recognised hazard, when most of the genes in a given organism are identified by sequence similarities – often weak – with those in other organisms (which could also be wrong!). We now believe that this supposed MenG is part of a larger complex of proteins involved in RNA degradation, and have identified another protein that is the probable true MenG.

PAE2754 – a conserved hypothetical protein

This protein provides an example of how functional hypotheses can be developed from a protein structure. At the outset of our structural genomics programme we compared the genomes of the two organisms we were

interested in, *P. aerophilum* and *M. tuberculosis*, and identified 250 pairs of genes that were very similar. PAE2754 seemed particularly interesting, as *P. aerophilum* had four close homologues, of which PAE2754 was one, and *M. tuberculosis* had another four, yet all were of unknown function, and structure prediction methods failed to identify any likely structure.

The crystal structure (Fig. 1D) showed that PAE2754 forms a tetramer, in which each monomer has four acidic amino acids clustered in a small pocket.[21] A search of sequences in other genomes finds many similar proteins, all with the same four amino acids, and we speculated that these must be at the active site and might be a metal binding site. We then discovered that another group of proteins, which were known to be exonucleases, *i.e.* they degrade the ends of DNA or RNA, have the same cluster (even though their sequences appear at first sight to be unrelated) and that this cluster is used to bind Mg^{2+} at the active site. We have concluded that PAE2754 and its relatives are part of a novel group of enzymes involved in DNA or RNA editing.[21] From the observation that the four active sites of the tetramer are only accessible through a tunnel we also conclude that they act on single-stranded overhangs from double-stranded DNA – and this has now been shown in biochemical assays!

Conclusions

The examples given above show that the face of structural biology is changing fast. Advances in crystallographic methods, including more systematic crystallization, crystal freezing, powerful and more automated computational approaches and in particular the use of synchrotrons, all

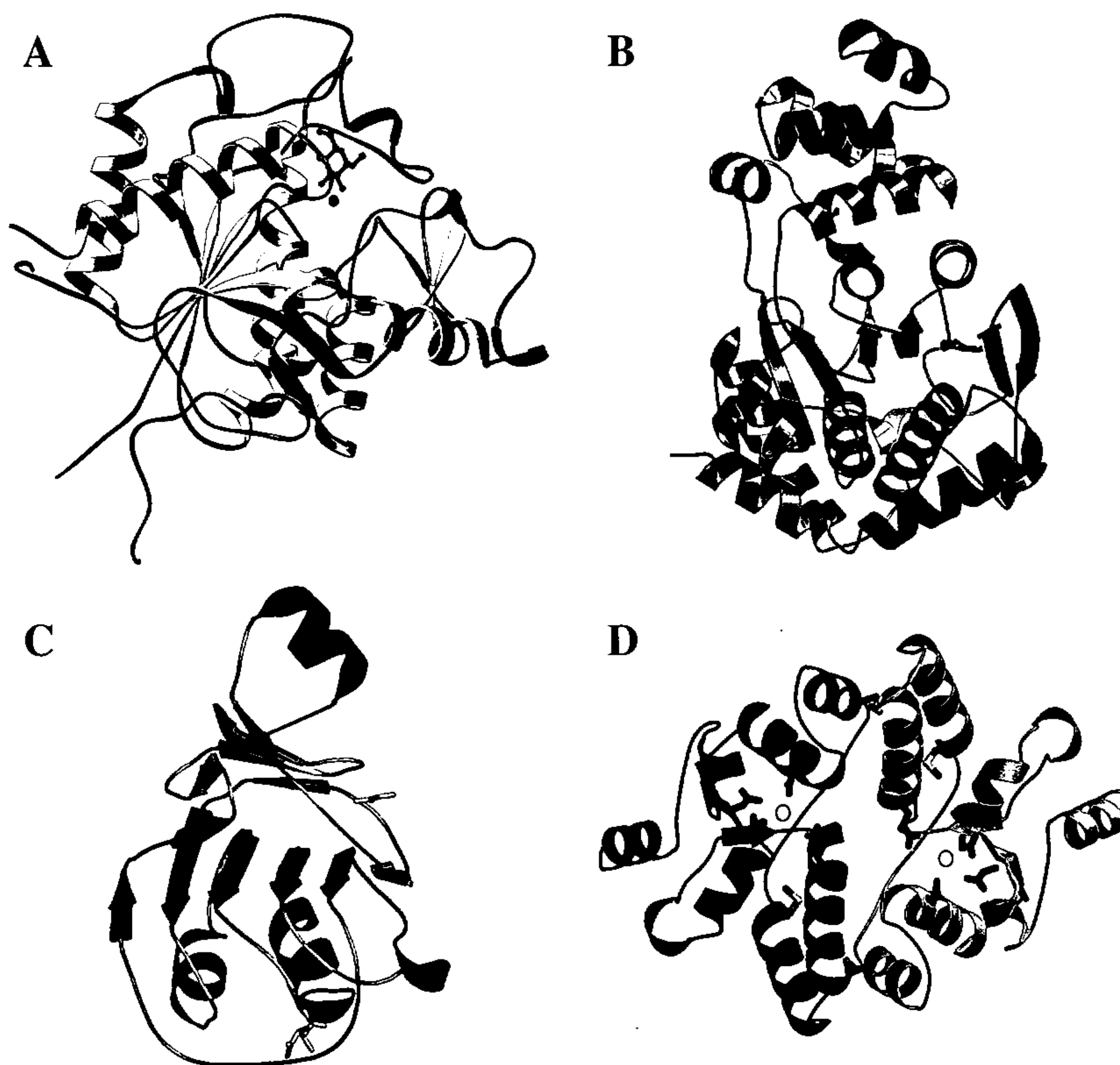

Figure 1. Structures of a representative group of proteins, determined by X-ray crystallography (see text). In each case, the polypeptide conformation is depicted by showing α-helices as coils and β-strands as arrows, joined by loop regions. **A.** MshB: A glucose moiety, from the β-octylglucoside detergent used as a crystallization additive, marks the active site, adjacent to a bound Zn^{2+} ion (black sphere). **B.** TrpD: The enzyme forms a dimer but only the monomer is shown here. **C.** Protein previously thought to be MenG. Three monomers with the structure shown here form a tightly associated trimer. Two small molecules, tartrate and glyoxalate, are found bound to the protein and may give clues to its true function. **D.** PAE2754 previously of unknown function. The protein is tetrameric but a dimer is shown here. Conserved acidic amino acid side chains mark probable binding sites for Mg^{2+} ions (shown by small open circles) and suggest a role in DNA or RNA editing.

combine to make the processes of structure determination very much quicker and easier. Structural genomics initiatives such as that described above will also help to increase the ease with which any protein of interest can be produced in the laboratory. The result should be a bonanza for chemists, in addressing structure and mechanism in biology, designing inhibitors or drugs in the context of their true protein targets, or using proteins for other creative purposes.

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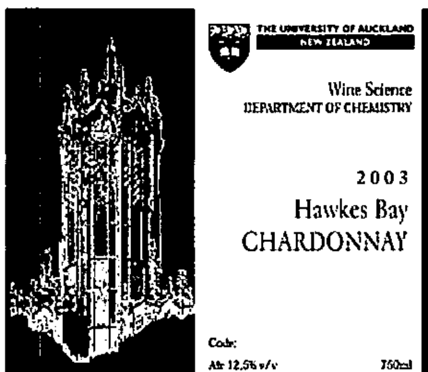
Unlocking The Aromatic Chemistry Of New Zealand Wines

Paul Kilmartin, Laura Nicolau and Stuart Dykes
Wine Science Programme, Chemistry Department, The University of Auckland, Private Bag 92019, Auckland

Introduction

Research into the chemistry of aromatic compounds was taken in new directions in 2003 at The University of Auckland with the introduction of a two-year MSc programme in Wine Science under the auspices of the Chemistry Department. In the first year students take courses on the science of grape production and winemaking, along with analytical and sensory techniques.[1] They also have the opportunity to learn about the organisation and operation of wine businesses and the industry as a whole. A highlight of the first year is the 20-litre scale *micro-vinifications* where each student begins with grapes (hand-picked in West Auckland) to be crushed, pressed, fermented, stabilised, and eventually bottled (with either corks or screw-caps) at the winemaking facilities at the Tamaki campus – the students have many decisions to make about how the wine is handled based upon the analytical data obtained as the wine develops. Additional Chardonnay grapes from Hawkes Bay contributed to the first ever The University of Auckland vintage that was bottled in November of 2003.

Above: Pressing of the first Chardonnay grapes in April 2003. L to R: Stuart Dykes, Simon Sharpe, Greg Rowdon, Chris Ward, John Bradbury, Ian Trembath (Nobilo Wines), and Jan Robertson (Wine Science Technician).



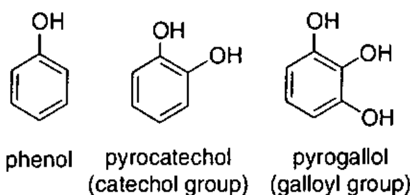
Above: The University of Auckland wine label - 2003.

The second year involves a research project in wine science, and this is where the chemistry of aromatic compounds is becoming a feature. The *aromatic chemistry* referred to here encompasses both the phenol-based chemistry that is responsible for red wine colour and mouth-feel (which fits the chemist's definition of *aromatic* or benzenoid-based chemistry) and the chemistry of the volatile components which make up the wine bouquet – the enologist's definition of *aromatic*. Projects involving polyphenols in red wines have been a feature of Kilmartin's research over the past five years, and current projects include the effect of micro-oxygenation on red wines such as Cabernet Sauvignon and Merlot (in association with Mission Estate Winery) and the development of polyphenols in grape seeds and skins in New Zealand's quality Pinot Noir wines (with Montana Wines). The development of gas chromatographic and sensory procedures for wine volatiles, particularly those characteristic of New Zealand's leading export wine with an established international reputation, the Sauvignon Blanc, are a further focus.

Red wine polyphenols

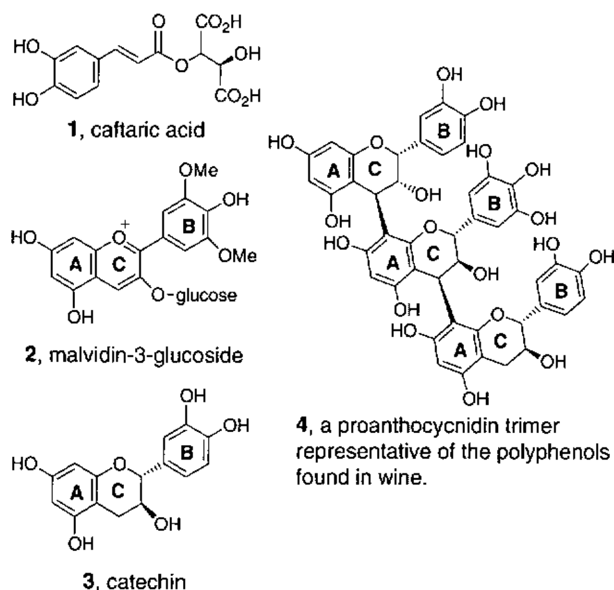
Polyphenols are responsible for the difference between red and white wines, and for differences between young and aged wines.[2] As antioxidants they may also be beneficial for human health – their ability to prevent the oxidation of low density lipoprotein (LDL) may assist in reducing heart disease.[3] Many polyphenols are also tannins. These are substances capable of producing stable combinations with proteins from interactions with saliva glycoproteins to produce a sensation of astringency - but only those that are relatively bulky (M_r 600-3500).

Basic phenols (not found in wine)



Of the polyphenol compounds present, wine is rich in substances with a catechol (*o*-dihydroxybenzene) group. The catechol and pyrogallol (1,2,3-trihydroxybenzene) groups react very readily with enzymes and with oxidants such as reactive oxygen species to form a stable semi-quinone radical that does not abstract hydrogens from other substances to propagate free-radical chain reactions. Instead, it persists to react with another semi-quinone radical or further oxygen species to form a quinone.

There is considerable variability in type and amount of polyphenols with the grape variety, climate, and other growing and processing conditions.[4] White wines are made by quickly pressing the juice away from the grape solids, whereas with red wines the juice is fermented in the presence of the grape solids for several days to enhance phenolic extraction. The level of total phenols in white



wines is typically ~200 mg/L, made up principally of hydroxycinnamic acids such as caftaric acid, **1**.

In red wines the total phenols commonly exceeds 2000 mg/L and consists mainly of polyphenols with a three-ring flavonoid structure. Anthocyanins such as malvidin-3-glucoside, **2**, give wine a red colour (or bluer at a higher pH) as the fully conjugated 10π A/C rings of the protonated form absorbs light at *ca.* 520 nm. Monomeric anthocyanins begin to disappear even before fermentation is complete and few remain after one year in the bottle, while those that become incorporated into oligomeric polyphenols or as other stable derivatives provide for the longer-term colour of the wine.

The most abundant polyphenols in red wines are the flavan-3-ols, *e.g.* **3**, also called the *catechins*. These originate in the seeds and skins of the grape, and are found mainly as oligomeric structures termed proanthocyanidins or condensed tannins (see **4**). Processing steps that incorporate oxygen can yield *o*-quinone products from the catechol groups that react to form new bonds and higher molecular weight compounds.

The measurement of sugar levels in grapes (as °Brix) remains the main chemical analysis procedure used to determine when grapes are harvested. Questions are being raised about how we can determine the optimum harvest time for *phenolic ripeness*. The anthocyanins in particular accumulate to a maximum, which may or may not match sugar ripeness. In Marlborough (and in Australia) sugar ripeness tends to be reached before phenolic maturity whereas in Auckland phenolic maturity may be reached before sugar ripeness. On the other hand, seed proanthocyanins are in decline leading up to ripeness in a variable manner – they move from a lower degree of polymerisation to become larger molecules with marked astringency (90% of dimers and trimers are lost during this time). Complex skin tannins increase in concentration leading up to ripeness, but there is little variation in the degree of polymerisation. Pinot Noir has high seed tannins and low skin anthocyanins while Cabernet has the reverse. The concept of phenolic maturity also needs to include the capacity for extraction of polyphenols as maturity factors control the breakdown of skin cells and ultimate release of anthocyanins, and thus wine colour (squash a grape and look at the juice colour!).

The uptake of oxygen by red wines is a key part of wine maturation prior to bottling, and the main substrates of oxidation in the wine are the polyphenols. This is in contrast with white wines where polyphenol oxidation leads to browning of the wine and is largely avoided. When a red wine undergoes (expensive) barrel aging *ca.* 20 mL of O_2/L wine is added through normal processing operations such as the topping up of barrels. This oxygen helps intensify the colour of red wines through tannin-anthocyanin bonding, and it can also soften the tannins present.

With wine stored in stainless steel tanks, the necessary oxygen is often introduced by pumping the wine over periodically. A new approach initiated by Patrick Ducournau in France and developed in the 1995 research work of Thierry Lemaire at Montpellier[5] makes use of micropore ceramic outlets to deliver 0.5-10 mL of O_2/L/month to wines on a continuous basis. A system was released commercially in 1996 and it is now widely used in Italy, California, Australia, New Zealand, and Chile. The amount of oxygen that enters the wine is kept small enough to avoid build-up of dissolved O_2, so that favoured polyphenolic polymerisation processes are promoted. Changes in wine during micro-oxygenation have been divided into a number of phases as shown in Figure 1.[6] During the *structuring phase* (1-6 months) there is actually an increase in aggressiveness and intensity of tannins on the palate, and a decrease in aromatic complexity. This is followed by the *harmonisation phase* in which there is an increase in favourable tannin softness and wine complexity. Micro-oxygenation should be concluded when these reach a maximum and before over-oxidation leads to a perception of tannin dryness and a loss of wine freshness.

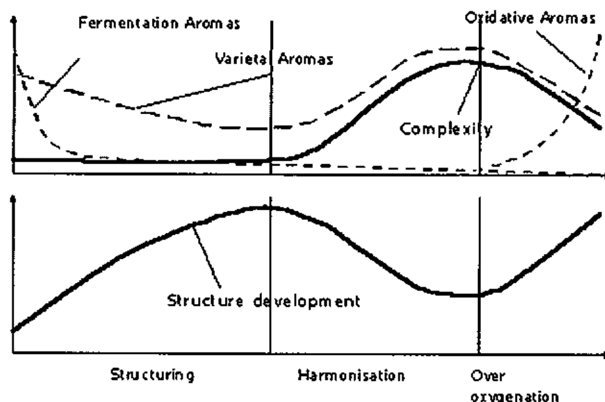


Figure 1. Phase diagram for wine micro-oxygenation adapted from ref. 6.

Our sensory panels and the polyphenol analyses have confirmed a number of these trends. In particular, the mean chain length of the proanthocyanidins, measured using a thiolysis procedure followed by HPLC detection of the constitutive units, has shown that there is an initial increase followed by a decrease in mean degree of polymerization (mDP) of the proanthocyanidin units. These correspond to the structuring phase and harmonization phase, respectively. The particular wine was taken to the over-oxygenation stage where an increase in mDP was also observed.

Recent studies have shown a strong correlation between astringency and the mDP of proanthocyanidins[7] that supports our results. The reason for the decrease in mDP during the harmonization phase is unclear. However, evidence of proanthocyanidin shortening in the presence of nucleophilic species, such as monomeric flavan-3-ols or anthocyanins, has recently been presented and this may provide clues to a possible mechanism.[8]

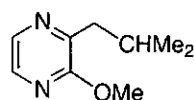
Sauvignon Blanc aroma

What makes New Zealand Sauvignon Blanc wines so distinctive, with characteristic odours that are prized worldwide? In the increasingly competitive international wine market, the quality advantage which Marlborough Sauvignon Blanc, in particular, has established needs to be maintained and enhanced if the New Zealand industry is to keep expanding at its present rate.[9] A scientific understanding of the chemistry of New Zealand Sauvignon Blanc aroma is seen as a key step to accompany research into viticultural and winemaking practices to improve wine quality.

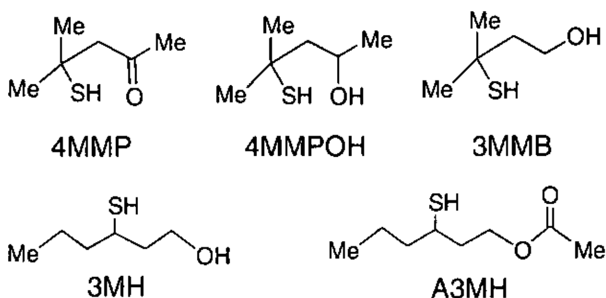
The aroma profiles of many wines are due to a large number of esters, higher alcohols, and further volatile compounds with a complexity that has eluded wine scientists. In contrast with other wine varieties Sauvignon Blanc aroma is dominated by a small number of volatile compounds. The main terms used to describe Sauvignon Blanc wines are associated with plants and fruits and are as follows: green bell pepper, asparagus, grassy, gooseberry, box tree, broom, blackcurrant buds, eucalyptus, grapefruit, passion fruit, *etc.* International research has shown that volatile thiols (RSH) and methoxypyrazines, *e.g.* **5** (Chart 1), are the most important components of Sauvignon Blanc aroma. Many studies have been conducted on the methoxypyrazine contribution to Sauvignon Blanc aroma and the importance of this group of components to the typical herbaceous quality of this wine variety.[10] In general, higher methoxypyrazine levels are observed under cooler ripening conditions.

An additional group of compounds, all volatile thiols, have been identified[11] in the laboratory of Dubourdieu as being responsible for a range of characteristic Sauvignon Blanc aromas. The individual thiols, the pyrazine, their sensory descriptors, and perception thresholds are given in Table 1. While these thiols are present in extremely low concentration (ng/L or µg/L), 4MMP, A3MH, and 3MH (Chart 1) are generally present in much higher concentrations than their respective perception thresholds.

Chart 1. Volatile thiols - see Table 1 for details.



5, 2-methoxy-3-(2-methyl-prop-1-yl)pyrazine



Thus the compounds of Table 1 contribute towards the aroma of French Sauvignon Blanc from Bordeaux and Sancerre, and their quantity varies between different wine samples from different Domaines in the same region and also between regions.[12]

The thiols are present in grapes and must as odourless precursors in the form of *S*-cysteine conjugates. Thus the grape juice lacks the distinctive odours that the wine will eventually take on. However, the grape-grower is not working entirely blind in following the development of the grapes on the vine since crushing a grape in the mouth will release the volatile thiols from their *S*-cysteine conjugates and these will react with enzymes present in the mouth with the flavour emerging after an initial blander taste. In Sauvignon Blanc winemaking the volatile thiols are likewise released by the degradation of the *S*-cysteine conjugates by yeast. The yeast-strain has also been shown to play a key role in the amounts of volatile thiols that are produced in the wine.[13]

In 2004 we aim to establish gas chromatographic methods for the detection of methoxypyrazines, volatiles thiols, and their odourless precursors in grapes. These techniques will be applied to survey the aroma profiles of New Zealand Sauvignon Blanc wines as well as in further projects such as the changes which occur to the aroma of red wines during micro-oxygenation. The procedures will also be applied in collaborative projects with our biological scientists on the yeast genetics of Sauvignon Blanc ferments, and with viticulturalists and sensory scientists from HortResearch and the Marlborough Wine Research Centre of Excellence in Blenheim.

Table 1. Volatile thiols and a methoxypyrazine identified in Sauvignon Blanc wines and their perception thresholds (ng/L) in an aqueous alcohol solution (12 v/v%).

Compound	Abbrev.	Perception threshold	Olfactory description
4-methyl-4-sulfanylpentan-2-one	4MMP	0.8	Broom
3-sulfanylhexyl acetate	A3MH	4	Passion fruit
3-sulfanylhexan-1-ol	3MH	60	Grapefruit
4-methyl-4-sulfanylpentan-2-ol	4MMPOH	55	Citrus zest
3-methyl-3-sulfanylbutan-1-ol	3MMB	1500	Cooked leek
2-methoxy-3-(2-methyl-1-propyl)pyrazine	**5**	2	Green pepper

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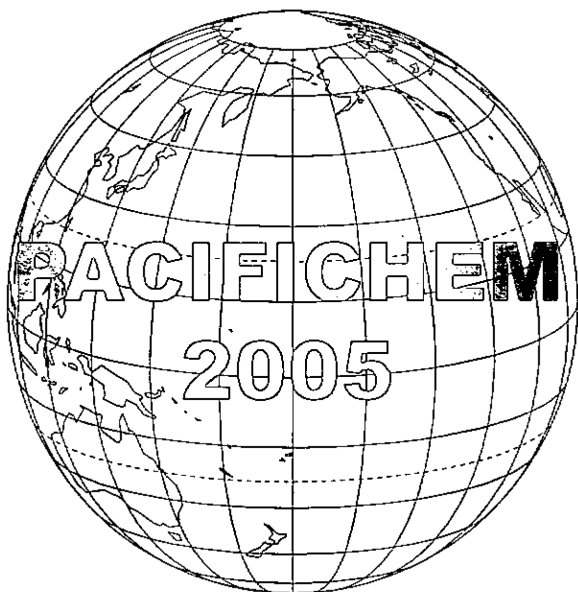

Update

www.pacifichem.org

The 2nd round of submissions for the 2005 symposia closed on March 25 and these will be assessed for their merit and suitability to add to the 140 or so symposia already accepted. The final round of submissions will close later in the year and the full program is expected to be available on-line shortly thereafter. The current list of accepted symposia and all relevant details are available on the website as detailed above. As at the date of writing some 35 NZIC members have been identified as possible speakers/organisers and a strong contingent is anticipated at the meeting which will take its customary format in the hotels along the Waikiki beachfront in Hawaii.

The Organising Committee have approved a package worth in excess of $US 60,000 to support attendance at the Congress by chemists from the developing regions of the Pacific Basin. A list of the designated countries and the requisite application materials are available on-line at the Pacifichem site (see above). ***If you have a colleague working in one of these countries, encourage him/her to apply - grants are for $US2000 each plus complimentary Congress registration, and application close on 15 January 2005.***

Now is the time to be thinking of presenting a paper, raising the travel funds and planning on taking just a little time on the beach between symposium sessions!

Brian Halton (Pacifichem Representative).

Chemistry In ESR, Auckland

Keith R. Bedford and Jim W. Mitchell
ESR, Private Bag 92-021, Auckland
Email: Keith.Bedford@esr.cri.nz

History

The Institute of Environmental Science & Research Limited (ESR) was constituted as a Crown Research Institute (CRI) in 1992 in the course of the restructuring of government science and research in New Zealand and as a consequence of the coming into effect of the Crown Research Institutes Act. ESR was formed from parts of the former Department of Scientific and Industrial Research (DSIR Chemistry), the former Department of Health (Communicable Disease Centre) and regional Public Health Laboratories. As a CRI, ESR is incorporated as a private company under the Companies Act 1955. The shareholders of ESR are the Minister of Crown Research Institutes and the Minister of Finance. ESR is, for the purposes of the Public Finance Act 1989, a Crown Entity.

ESR can trace its origins through predecessor organisations, including Chemistry Division of the DSIR, back to the establishment of the Colonial Laboratory in Wellington in 1865. Forensic work and food and drug analyses formed a significant part of the work of the fledgling organisation from its very early days.[1] In due course regional laboratories were established, including one in Auckland, each headed by a *Government Analyst*.

ESR Evolves

ESR began operations with a disparate collection of responsibilities and with organisational cultures and practices reflecting the varied origins of its parts; it serviced mainly government clients. According to one source, there was doubt at a high level about whether ESR would survive as a viable CRI.[2] The years immediately following establishment were marked by a significant amount of restructuring, reorganisation and consolidation of activities. In Auckland this process ultimately led to the closure of the Mount Eden site, inherited earlier from the then Department of Health, and the exiting of air pollution and waste monitoring activities based at that site. From the turmoil of those years the present structure evolved, based on three Business Groups: Environmental Health, Forensic, and Science Information Management Services (SIMS). Of the three, the Environmental Health and Forensic Business Groups have significant activities at the ESR Mount Albert Science Centre, which serves as the base for approximately 100 staff.

In the forensic disciplines, it became apparent that separate *full service* laboratories, set up in Auckland, Wellington and Christchurch for historical reasons, could not be sustained. This was because of the increasing cost of instrumentation required to deliver a service to international best practice standards and because of the cost of maintaining expertise. In 1997 a radical restructuring of forensic science services was undertaken. Forensic Service Centres in Auckland, Wellington, and Christchurch became bases for scene examination experts. The scientists and technicians at these centres work with Police at serious crime scenes, provide consultancy and training services, are the point of contact for law enforcement agency clients, and case-manage major investigations. They in turn, refer selected items and samples to specialised analytical laboratories and re-integrate the results into the context of the full investigation. A number of these specialised analytical laboratories are based in Auckland and are described later.

Environmental Health

The main environmental health interests of ESR are communicable disease, population health, food safety, and water quality, and are spread over three sites (Mt. Albert, Kenepuru and Ilam). The Mt. Albert site contains part of the food safety group (including microbiology and chemistry) which is led by Professor Ian Shaw at Ilam, Christchurch.

The Mount Albert food chemistry staff of nine has a varied diet of food analysis, consultancy, and research. Going back to earlier days as DSIR Chemistry, the emphasis was on provision of scientific services to government departments such as Health and Customs, and today much of that government-related work continues, notably for the NZ Food Safety Authority (NZFSA). Current NZFSA projects include:

- Scientific support for District Health Boards that requires analysis of food complaints, *e.g.* taints, foreign matter, adulteration, *etc.* *Food forensics* is highly challenging and requires access to a wide range of professional and instrumental expertise in external organisations. Examples are insect and small animal experts, mycologists, forensic dentists for examination of tooth fragments from food, and isotope ratio analysis and scanning electron microscopy.
- Wine certification of all wine exported from New Zealand is mandatory. Thus it is analysed for compliance with local and some overseas regulatory requirements.
- Nitrite and nitrate analyses of various food samples provide data for New Zealand dietary exposure and risk assessment.

Work for commercial clients includes:

- Food complaint work for manufacturers.
- Import/Export certification analyses for foods and utensils.
- Authenticity (*true to label*) studies for clients such as the Commerce Commission, Consumers Institute, the Wine Institute, and the New Zealand Juice and Beverage Association.
- Identification of polymeric materials and additives in food packaging migration testing.

The research interests here are presently focused on antimicrobial properties of food components, with recent projects on wine and grape juice in collaboration with The University of Auckland.

Forensic Science

The Auckland ESR site is the base for the Auckland Forensic Service Centre and specialised analytical laboratories deal with drugs, including clandestine laboratories, physical evidence, and biology/DNA.

The Auckland Forensic Service Centre provides around-the-clock response to Police in the top half of the North Island. Several staff members have developed specialist expertise

in fire investigation (cause and origin) and may be called upon to provide assistance in other parts of the country.

ESR uses the forensic accreditation administered by the American Society of Crime Laboratory Directors Laboratory Accreditation Board (ASCLD-LAB). This scheme has been extended to include the discipline of crime scene examination. It is of note that in 2001 the three ESR Forensic Service Centres were among the first six laboratories to be awarded this accreditation and were the first outside of North America.

The range of drugs submitted to the Drugs Laboratory has varied with time. A current issue is the huge increase in methamphetamine seizures resulting from the proliferation of clandestine laboratories (*clan labs*), often referred to as *P labs*. The *P*, for *pure*, refers to the percentage purity of the product. Prior to local manufacture, street drug samples of methamphetamine were usually heavily diluted or adulterated, and were often only 3–5% pure. *P* is the manufactured product and whilst it may be contaminated with by-products or impurities, is usually of the order of 80% methamphetamine, generally as the hydrochloride.

The investigation of *clan labs* has significantly increased the need for specialised *clandestine laboratory investigating chemists* and ESR has struggled to recruit and train sufficient people in this area to keep pace with the increase. An idea of the magnitude of the issue can be drawn from the fact that New Zealand's first methamphetamine laboratory was found in 1996. Since then the number of *labs* discovered has escalated dramatically. Police found 9 *labs* in 2000, 41 in 2001, and 147 in 2002. In the first 10 months of 2003 Police had already shut down 146 *laboratories*. The training required for a *clandestine laboratory investigating chemist* can take two years and includes aspects of hazard assessment and health and safety advice, as well as evidence recovery and crime scene interpretation. The need for this intensive training is highlighted by reports from the US that one in five *clan labs* is discovered because of a fire or explosion.

The Physical Evidence team deals with a very wide range of examinations and analyses that include toolmark and firearms examination, glass, fibres, paint, and hydrocarbon residues (from suspicious fires) analyses, physical fitting, and a wide range of one-off examinations. Significant investment has gone into recently equipping this laboratory with modern analytical instrumentation including a pyrolysis GC-MS, microspectrophotometer, automated fibre-searching instrument, as well as FT-IR with microscope and automated glass refractive index measurement (GRIM). This group has won international recognition for work in evidence interpretation, particularly applying a Bayesian statistical approach.

The area of forensic science that has changed at an astonishing rate over the past decade is that of Forensic Biology. The discipline is now synonymous with DNA. The technology involved has gone through several *generations* and continues to develop rapidly. In 2003 New Zealand celebrated *DNA 50*.[3] At the establishment of ESR in 1992 forensic DNA testing was in its infancy while in 2003 at ESR about 35 people were employed in our national Forensic DNA facility; they processed an estimated 40,000 samples of all types. The new, state-of-the-art DNA Facility was opened in May 2002 and includes the New Zealand National DNA Databank as well as separate laboratories for casework analysis.

The New Zealand DNA Databank consists of two databases. The first is the National DNA Database (NDD), which is comprised of DNA profiles from individuals who have supplied reference samples either voluntarily or compulsorily. As at December 2003 there were more than 37,000 DNA such profiles on this database. The second is the Crime Sample Database (CSD), on which more than 7400 DNA profiles from crimes have been entered. The two databases are functionally separate. However, a matching facility allows each of the databases to be compared against itself as well as against the other database. In this way crime-to-crime links and crime-to-person links are generated. Currently, the crime-to-person hit rate between the two databases is 50%. This means that half the profiles obtained from crime scene samples produce a link to a known individual on the NDD thus providing intelligence information for the Police. Crime-to-crime links have currently been identified in 33% of case profiles loaded. The highest number of crime scenes linked to a single, but as yet unidentified, individual is 18.

Forensic Research

External funding for forensic science research in New Zealand has historically been almost non-existent, due largely to the misconception that forensic science is "police science" and should be funded by the end-user. More recently Science and Research funding agencies have begun to appreciate that forensic science includes public good science and some progress has been made with funding projects that explore the relationship between the contribution of forensic science to society and social science issues. However, the most significant impetus to the development of forensic science research in ESR has been the establishment of a partnership between The University of Auckland and ESR in the Postgraduate Forensic Science Programme that commenced in 1996. Studies are at a variety of levels and now include PhD research. Projects undertaken by the students in the eight years since the programme began have made a very significant contribution to published work, and to what is described as *operational research*, often with Police capability development programme support. There is collaboration with other tertiary institutions in the forensic area, *e.g.* with toxicology work based in Wellington, but this is beyond the scope of this article.

Conclusion

The Mission Statement of ESR refers to the application of *specialist science solutions* to protect people and their environment. Applied chemistry in the environmental health and forensic work undertaken by ESR makes a major contribution to fulfilling that mission in Auckland.

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AN INTERVIEW WITH LEIV K. SYDNES - IUPAC PRESIDENT 2004-2006

Leiv Sydnes (Professor of Chemistry, University of Bergen, Norway) has been a personal friend of Professor Brian Halton, Editor of *Chemistry in New Zealand* for many years. He has visited New Zealand several times and stayed with Brian Halton in Wellington each time. What follows arose from discussions on the occasion of his last visit. Since then Leiv has been awarded the 2003 Federation of European Chemical Societies Award for service. Apart from his work for IUPAC, this recognition is for his bringing the value and excitement of chemistry to the schoolroom and to society in general - and this includes prime time TV programmes on relevant chemistry related topics as well as numerous public lectures.

Ed. Do you think that most chemists are adequately aware of the International Union of Pure and Applied Chemistry (IUPAC) and its activities?

LS. In general, no. I have not yet met a qualified chemist who does not associate IUPAC with nomenclature and perhaps meetings, but these activities are just a part of our preoccupation. IUPAC is involved in a large number of other areas and we are trying to inform the chemical community about initiatives and actions taken. While I have to admit that our information does not come across the way we would like, I am sensing an increased willingness in the chemical community to serve and become engaged in IUPAC activities.

Ed. What do you see as the most important goal of your Presidency?

LS. My most important task is to increase the profile of IUPAC and a better awareness of the Union in the chemical community and ensure that its core activities are in good shape. The President, therefore, has to act as a facilitator of initiatives and actions in a range of areas that advance worldwide aspects of the chemical sciences and contribute to the application of chemistry. As many new initiatives are required there should be lots of room for interested chemists to participate in IUPAC activities.

Ed. But these are very general comments. Can you be more specific?

LS. Firstly, the very existence of the Union is dependent on its projects that address scientific issues requiring either international standardization (*e.g.* of nomenclature, terminology, and quantities) or the evaluation and critical assessment of quantitative data in the service of Mankind. In this work the various IUPAC divisions must play an active role under the leadership of the division committees. Generally speaking, those countries with higher representation in the IUPAC governing bodies have by far the most members in the various project task groups. As pointed out by a number of participants at the 2003 General Assembly, this imbalance of the geographic distribution of the IUPAC workforce needs to be corrected. This is easier said than done, but it is my hope that by improving the contact between the divisions and the chemical communities in the IUPAC member countries, through the work of the Union Advisory Committee, and by meeting with the National Adhering Organizations in the Union's member countries, the geographic base of the task groups will broaden considerably. I hope that we can meet this challenge and be active in the search for qualified volunteers willing to serve IUPAC.

Secondly, I would like to see that IUPAC becomes more visible at conferences and utilize them more actively to make the Union better known in the chemical profession. In doing this, recommendations, technical reports, and reports from task groups can be disseminated and new projects likely will evolve.

Ed. But how do you see this? Currently IUPAC Affiliates receive a 10% discounted conference fee for such sponsored conferences but there seems to be little other involvement other than a conference report in *Chemistry International* from those that provide one.

LS. In order to achieve IUPAC support of a conference there should be some strings attached so that at least one session during the meeting is dedicated to discussion of exciting scientific topics related to the conference theme and those appear likely to emerge, and to assess the need for standardization or critical assessment of quantitative data within the conference theme, or to explore if new.

Ed. Do you think that conference organisers will accept what they may see as an intrusion?

LS. If organized skilfully by the appropriate division(s), with a global perspective and the young chemist in mind, such sessions would gradually prepare the next generation of practitioners of the chemical sciences to meet the challenges of the future, say, to invent new materials, to develop new devices with extraordinary properties, and to work out new synthetic pathways of compounds for specific medical purposes in a sustainable fashion. In this way IUPAC will contribute to sustainable development and improved quality of life in a global perspective, which are among the important aims for the Union.

Ed. What other facets of chemistry do you see as important?

LS. In today's media-sensitive society, rating, standing, and reputation are important issues that we must consider. In doing so we realize that the chemical enterprise suffers from a dubious public image. Chemicals are too often associated with bad things happening to people and the environment, and in recent years, with chemical warfare. Yet the positive contributions from chemistry and chemical engineering that happen to society (and every one of us) on a daily basis, are barely publicized; this in spite of the fact that these sciences are instrumental in feeding us, clothing us, housing us, healing us, and even entertaining us. For everyone engaged in the chemical sciences this is of course frustrating. This situation presents a significant challenge that industrial and chemical organizations on an individual basis have made efforts to meet in the past, and continue to meet today by trying to present a balanced picture of the benefits and risks of chemicals. Considering the relatively low return on all these efforts and the expenses involved it seems reasonable to ask whether some coordination of the activity would be beneficial, and then, whether information presented through IUPAC, an organization with a global reputation of providing authoritative and unbiased information in the field of chemistry, would have added value and be regarded as more trustworthy than facts and figures furnished by others. In order to try to answer these and a number of related questions, IUPAC has funded a feasibility study *Chemistry's Contributions to Humanity*. The task group, chaired by Dr. Edwin D. Przybylowicz, has wide-ranging membership including several chemists from the Committee on Chemistry Education under the leadership of Professor Peter Atkins. The project group has an enormous task, and any input to make the feasibility study more complete would be very much appreciated. I urge your readers to read the project description, see:<www.iupac.org/projects/current_projects.html> and supply information to Dr. Przybylowicz or the IUPAC secretariat, as they are able.

Ed. Many of my colleagues feel that IUPAC misses out in its interaction with the individual chemist because IUPAC does not communicate directly with NZIC and therefore our chemical community; instead, the Union communicates with the Royal Society of New Zealand, which is designated by government to represent New Zealand. Do you have any comments?

LS. We are aware of this situation, which is not unique for your country. In order to improve the situation the IUPAC secretariat has worked hard to improve our webpage, which I find so good that I am sure many will find it very useful. One thing that is obviously missing, however, is a complete set of links to the homepages of the chemical societies in the IUPAC member countries. Such links, asked for by chemists in academia and the chemical industry as well as representatives at the last General Assembly, are very useful, but not always easy to get hold of because one may need to know the name of a chemical society in a foreign country the language of which one does not know. The means to do the job have been available for a long time, but the chemical community has not performed well enough. In this regard it is time for individuals to take action and check what appears at 'www.iupac.org' as information for his/her country. If the information is incomplete then encourage the appropriate individual in your society to furnish the necessary information. IUPAC deserves global support in this endeavour; after all, it is done to facilitate networking among us for the benefit of all.

Let me also mention that we hope to improve the communication with the chemical community in all member countries through the Union Advisory Committee (UAC), which is a new committee established by Council at the meeting in Ottawa in August last year. Each member country has a member in UAC, and this individual is supposed to know and interact with the chemical community in his or her country. The member from your country is Professor Kip Powell of the University of Canterbury.

Ed. Do you have any final comment?

LS. I regard *facilitation* to be an important task for the President of the Union. In order to help the chemical community to use IUPAC more actively adequate input is required. Of course a fair amount of information comes through the divisions, active task groups, *etc.*, but IUPAC Officers would appreciate constructive comments and ideas from individuals that care to write. I therefore challenge your members to write me (Email: leiv.sydnes@kj.uib.no) (or to any other IUPAC officer) and present their comments and what they have in mind for attention during my presidential period. I look forward to a fruitful relationship with New Zealand for the benefit of IUPAC!

Ed. The NZIC wishes you and your IUPAC colleagues every success in achieving your objectives that will benefit us all.

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EDITORIAL

Andrew Brodie, President

If you attended the New Zealand Institute of Chemistry Conference in Nelson in December 2003 you would have gained the impression that our Institute is alive and well. The meeting was an outstanding success and Andrew Abell with his committee are to be congratulated on their achievement. Around 400 delegates attended, with a strong contingent of younger researchers from the universities and other organisations. The interdisciplinary themes (as highlighted by the plenary lectures), the enthusiastic presentation of posters by students and a great conference dinner, all added up to a vibrancy and excitement in our discipline. The big question is - how do we capture the energy of our conferences into our Institute as a whole?

The NZIC is not a vibrant organisation. The AGM at Nelson could not even attract a quorum! How many, apart from the branch committee, attended your last branch meeting? Did you? If we continue in our present form, the eventual demise of the NZIC is highly probable. You think I exaggerate? Let's look at some the problems:

- *An aging and declining membership* - about half of you are over 50.
- *Few student members stay on as full members* - of the 52 students who joined in 1998, only 2 are now full members.
- *Membership skewed towards the professional chemist* - about half of you have a PhD.
- *Industry is not well represented in the organisational structure* - few fellows (FNZIC) are from industry.
- *Expenditure is greater than income from members' subscriptions* - we have survived financially because other NZIC activities such as conferences and *CHEM NZ* have made a profit.
- *We feel we are busier than we used to be* - changing organisational structures in universities and research institutes such as the CRIs mean that chemical scientists do not feel they have the time or workplace support to assist with NZIC administration, either at branch or national levels.
- *Communication within the Institute is poor* - we all have to make more of an effort to improve this.

As our immediate past president, David Bibby, said during his branch visits last year, chemical science has been a victim of its own success. Chemistry is still an important core scientific discipline but its concepts and techniques are being used by emerging areas. Materials science is built on the traditions of inorganic and physical chemistry. Chemical biology is a merging of chemical and biological science. Scientists prefer to join more specialised societies rather than a broad brush one representing all the chemical sciences. To a large extent, the American Chemical Society has been successful in catering for a wide range of interests, but it is much larger than the NZIC.

Council has started discussing various strategies and will be considering all options. As part of the process, First Vice-President, Graham Bowmaker and I visited our administrative office at ANCAT Holdings Limited in Auckland. As David Bibby has said, one option is to do nothing. The other extreme is to terminate the NZIC and dispose of its assets! Options such as ceasing publication of *Chemistry in New Zealand* or substantially increasing subscriptions, do not face the core issues but delay the inevitable. However, as an interim measure, issues of the journal will contain less material and branch grants are likely to be the same as 2003. This is unfortunate but it would be fiscally irresponsible of your Council to allow expenditure to exceed income.

As Stephen Mann[1] said, when referring to University chemistry in the UK but the same comment applies to the NZIC, "We need a collective 'Viagra-like' remedy rather than a life-science support machine, to restimulate the imagination ... " What sort of Institute do you want? For the NZIC is not Council or its Executive but you - the grass roots membership in the branches. That is where the action must be if we are to do more than limp along in the next decade. I am confident that together we can work through the issues for chemical scientists have always been good at facing challenges and seizing the opportunities that they present. It is important that we hear from you, whether via your branch delegate or email to me on A.Brodie@massey.ac.nz. I ask you to think beyond the square and apply the creativity you apply to your job - whether it be in the industrial plant, research laboratory or classroom - and come up with some solutions. The future of the NZIC lies in your hands!

1. Mann, S. *Chemistry in Britain*, **2003**, *39*, 3.

CHEMICAL EDUCATION TRUST 2004 DISTRIBUTION

Applications are invited from secondary school chemistry teachers (senior chemistry teacher via Head of Science) for grants from the NZIC Chemical Education Trust to promote the teaching of chemistry in their school. For the 2004 distribution, grants of about $400 are envisaged but greater or lesser amounts can be applied for. Applications should be received no later than 1 August 2004 and should be addressed to:

Dr. P. T. Holland
NZIC Chemical Education Trust
Cawthron Institute
Private Bag 2
Nelson
Email: patrick.holland@cawthron.org.nz

NEWS

New Year Honours

We congratulate:

Dr. Allan Limmer (Stonecroft Wines, Hastings) on election to ONZM for services to the wine industry and **Professor Margaret Brimble** (The University of Auckland) on election to MNZM for services to science.

Andrew Brodie 2004 President, NZIC

Above: Andrew Brodie.

Andrew Brodie discovered he enjoyed chemistry more than any other subject when he was a youngster in the third form at Cashmere High School in Christchurch. He says, *I can still remember the experiment that hooked me onto the subject – heating up blue hydrated copper sulfate and watching it turn white as it dehydrated.* His study of chemistry continued at the University of Canterbury, where he completed a BSc (Hons) degree (1965) and PhD in 1968 (under the supervision of Cuth Wilkins and Gordon Rodley).

After two years of postdoctoral work at University College, London with Jack Lewis (now Lord Lewis), he returned to New Zealand at the end of 1970 to a lectureship at Massey University. Apart from a year at Osaka University (1976) and two years at Cambridge University (1982 and 1989), he has remained at Massey. He has enjoyed the various challenges as he moved up through the ranks from lecturer to Professor of Chemistry and Head of the Department of Chemistry and Biochemistry, and then the separate Department of Chemistry. He now heads the Chemistry group in the Institute of Fundamental Sciences.

With colleague Eric Ainscough, his research is focussed on the Marsden-funded project, *New Metal-Rich Molecular Scaffolds*, which is concerned with the reactivity of multi-modal phosphazene ligands, including polymers, towards transition metals. Other research areas have included the structure and reactivity of compounds containing metal-sulfur bonds, spectroscopic studies on the metallo-protein, human lactoferrin, and the synthesis of new multidentate and phosphorus-nitrogen ligands with the aim of producing novel reactivity patterns when these are bound to transition metals.

He believes it is important to support one's professional organisation and been involved in a multitude of NZIC activities including being a member of the Manawatu Branch committee (1972-74 and 1998-present) and it's chairman in 1979. He also chaired the 1988 NZIC Conference organising committee, co-chaired IC'99 (a joint conference with the RACI Inorganic Division), edited CHEM NZ (1983-87), has been on the NZIC Council since 1999 and the RSNZ Council (representing the Physical Sciences and Technologies) since 2002. He relaxes by swimming, tramping, gardening, and playing the clarinet in the Manawatu Concert Band.

New Royal Society Fellow

Geoffrey Brind Jameson (Professor in Structural Chemistry and Biology, Massey University, Palmerston North) was elected FRSNZ in November 2003.

Above: Geoff Jameson at work.

Geoff Jameson is a graduate of the University of Canterbury [BSc(Hons) and PhD] with research on picket-fence porphyrin models for O_2-binding haemoproteins (under Ward Robinson and the late Gordon Rodley). Following his PhD in 1977, he was a postdoctoral with James Ibers (Northwestern University, IL, USA). He moved to Switzerland (University of Zurich) and after two-and-a-half years of research in solid-state chemistry and mechanisms of topotactic reactions, he returned to the USA (Georgetown University, Washington DC) in 1982 gaining tenure in 1987. There, research focused on model systems for non-heme iron proteins and the first model systems that captured the asymmetry ubiquitous to the Fe-O-Fe moiety in proteins were synthesised. Structural studies were complemented by a variety of magnetic and spectroscopic techniques in order to understand unique properties of these systems.

He returned to New Zealand to Massey University in 1994 and research has increasingly focused directly on biological systems, taking advantage of molecular biology techniques that allow a degree of control that is very difficult to achieve

with model systems, in order to establish relationships between structure and function. He has contributed new insights into structure-function relationships in lactoferrin, in particular the role of surface free energy in the opening and closing of the metal-binding cleft, a theme recently pursued in understanding how the milk protein, β-lactoglobulin, maintains a large solvent-exposed, water-hating (hydrophobic) pocket. β-Lactoglobulin has been intensively studied to determine also the structural basis of a host of pH-dependent properties, and the structural basis of the different heat sensitivities of different naturally occurring variants (a subject of considerable interest to the dairy industry). The Massey group was the first to determine unequivocally the mode of binding of fatty acids to this molecule, resolving a controversy that had been vigorously debated for many years.

The major thrust of recent research has been on iron and manganese superoxide dismutases, a key component of biological defences against the hazards of life in aerobic and transiently aerobic environments. Here, work focuses on a manganese superoxide dismutase whose structure has been determined to an unprecedentedly high resolution of 0.90 Å, a resolution that allows hydrogen atoms to be seen.

Geoffrey Jameson has contributed to the practice of X-ray crystallography through developing methodology (now incorporated in the SHELXL program for handling structure determinations of small molecules through to proteins when the X-ray diffraction data are harvested from twinned crystals) and in handling *pathological* small-molecule structures both for molecular structure and, equally importantly, the relationships between structure and function of the compounds. The focus of his work can be summarised as providing that structural framework necessary for understanding form and function in chemical and biological systems. He applies the techniques of X-ray diffraction, the ultimate microscope into the structure of matter, to determine three-dimensional structures of molecules, ranging from substances that contain only a few atoms to large protein molecules.

Geoff has published over a 100 scientific papers (in peer reviewed journals) as well as 5 book chapters. He is a frequent keynote speaker at large international conferences in crystallography and bioinorganic chemistry. He gained a personal chair in Structural Chemistry and Biology (2002) and is known to many members for his 2003 RSC Australasian Lectures. He was awarded the 2003 SGS prize of the NZIC.

Easterfield Medals Awarded

The December 2003 NZIC Conference saw the presentation of both the 2001 and 2003 Royal Society of Chemistry/NZIC Easterfield Medals by NZIC President **Professor David Bibby**. *Associate Professor* **Carol Taylor** (Massey University) received the 2001 medal and gave her lecture *Hydroxyprolines: Molecular Engineering par excellence* while **Dr. Kate McGrath** (University of Otago) was awarded the 2003 medal and delivered her lecture *Moving Away from Rhombohedral Calcite: Controlling Crystal Morphology.*

Above: The Easterfield Medal (top) with presentations to Associate Professor Carol Taylor (middle) and Dr. Kate McGrath (bottom). Presentations were made by Professor David Bibby. (Photo Credits: Professor Brian Halton).

BRANCH NEWS

WHO'S WHO IN THE BRANCHES - 2004

(C - Chair; S - Secretary; T - Treasurer; D - Council Delegate; E - Branch Editor; Com - Committee member)

AUCKLAND
C/D: Gordon Rewcastle; S: Gordon Miskelly; T: Alistair Nielson; E: Mark Paton. Com: Alastair Bingham, Brent Copp, Sanjaya Sena (Student Representative), Jadranka Travas-Sejdic, Anna Yee.

CANTERBURY
C/D: Jan Wikaira; S: William Lewis; T: Rob Lake; E: Cassandra Hinton; Com: Michael Edmunds, Rebecca Hurrell, Andy Pratt, Jonathon Slater.

MANAWATU
C/D: Jeremy Dombroski; S: Justin Bendall; T: David Shillington; E: Ben Mulchin. Com: Mike Boland, Grant Boston, Andrew Brodie, Dave Harding, Richard Haverkamp, Geoff Jameson, Emily Parker, Barry Scott.

OTAGO
C: Allan Blackman; S/E: Paul Fawcett; T: Kim Currie; D: Keith Gordon; Com: Sally Brooker , John Birch, David Cordes (Student Representative) Lyall Hanton, Jonathan Kim, Rachel Mortimer, Margaret Mills, Sigurd Wilbanks.

WAIKATO
C: Bob Wilcock; S: Kitty Lee; T: Michael Mucalo; D: Richard Coll; E: Michele Prinsep. Com: Shane Burggraaf, Carrick Devine, Bill Henderson, Paul Judd, Allan Langdon, Sir Don Llewellyn, Nick Lloyd (Student Representative), Brian Nicholson, Nathan Pritchard, Peter Robinson.

WELLINGTON
C: Ken MacKenzie; S: David Weatherburn; T: Alan Turner; D/E: Brian Halton. Com: Gary Ainge, Suzanne Boniface and Elizabeth Douch (shared Schools' Representative), Dan Brew, Tom Clarkson, Kirsten Edgar and John Ryan (shared Student Representative), Vince Gray, Ted Harvey, Kathryn McGrath and Peter Northcote.

CANTERBURY

The November meeting was addressed by **Dr. David J Newman** (Natural Products Branch, National Cancer Institute, USA). In his talk, *The Role of Natural Products in Drug Discovery*, David gave us his view on the vast extent to which pharmaceutical agents have been derived from natural products. He went on to explain how, with a little bit of forensic work on the chemical structure, what appear to be totally synthetic pharmaceutical agents can be shown to have been simple elaborations of compounds produced from a wide range of mother nature's chemical factories in the form of plants, microbes, animals, and even minerals.

The Canterbury Branch held its Annual General Meeting in December and, in addition to the formal proceedings, student recipients of Travel Grants to attend the NZIC Nelson conference provided a 5-minute presentation on what was to them the most significant seminar at the conference. Posters presented by Canterbury students were also on display at the meeting. The feedback from students was of a high standard and much enjoyed and appreciated. Many members who had attended the conference enjoyed the recap gaining another perspective on material presented. The students' feedback attested to the benefit of the conference, strong student participation in it, and Branch support for doing so.

The Branch congratulates University of Canterbury student **Anna McConnell** who gained our Student Prize for the Top Chemistry Student at 200 Level for 2003. Anna will be awarded her prize at the Student BBQ in March 2004.

ESR

Dr. Chris Nokes has been appointed as a Technical Expert on the Ministry of Health's Expert Committee on Drinking Water Quality. The Committee is being convened for the revision of the Drinking Water Standards for New Zealand to which Chris will add strong chemical technical input when considering the chemical and microbiological parameters.

Professor Ian Shaw has been appointed as Pro-Vice Chancellor for the College of Sciences at the University of Canterbury and will be leaving ESR in March. Though he will be very much missed, ESR wishes Ian well for his new challenge at the University of Canterburyand we look forward to our continued association with Ian in this new role.

ESR has entered collaboration with the Fiji Institute of Applied Sciences to undertake a Fijian Total Diet Survey (TDS). As with the approach taken in New Zealand, the TDS considers the constituents, including chemical, in foods as prepared and eaten by consumers. The two-year project headed by **Dr. Richard Vannoort** (ESR) is funded by NZAID. The Forensic Group benefited from the close proximity of the International Association of Forensic Toxicologists conference in Melbourne last November. **Shelli Turner, Sue Nolan, Stuart Dickson, Mike Heenan, Alexandra Park** and **Scott Hampton** all participated.

University of Canterbury

The Chemistry Department congratulates **Andrew Abell** who was promoted to a personal chair in Chemistry in December 2003. Student excellence has been recognized through the awarding of the Department's annual prizes as per:
Fenwick Prizes: **David Pearson** (400 level), **Andrea Cusiel** and **Sam Yu** (best 100-level demonstrators).
Haydon Prize: **Jocelyn Starkey** (300 level).
Ralph Earle Prize: **Liesl Marsh** (2nd-year PhD review seminar).
Roper PhD Scholarship: **David Pearson** (best 400-level student in science).
University PhD Scholarships: **Sam Yu, Nick Weng, Jennifer Zampese, Emma Turner**, and **Kaleem Syed**.
University of Canterbury Research Award: **Greg Francis**.

David Pearson has also been awarded a Lord Rutherford Memorial Research Fellowship, the second such award to a chemistry student in the last 12 months; **Tim Heaton-Burgess** was awarded in 2003. **Nick Weng** has also been awarded a Sims Empire Scholarship.

James Gardiner has completed his PhD but will stay until mid-2004 as a postdoctoral fellow with Abell's group before he heads off to ETH in Zurich. More recently, **Mark Bart** presented his final PhD seminar in February and gave an excellent performance in the subsequent oral examination. The Harland group will miss Mark but will regain floor space around his desk sufficient to accommodate a couple of new graduate students!

New international postgraduate students include **Steven Aitken** (MSc East Anglia and Calgary and Postgraduate Certificate in structure-based drug design - University of Nottingham). He then worked at SmithKline Beecham in Harlow, UK and the Fosters Group in Melbourne and joins the Abell group. **Jana Vieth** from Germany is to do a year's research in organic and organometallic chemistry and **Annabel Murphy**, a Sheffield graduate is a new PhD student from Britain. **Dr. Matthew Jones** is a new postdoctoral with Andrew Abell and Jim Coxon. Matt received his PhD from the University of Birmingham last year. **Dr. Barry Prince**, a PhD graduate of the Chemistry Department, University of Canterbury has returned from postdoctorals in the University of Wisconsin (Milwaukee) and the ANU to work with **Professor Murray McEwan**; he is to develop solid-substrate-absorbate technologies for the application of SIFT-MS. **Dr. Todd Clements** who has returned to San Diego.

As usual the Department is benefiting from, and enjoying, the collaborations of a number of visitors and guests. **Professor Bill Davison** (Environmental Sciences, University of Lancaster) and **Dr. Finian Leeper** (University of Cambridge) are Erskine Fellows. **Dr. Hao Zhang**, also from Environmental Sciences at Lancaster, is visiting. She has 20 years of experience in chemical speciation and the biogeochemistry of trace metals, has published more than 60 research papers, and serves on the Natural Environment Research Council peer review college. **Professor Dilip de Silva** (University of Colombo) is working with **Professors Murray Munro** and **John Blunt**. His research interests are in biologically active marine natural products and marine ecology.

Dr. Colin Freeman and **Professors Peter Harland** and **Bryce Williamson** attended the RACI/NZIC Physical Chemistry Conference in Hobart, Australia. **Dr. Jan Wikaira** was awarded a TLC grant to attend the Hobart RACI Chemical Education Conference where she talked about the stage-one mentoring programme.

The Chemistry Department spin-off company, **Syft Technologies**, under Geoff, Murray and his team has been awarded a $2.4 million TBG grant. This means that the government will subsidize planned expenditure up to $1.2 million.

MANAWATU

The 2004 Branch Chairman is **Dr. Jeremy Dombroski**, the CIO at HortResearch (Palmerston North). As CIO, Jeremy's role is to provide organization-wide leadership for all Technology and Information Systems functions and related areas. His responsibilities include the development and implementation of a technology vision and both the short- and long-term planning for systems development and technology-related initiatives.

Massey University

Late last year John Ayers retired from Massey University after 33 yearts of service. John can look back at pride with what he has achieved in his career, especially in the development of ion exchangers for use by the dairy industry. These exchangers have been based on cellulose in the form of either spherical beads or ground particles which, after extensive modifications and the synthesis of new derivatives, have proved to be both inexpensive and strong enough to stand up to the rigours of large scale industrial use. They are now used extensively around the world in a number of industries and have generated in excess of $2.5M in royalties for Massey University. At his farewell John was presented with a New Zealand Science & Technology Bronze Medal.

Above: John Ayers being presented with his New Zealand Science & Technology Bronze Medal by Royal Society Council member and NZIC President, Andrew Brodie. (Photo credit: Paul Buckley).

Congratulations to Carol Taylor and Simon Hall upon their promotions to Associate Professor. Carol is recognised for her inspiring leadership and excellence, especially for her exceptional research successes. Simon is Director of the new Massey Anzode Research Centre (see below), a significant development that indicates his particularly successful research on batteries.

Massey University has just signed its biggest commercialisation deal yet with United States-based company Anzode Inc. to take a revolutionary zinc battery technology, developed by **Simon Hall** and **Michael Liu**, to the international market. Massey has granted Anzode an exclusive global licence to the zinc battery technology, which has been patented in more than 30 countries and territories, at a cost of more than US$100,000, paid for by Anzode. Already the battery has attracted interest from the US military, a major US consumer electronics firm and the world's largest manufacturer of electronic components for cellphones and laptops, among others. The Anzode Research Centre has recently received $999,883 from Technology for Business Growth funds.

Richard Haverkamp was pleased to have his new scanning probe microscope officially 'turned on' by the **Vice-Chancellor, Professor Judith Kinnear**, late last year. The purchase of the $450,000 machine was made possible by a capital grant from the MacDiarmid Institute for Advanced Materials and Nanotechnology, of which Massey is a partner. The scanning probe microscope is capable of looking at individual atoms and measuring the tiny forces between particles and is being used by researchers from across the University, helping in a variety of research including bacteria and the development of new materials and technologies such as batteries, solar cells, biosensors. Richard has recently been elected to the Chair of the Manawatu Branch of the Royal Society of New Zealand.

Above: Vice-Chancellor, Judith Kinnear looking at the new scanning probe microscope with Richard Haverkamp (rear) and David Officer (front). (Photo credit: *Massey News*).

David Officer (Director, Nanomaterials Research Centre) recently participated in the opening of the Nanostructured Electromaterials Centre at the University of Wollongong. David is a collaborator in the new research centre, which recently received $A7.3 million from the Australian Research Council to carry out research to help invent and improve energy conversion systems as well as medical applications such as improving bionic ear function.

WAIKATO

A strong representation from Waikato attended the Nelson NZIC Conference and thoroughly enjoyed themselves. Congratulations especially to **Sally Gaw**, who won the student-paper competition with her lecture on the degradation of DDT in soils. **Brian Nicholson** and **Nick Lloyd** attended the OZOM2 conference in Adelaide in January, held to celebrate the 65th birthday of **Professor M. I. Bruce**.

The Branch seminar programme for the year got off to a great start with a lively lecture on *Polymer Colloids* from the RSC Lecturer **Professor Bob Gilbert** (University of Sydney).

Merilyn Manley-Harris will be spending part of 2004 at the University of Montana, where she will be able to catch up with MSc student **Bevin Jarman** who is spending his research year in the USA. **Alistair Wilkins** will be continuing his collaboration on naturally occurring toxins with Norwegian chemists during three months in Oslo. **Susanna Thwaite** has returned from a year working in Professor Schmidbaur's laboratory in Munich, and is currently completing her MSc thesis.

Richard Coll has been awarded visiting scholar status for sabbatical leave at the University of Cambridge (working with **Dr. Keith Taber** - Hommerton College) looking into the teaching and learning of chemical bonding, and research into graduate science supervision. Richard will travel to the UK via Vancouver where he will present a paper at the annual meeting of the USA-based National Association for Research in Science Teaching and, at the end of his sabbatical, he travels to Trollhatten in Sweden to deliver the keynote address at the World Association for Cooperative Education Annual Symposium.

WELLINGTON

The Branch began the year with a lecture from the 2004 RSC Australasian lecturer **Professor Bob Gilbert** (University of Sydney). His valuable and entertaining discourse on Starch: *Understanding Biochemistry Through the Techniques of Synthetic Polymers* attracted a reasonable audience considering the date change.

Victoria University

Dr. John Hoberg left VUW in December for his new position in Wyoming while **Dr. Kate McGrath** has successfully transferred from Otago in mid-January. **Dr. Joanne Harvey**, a VUW/ANU graduate, has been appointed lecturer in (organic) chemistry and will take up her appointment in late May. **Professor Jim Johnston** has been appointed Director of a new VUW Institute for Applied Chemistry & Technology Development; he will relinquish his position as Head of Chemical & Physical Sciences, which has already been advertised.

Dr. Peter Northcote has been in the USA both to visit institutions and lecture on his marine natural products work as well as assess NMR spectrometers for the replacement of VUW's 300 MHz Varian Inova instrument. **Ghislane Cousins** successfully defended her PhD thesis on the day before Dr. Hoberg left for the USA.

Industrial Research Limited

Fresh from a PhD on Nuclear Wastes Containment from the University of Aberdeen **Dr. Dan Brew** joined the

geopolymer-research team headed by **Dr. Catherine Nicholson** in November 2003 on a two-year postdoctoral position. **Dr. Ian Brown** (Material Technologies) attended the December meeting of the Materials Research Society in Boston; this has enabled him to build collaborations and to assess the status of hydrogen economy research and technologies. Afterwards he visited the new hydrogen research programme at ORNL (near Oakridge, Tennesse). **Associate Professor Ken Mackenzie** (Materials Technologies) has completed 7-weeks as a Centre of Excellence foreign academic visitor at the Tokyo Institute of Technology (Tokyo campus). This also involved lecturing at a number of Japanese universities and advising on their research programmes. He gave invited papers at the PacRim conference in Nagoya in October 2003 and the Reactivity of Solids Conference in Kyoto in November 2003.

Some eight students were working over the summer vacation in Materials Technologies together with graduates **Lily Campbell** (VUW - who gave birth to a healthy daughter, Bethany Mara, in mid-January), **Nick Long** and **Liz Jenkins** (Canterbury). In December IRL hosted a visit of 33 Regional finalists from the various New Zealand Science Fairs.

Industrial Research Limited has received some favourable press over the summer highlighting the (final) granting of it's 2223 composition of matter superconductor patent (**Professor Jeff Tallon, Drs. Bob Buckley & Murray Presland**), and the development and trialling of the IRL Carbohydrate Team (under **Dr. Richard Furneaux**)/Albert Einstein College of Medicine team (under **Professor V. Schramm**), T-cell cancer drug which has been accelerated to human testing by the United States Food and Drug Administration. Called Immucillin H, the drug has been licensed to BioCryst Pharmaceuticals for clinical trials.

IRL's Programme/Technology Platform Leaders (including **Ian Brown, Cees Lensink, Andrew Kay/Tony Woolhouse, Jeff Tallon,** and **Nick Long**) are working through the proposals and the various checks and balances for the FRST contract (2004-) proposals. At this stage, the job prospects for some IRL NZIC members look rather bleak, while the grounds for total rejection involving bids with some experienced researchers and collaborators that provide the necessary science quality are hard to interpret. To say there are some misgivings about the proposal/review/refereeing process and the logic of certain decisions would be an understatement although the process is not complete at the time of writing.

An International Workshop on Traceability and Uncertainty in Wellington, 3-5 May 2004, and organized by the Measurement Standards Laboratory (**Dr. Laly Samuel**) is sponsored by IRL. For full details see: <http://www.irl.cri.nz/msl/si-units/chemical/citac_workshop.html>.

International News

Clive Seymour Joins Bruker Daltonics As Vice President for Asia-Pacific

Bruker Daltonics Inc., an operating company of Bruker BioSciences Corporation (NASDAQ: BRKR), announces that Clive Seymour has been appointed as the Company's Vice President for Asia-Pacific, effective March 1st, 2004. Bruker Daltonics' business in Asia-Pacific has grown rapidly in recent years, based on innovative, high-quality products, and a strong commitment to customer service and support. Mr. Seymour will be responsible for the management of the Company's life-science sales and marketing, as well as all associated customer service and support operations in the Asia-Pacific Region, including Bruker Daltonics organizations in Australia, China/HK, Singapore/South-East Asia (Malaysia, Thailand, Vietnam, Laos, Cambodia, Philippines, Indonesia) and Taiwan, in addition to distribution channels covering South Korea and New Zealand. Bruker Daltonics K.K. is managed separately from its Yokohama, Japan headquarters.

Frank Laukien, Ph.D., President and CEO of Bruker Daltonics, commented: "We are very pleased to have Clive join our team to manage the further growth in this large and diverse region. Clive brings a wealth of experience from a decade of managing life-science businesses in the Asia-Pacific region, and he has an excellent track record for sales growth and business expansion in developing markets. With a broad range of innovative life-science products and high-level customer support in the region, we are well positioned to offer our Asia-Pacific customers the tools needed for advanced drug discovery and development, as well as for cutting-edge molecular biology and clinical research."

From March 1997 to February 2004, Mr. Seymour was Vice President Region Asia Pacific at Biacore AB, also assuming the roles of Head of Life Science Research Business Unit (2000) and Vice President Corporate Communications (2002). Previously, from 1992 to 1997, he was European Market Area Manager for Pharmacia Biotech Europe, and Regional Marketing and Sales Manager for Pharmacia Biotech Asia Pacific.

"I am delighted to be joining Bruker Daltonics at such an exciting time in the growth of life-science applications of mass spectrometry," commented Clive Seymour. "Asia-Pacific offers enormous potential for life-science solutions providers, with research and pharmaceutical development of high priority in the region. Bruker Daltonics has clearly demonstrated its commitment to innovation and customer support, and is delivering comprehensive applications-focused platforms to a number of fields, including expression proteomics, biomarker discovery, clinical proteomics and metabonomics. I am looking forward to joining this impressive organization to further develop and expand its Asia-Pacific operations."

Conferences & Seminars

17-21 May 2004
7th World Biomaterials Congress
Darling Harbour, Sydney, Australia
http://www.tourhosts.com.au/biomaterials/invit.html

18-19 May 2004
FDA Science Forum
Washington DC, USA
http://www.dcscienceforum.org/

30 May-3 June 2004
52nd ASMS Conference On Mass Spectrometry
Nashville, Tennessee, USA
Email: office@asms.org
http://www.asms.org/confASMS.php

6-9 June 2004
Bio 2004
San Francisco, USA
http://www.bio.org/events/2004/reg/

8-11 June 2004
7th International Exhibition And Conference On Instruments, Control And Automation
Kuala Lumpur, Malaysia
Email: assoc@gambica.org.uk
http://www.gambica.org.uk

10 June 2004
Royal Society Council Meeting
Wellington

28 June - 1 July 2004
Industrial Scale Protein Purification Workshop
Department of Materials and Process Engineering, University of Waikato, Hamilton
http://mape.waikato.ac.nz/industrycourses/

3-11 July 2004
2004 New Zealand International Science Festival - Emerging Technologies
http://www.scifest.org.nz/scifest/sci_frame.html

4-8 July 2004
SCICON 2004 NZASE Biennial Conference For Teachers Of Science
Canterbury University, Christchurch
Contact: Richard Rendle
29 Mappleton Avenue, Christchurch 5
Phone: (03) 3597275 Fax: (03) 3597248
Email: rendle@clear.net.nz

6-9 July 2004
International Conference On Sustainability, Engineering And Science
Auckland, New Zealand
Contact: Vicky Adin, Conference Manager
Conference SES, P O Box 272.1460, Papakura, Auckland
Phone: (09) 299 7538
Email: vickya@kiwilink.co.nz
http://www.nzsses.org.nz

1-6 August 2004
The SPARC 3rd General Assembly
Victoria, British Columbia, Canada
Email: norm.mcfarlane@ec.gc.ca
http://sparc.seos.uvic.ca/

10-13 August 2004
Forest Entomology And Biosecurity Under The Auspices Of The International Union Of Forestry Research Organisations
Heritage Hotel, Hanmer Springs, New Zealand
Contact: Eckehard Brockerhoff
Phone: (03) 364 2812
http://www.forestresearch.co.nz/iufro2004

24-28 August 2004
New Zealand Institute of Medical Laboratory Science Annual Scientific Meeting & Conference and Immunology SIG Meeting
Kingsgate Hotel, Hamilton, New Zealand
Contact: Robin Allen
Email: allenr@waikatohb.govt.nz
http://www.eenz.com/waikato_info.htm

4-9 September 2004
8th International Global Atmospheric Chemistry (IGAC) Conference
Christchurch Convention Centre, Christchurch
Contact: Kim Gerard
Email: kim@conference.co.nz
http://www.IGAConference2004.co.nz

5-8 September 2004
2004 International Conference On Bioinformatics (InCoB)
Aotea Centre, Auckland
http://www.incob.org

9 September 2004
Royal Society Council Meeting
Auckland

28 November 2004 - 1 December 2004
The 14th Annual Queenstown Molecular Biology Meeting
In conjunction with the New Zealand Society for Biochemistry and Molecular Biology
"Molecular Mechanisms in Cell Biology"
Rydges Hotel, Queenstown
Contact: Billie Masters, Conference Organiser
Email: billie@jsmasters.co.nz
http://www.qmb.org.nz/

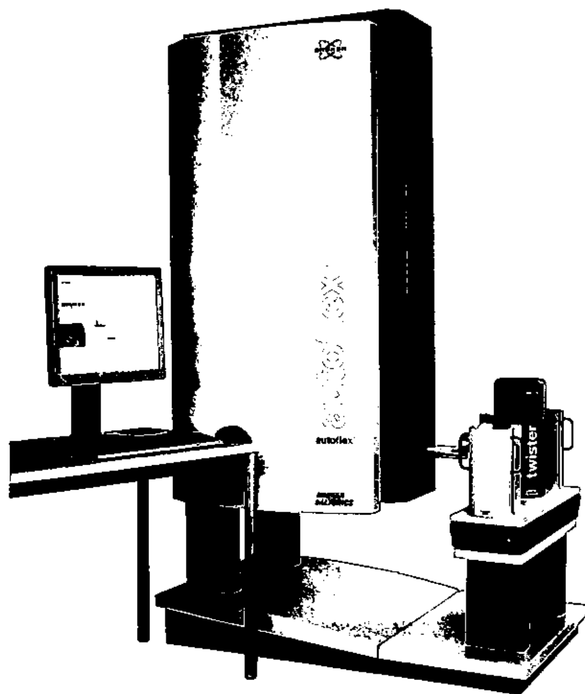
9 December 2004
Royal Society Council Meeting
Wellington

10-14 July 2005
7th World Congress Of Chemical Engineering
Glasgow
http://www.chemengcongress2005.com

NEW PRODUCTS

Bruker Daltonics Announces autoflex II MALDI-TOF And TOF/TOF Systems For High-Success Expression Proteomics And Clinical Proteomics

New autoflex II TOF/TOF Offers Seamless Biomarker Profiling and Subsequent Biomarker Identification by TOF/TOF for Demanding ClinProt™ Researchers.



Bruker Daltonics Inc., an operating company of Bruker BioSciences Corporation recently released the autoflex™ II series of MALDI-TOF and novel high-performance MALDI-TOF/TOF mass spectrometers. The systems were exhibited in March at the 2004 Pittcon in Chicago.

The autoflex II TOF/TOF is the first vertical TOF/TOF system, and offers almost all of the high-performance MS/MS characteristics of larger TOF/TOF systems, yet requires much less lab space, and is priced competitively. The autoflex II MALDI-TOF is the successor of the well-established autoflex system launched in 2000, the first industrial high-throughput MALDI-TOF MS system. The autoflex II incorporates various performance, electronics and software enhancements, and can be optionally upgraded on-site to full TOF/TOF capabilities.

The autoflex II now operates under Bruker Daltonics' integrated Compass™ software environment for easy operation in multi-instrument laboratories, and with the most comprehensive proteomics analysis software suite in the industry. For pharmaceutical customers, the autoflex II system offers software to support customers in achieving 21 CFR Part 11 compliance. Bruker Daltonics also offers IQ, OQ and PV support for FDA-regulated customers.

The autoflex II series benefits from Bruker Daltonics' unique AnchorChip™ MALDI sample targets which use the industry-standard microtitre plate (MTP) format, and are thus easily compatible with most common microbiology or clinical diagnostics sample preparation robots. An optional robotic arm is available for unattended 24/7 operation. Bruker Daltonics' patented AnchorChip technology provides exactly-positioned samples on the MALDI-target for robust and fast automation, as well as a sensitivity boost by up to two orders of magnitude.

Applications to Expression Proteomics and Advanced Clinical Proteomics

Since the introduction of Bruker Daltonics' ultraflex™ TOF/TOF system in 2001, MALDI-TOF/TOF technology has been adopted rapidly by the expression proteomics community. Key advantages of MALDI-TOF/TOF technology include superb MS/MS sensitivity, sequence coverage and information content due to the inherent high-efficiency MS/MS fragment generation. TOF/TOF tandem mass spectra can be acquired with unprecedented throughput and ease of use, and the MS/MS results from the ultraflex and autoflex TOF/TOF systems are straight-forward to interpret.

The autoflex II TOF/TOF provides the power of MALDI-TOF/TOF in a compact and affordable research system for proteomics applications. The system enables fast, automated protein identification by MALDI-TOF peptide mass fingerprinting, immediately followed by more detailed TOF/TOF protein structural characterization. The autoflex II system can be an integral module of either Bruker Daltonics' Proteineer™ solution for high-success expression proteomics, or ClinProt solution for advanced biomarker discovery and robust clinical proteomics.

ClinProt is an integrated set of tools for peptide and protein biomarker discovery and clinical proteomics research, including automated magnetic bead based sample preparation methods, and ClinProTools™ comprehensive analysis, visualization and statistical model building software tools. In an advanced biomarker discovery workflow, after magnetic bead sample preparation, profile spectra are acquired in either linear or in high-resolution reflector mode, which can differentiate potentially overlapping peaks. The superior sensitivity and excellent resolution of the autoflex II generate mass spectral profiles of potential biomarkers with high information content in a robust and reproducible manner. Moreover, putative biomarkers can often be identified immediately by a subsequent TOF/TOF measurement on the same sample using the autoflex II TOF/TOF.

Frank H. Laukien, PhD, President and CEO of Bruker Daltonics, commented: "Mass spectrometry is rapidly growing in importance in clinical and diagnostics research. However, the biomarker discovery community, as well as the FDA, have recently become concerned about the robustness and reproducibility of earlier mass spectrometry technologies in clinical proteomics publications. There is now a clear trend away from developing potential diagnostic assays just based on MS patterns or "barcodes", and towards a clear scientific understanding of the biomarkers. We believe in particular that biomarker identification is indispensable to query the scientific literature in order to assess the potential biological and medical relevance of putative biomarkers. The new autoflex II TOF/TOF, which allows for seamless MALDI-TOF profile acquisition and subsequent biomarker identification by TOF/TOF, appears to be ideal for today's much more demanding biomarker discovery environment."

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Advances In Suppression Technology Improve Ion Chromatography Method Detection Limits

Ai Scientific has introduced the Dionex SRS ULTRA II Self-Regenerating Suppressor. Designed with new ultraclean components, the SRS ULTRA II provides unprecedented low background and low noise levels that improve method detection limits (MDLs) on the order of three to five times.

The SRS ULTRA II offers an extremely fast startup time,on its first use, and every day thereafter. Two types of SRS ULTRA II devices are available: the ASRS® ULTRA II (Anion Self-Regenerating Suppressor) for use in the determination of anions and the CSRS® ULTRA II (Cation Self-Regenerating Suppressor) for use in the determination of cations. Both types are available in two different size formats; a 4-mm standard bore format for use with 5- or 4-mm i.d. (internal diameter) standard-bore columns and a 2-mm microbore format for use with 3- or 2-mm i.d. columns.

The SRS ULTRA II Self Regenerating Suppressor utilizes AutoSuppression® with Reagent-Free ion chromatography (RFIC) technology produced by Dionex which is only available in Australia and New Zealand from Ai Scientific. The SRS ULTRA II provides electrolytic suppression to enhance analyte conductivity while decreasing eluent conductivity, resulting in a significant improvement in sensitivity and MDLs. AutoSuppression technology allows the SRS ULTRA II to regenerate itself continuously using recycled water. The SRS ULTRA II is ideal for suppression of potassium hydroxide eluents for anion exchange or methanesulfonic acid eluents for cation exchange used in the new Dionex ICS-2000 and ICS-2500 RFIC Systems.

Contact: Ai Scientific
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nPoint Introduces Enhanced Scanning Control Mode For AFM And Nanopositioner Stages

New Mode Offers High Scan Rates That Speed Data Collection and Metrology Without Sacrificing Accuracy or Stability.

nPoint, Inc., the global leader in ultra-precision motion and control nanopositioners for nanoscale research and manufacturing, has announced an enhanced scanning control mode for nanopositioning stages that incorporates digital signal processing (DSP) for precision response at high scan speeds. Nanopositioners are key components in scanning probe microscopes (SPMs) including atomic force microscopes (AFMs) and related instruments used in nanotechnology. The enhanced scanning control mode is part of the overall nanopositioner controller. The controller incorporates advanced closed-loop control to increase scan speed for faster data collection in AFM and metrology applications and rapid response for critical nano-location applications. The new advanced scanning mode is available for all nPoint nanopositioning stages, including the nPoint iC AFM Upgrade Kit, which provides closed-loop control and metrology capability to scanning probe instruments currently in use. The new scanning control mode provides faster scanning with minimal phase-lag between the commanded and achieved position, maintaining positioning accuracy at high scanning speeds. The iC Upgrade Kit with the new controller can be easily integrated into an existing AFM without the need for modification or custom installation. The enhanced DSP Controller and iC Upgrade Kit are available now.

nPoint, Inc. is the global leader in ultra-precision motion and control devices for nano-scale research and manufacturing. nPoint designs, manufactures, and sells the PiezoMAX series of nanopositioning systems and sensors, as well as ProbeMAX carbon nanotube scanned probe microscope tips. The positioning products enable rapid, precise, and repeatable motion in critical-dimension (CD) measuring tools, profilometers, atomic force microscopes, optical microscopes, and semiconductor and biotech lithography instruments for applications in life science and semiconductor research, as well as semiconductor manufacturing and testing. iC is a trademark of nPoint, Inc.

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Email: katerina.moloni@npoint.com
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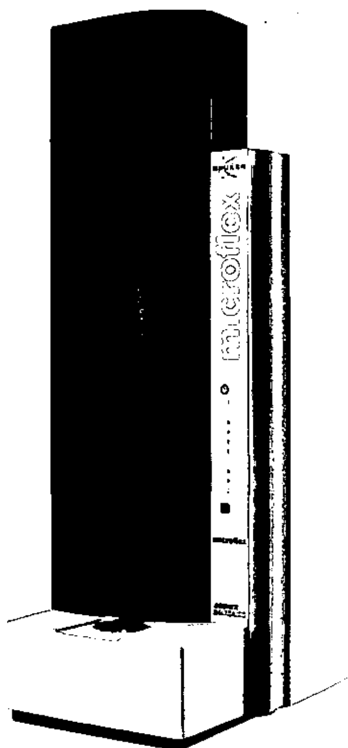
NEW PRODUCTS

Bruker Daltonics Introduces High-Performance Benchtop *microflex*™ MALDI-TOF For Proteomics Applications

New microflex Significantly Outperforms Other Bench-Top MALDI-TOFs.



At the recent 2004 Pittsburgh Conference in Chicago, Bruker Daltonics Inc., an operating company of Bruker BioSciences Corporation released the *microflex*, a high-performance research-grade bench-top Matrix Assisted Laser Desorption Ionization - Time-of-Flight (MALDI-TOF) mass spectrometer. Due to its unique gridless design of ion source and reflectron, this novel instrument provides superior 15k resolution, as well as excellent mass accuracy and outstanding sensitivity.

The *microflex* is designed as a compact and affordable bench-top package that is convenient for many life-science laboratories. The *microflex* operates under Bruker Daltonics' integrated *Compass*™ software environment for easy operation in multi-instrument laboratories, and with the most comprehensive bioinformatics analysis software suite in the industry. It can be applied to expression proteomics, clinical proteomics or functional genomics without compromise in performance.

The *microflex* benefits from Bruker Daltonics' patented AnchorChip™ technology which provides homogeneous, exactly-positioned samples on the MALDI-target for robust and fast automation, as well as a sensitivity boost by up to two orders of magnitude. The new *microScout*™ ion source uses targets plates of exactly one quarter the size of industry-standard microtitre plates (MTP). Therefore, four *microScout* targets can be processed simultaneously with most MTP-compatible laboratory robots.

Furthermore, the *microflex* system can be an integral module of either the *Proteineer*™ solution for high-success expression proteomics, or the *ClinProt*™ solution for biomarker discovery and clinical proteomics. *ClinProt* is an integrated set of tools for peptide and protein biomarker discovery and clinical proteomics research, including automated magnetic bead based sample preparation methods, and *ClinProTools*™ comprehensive analysis, visualization and statistical model building software tools.

For pharmaceutical customers, the *microflex* system offers software tools to support customers in achieving 21 CFR Part 11 compliance. Bruker Daltonics provides optional IQ, OQ and PV services to FDA-regulated customers.

Dr. Ulrich Giessmann, Vice President of Bruker Daltonics, explains: "With the *microflex*, we have introduced the first very high performance bench-top MALDI-TOF instrument to the life-science mass spectrometry market. By combining well-proven and new technology with a robust support concept, we have generated a next-generation system that can meet the demands of most life science researchers for no-compromise performance in a compact and easy-to-use system. We believe the *microflex* will appeal particularly to clinical researchers and pharmaceutical customers. The *microflex* has the potential to grow the bench-top MALDI-TOF market considerably."

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Portable Gasoline Analysis With Mid-FTIR

Ai Scientific has introducted the Grabner IROX 2000. IROX 2000 is an extremely compact, robust and user-friendly mid-FTIR spectrometer for the automatic measurement of the concentration of the most important components of gasoline. Thanks to an improved mathematical model and the use of a built-in density meter the instrument provides the most reliable results for the prediction of key properties such as Octane Numbers, Distillation Properties and Vapour Pressure. A large number of country specific calibration samples is stored. Outlier fuels can be easily added even without a PC. The use of a new calculation model in IROX 2000 results in a better correlation to the CFR-engines for the octane numbers RON, MON. The determination of other fuel properties such as D86 distillation temperatures and vapour pressure have significantly improved, and the application of this new model to determine the Total Aromatics content yields measuring results that are closer to those of the official methods. Another recent development concerns the introduction of the Oxygenate TAME in the calculation model to improve the determination of RON/MON. The portability and vehicle battery options are ideal for on-site field use.

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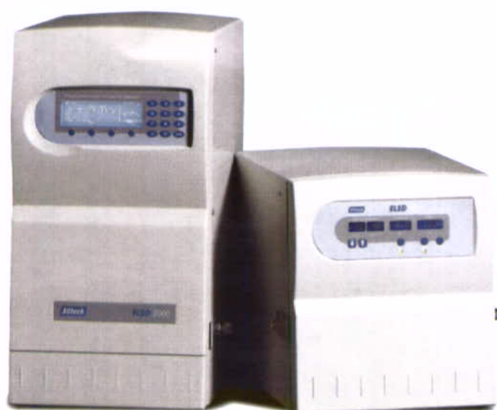

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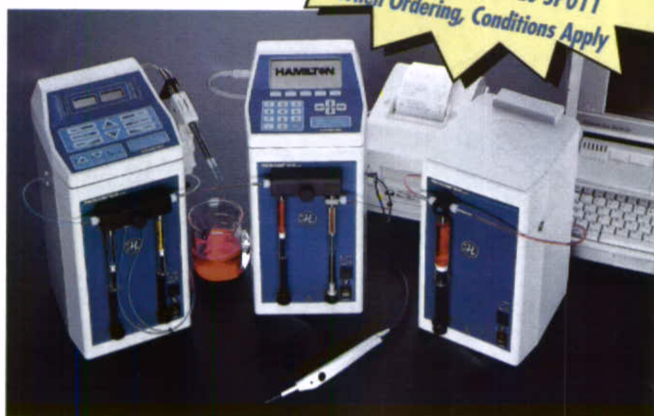

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