A Short History of Natural Product Research in the CSIR

DR D WALWYN[1] AND PROFESSOR P STEYN
CSIR, PO Box 395, Pretoria 0001
[1]Email: dwalwyn@csir.co.za

Natural product research has been a major component of the CSIR's bioscience activities for its entire history, and particularly in the 1960s and 1970s. This type of work is also strongly aligned with one of the objectives of the CSIR, namely to develop the country's natural resources. This poster is a short summary of this research; it outlines some of the main areas and attempts to link the research outputs with its broader impact.

NATURAL PRODUCTS AND THE MEDICAL SCIENCES

By exploring the hidden properties of our plant and animal resources, it was anticipated that the CSIR would discover new drugs and new chemical structures that could be used to tackle major diseases in South Africa. We know today that this is a 'needle in the haystack' approach; however the organisation did find several new chemical entities, which are today licensed and in the advanced stages of development prior to commercialisation. The most well-known is the anti-obesity product, referred to as P57 and derived from the Hoodia plant. Research into Hoodia began in the 1960s as part of the CSIR's investigation into the nutritional value of veld foods. Following early observations around appetite loss in laboratory animals and subsequent failed attempts to identify the chemical substance responsible for the anorectic effects of extracts of the Hoodia plant, the project was moth-balled. It was only some 20 years later when technological advances in nuclear magnetic resonance spectrometry led to the re-launch of the investigation, that the active component was identified, patented and licensed. The licence is presently owned by Unilever who is undertaking final product development on the product.

In addition to the work on P57, interesting natural product research on snake venom was also undertaken within the National Chemical Laboratories. Mostly the work was focused on the isolation, characterisation and sequencing of the active proteins of such venoms. Today, there is an active market in these products, but none of this commercial activity can be linked to the work of the CSIR. Although the organisation undoubtedly contributed to the public knowledge in this area, it failed to evolve the work beyond characterisation and sequencing.

FOOD SCIENCE AND NATURAL PRODUCTS

The CSIR initially undertook a great deal of nutrition research, but this was later incorporated into the MRC upon its formation in 1969, with the notable exception of food science and technology, which formed the basis for the National Food Research Institute (NFRI). The latter's research included programmes in food safety and food spoilage (see below), and the commercialisation of sorghum beer.

Sorghum beer research, in fact, began in 1954, following an approach from certain municipalities that were experiencing problems with the large-scale brewing of sorghum beer. The work was initially undertaken in the Biochemistry Division (possibly the parent of CSIR biosciences), but was transferred to the Sorghum Beer Research Unit following its establishment in 1962. It could be argued that sorghum beer was a highly successful example of the commercialisation of an indigenous food. Within 20 years, sorghum beer production had been transformed from a cottage to a commercial industry, whose output at 1 billion litres per year exceeded that of the South Africa Breweries (800 million). The initial technical challenges, namely to improve the production process in order to ensure product consistency and safety, and to extend the product shelf-life, were certainly overcome, and the solutions brought improved safety and nutritional value to a widely consumed 'food' product.

Research in food safety and the problems of food spoilage was driven by an interest in the spoilage products mycotoxin, and in particular, aflatoxin. Contamination of agricultural products was a major problem in South Africa and limited the post-harvest yield of food crops. The work resulted in the discovery and classification of a broad range of food spoilage organisms (especially yeasts and other fungi; **see box**) and established a platform of expertise that was used to improve post-harvest technologies within the food-processing industry, including approaches such as Hazard Analysis and Critical Control Point (HACCP) methodologies. Even today, the CSIR retains some of this expertise and continues to assist the food industry in the identification of spoilage organisms and the implementation of quality assurance procedures.

NATURAL PRODUCTS AND AGRICULTURAL SCIENCES

Rumen digestion had been a research topic of the CSIR at Onderstepoort from the 1950s. The DMR Unit (Digestion and Metabolism in Ruminants) included both CSIR and ARC staff, and was world renowned from an academic perspective. It was argued that if we understood more about the nature and mechanism of rumen bacteria, it should be possible to increase South Africa's agricultural output through improved feed utilisation and meat production for ruminant animals. The Onderstepoort work did not initially cover any molecular biology; it was only later at the LMCB that research began on the genetics and cellular pathways of rumen bacteria. Initially, the LMCB focused on the identification and characterisation of cellulolytic enzymes (responsible for the breakdown of plant cellulose), but after the closure of the LCMB and the transfer of the programme to AECI, further work was done on attempting to enhance such bacteria through mutation. In retrospect, one could describe this work as naïve, since it was commissioned with insufficient regard for the associated issue of cost-effectiveness, but it was driven by a research curiosity of the kinetics and microbiology of rumen digestion.

CONCLUSION

In conclusion, the history of natural product research at the CSIR is rich with projects covering a wide range of application areas and technologies. Over the last 60 years, there have been both successes and failures in research, as may have been expected. Further research into our natural products is planned in the future, addressing such key challenges as finding novel treatments for infectious diseases and improving food security.

The CSIR's Mycotoxin Research

The CSIR's research in mycotoxins was mainly directed to structural determination by the application of advanced physicochemical techniques and occasionally the principles of biosynthetic architecture. The research team developed top class expertise mainly in the application of very high field NMR spectroscopy, particularly the $\{^{13}C\}^{1}H$ Selective Population Inversion technique, commonly called SPI. These endeavours led to the isolation and characterization of several totally new and structurally novel fungal metabolites, e.g. the ochratoxins (nephrotoxins, carcinogens and teratogens), aflatoxins (hepatocarcinogens and terato¬gens), penitrems (neurotoxins), phomopsins (hepatotoxins), rhizonin (hepatotoxin) asteltoxins and several more as well as to the unravelling of the complex biosynthetic processes involved in their formation.

The ochratoxins are second in importance to the aflatoxins; the most important group of mycotoxins, in fact more than 5 000 publications dealing with the ochratoxins have already appeared. Ochratoxin A (OTA) is produced essentially by fungi of the *Aspergillus* genera and by *Pennicilium verrucosum*. Among the mycotoxins of human and animal health concern, the ochratoxin involvement is increasing, as it is the cause of Danish porcine nephropathy (DPN), a disease amongst pigs in Denmark. OTA is implicated in Balkan endemic nephropathy (BEN) in the Balkan countries as well as in chronic interstitial nephropathy (CIN) in North Africa owing to its occurrence in feed and food. OTA is regarded as a possible carcinogen in humans (classified 2B) by the International Agency for Research on Cancer in Lyon, France. The contributions of the CSIR involved the isolation and structural elucidation, including the absolute configuration determination of OTA total synthesis; the biosynthesis employing initially radio-labelled precursors and subsequently stable isotope labelling and the quantification of enrichment by advanced Nuclear Magnetic Resonance (NMR) techniques, and the origin of the carboxyl group linked to L-ß-phenylalanine as from the methyl group of L-methionine: structure/function studies; the X-ray structure of OTA, and the analysis of OTA. A research team in Utrecht, Netherlands named one of the toxigenic fungi as *Aspergillus steynii* in honour of Dr Steyn for all his excellent work on OTA and other mycotoxins.

In addition, significant contributions were made to the studies of the aflatoxins, such as the isolation and characterization of aflatoxin M_1, the so-called milk toxin. The substance is produced by the metabolism of aflatoxin B_1 by mammals, e.g. cows in lactation. This discovery of this carcinogenic metabolite of aflatoxin B_1 led to severe international legislation to control the levels of the aflatoxins in animal feeds. In similar studies Steyn and co-workers identified 3-hydroxy-aflatoxin B_1, a new metabolite of the in vitro metabolism of aflatoxin B_1 by vervet monkey (*Cercopithecus aethiops*) liver. The most noteworthy contribution of Steyn and his research team in mycotoxin biosynthesis, in particular polyketide biosynthesis, was the detailed investigations on the biosynthesis of aflatoxin and its congeners. These studies, based on stable isotope labelling experiments [2-^{13}C]-, [1-^{13}C] and [1,2-^{13}C]-acetate ^{2}H- and ^{18}O-labelled acetate, the use of mutants, and specific enzyme inhibitors led to the formulation of the polyketide progenitor and its folding pattern.

Steyn established that the biosynthetic sequence involved the following precursors: norsolorinic acid, averufin, versiconal acetate, versicolorin A, and sterigmatocystin, leading to aflatoxin B_1. These studies refuted the earlier hypothesis aflatoxin biosynthesis of George Büchi at MIT, based on the involvement of a C_{18}-polyhydroxy¬naphthacene.

The structural studies of the complex class of mycotoxins continued to offer several challenges, e.g. the study of novel peptides, such as the phomposins and rhizonin. The structural elucidation of the phomopsins involved collaboration with the research team of Claude Culvenor at the Australian CSIRO, Melbourne. Phomopsin A, the main toxin isolated from cultures of *Phomopsis leptostromiformis* and the cause of lupinosis among sheep in Australia and South Africa, is a linear peptide containing several novel ß-dehydro-amino-acids. The sequence of the amino acids was established by heteronuclear $^{13}C\{^{1}H\}$ selection population inversion experiments and by fast atom bombardment mass spectrometry. The structure and absolute configuration of phomopsin A was unequivocally confirmed by X-ray crystallography. Rhizonin A, a cyclic heptapeptide isolated from the fungus *Rhizopus microsporus*, is a potent non-specific hepatotoxin. The fascinating molecule contains three N-methyl amino acids as well as two pairs of like amino acids with opposite alpha carbon stereochemistry, particularly noteworthy was the presence of the unique amino acids N-methyl-3-(3-furyl)-L-alanine and its counterpart containing the opposite absolute configuration. The conformation of rhizonin was established in solution (NMR studies) as well as in the solid state (X-ray crystallography).

A significant accomplishment in mycotoxin chemistry was the elucidation of the structures of the tremorgenic substances, namely the penitrems, janthitrems and lolitrems. The penitrems are relatively large molecules and contain some of the most complex features of natural products not solved by X-ray crystallography. The structural studies involved sophisticated high field ^{1}H and ^{13}C NMR studies and the unambiguous assignment of all the ^{13}C resonances; in this respect selective $^{13}C\{^{1}H\}$ decoupling experiments gave invaluable information. The biosynthetic study of the penitrems enabled the establishment of their biosynthetic origin, namely originating from an indole nucleus linked to a diterpene unit.

K-4570 [www.kashanprint.com]