Current Trends in Natural Products Research from the CBNP10 Symposium at Warwick

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Abstract *Natural products are compounds that are produced by living organisms. They have numerous applications in our everyday life, from antibiotics to herbicides. They possess great chemical and structural diversity, which gives them a leading position as a source of new drugs. Many institutions worldwide are focusing more and more on natural product research, with microorganisms and plants being the most common source for discovery of new compounds. On the 30th June and 1st July 2016, early-career scientists working in the field of natural products gathered at the University of Warwick for the 10th edition of the Chemistry and Biology of Natural Products Symposium (CBNP10). This critical reflection reviews, in the context of the current research in the field, the major considerations that arose from this meeting.*

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Background

Natural products are a vast pool of compounds that are biosynthesised by living organisms. Despite not being strictly needed for survival, they can offer a competitive advantage to the producing host, compared with organisms that live in the same environment that do not produce them. This is the case for compounds that possess antibacterial, insecticidal, herbicidal, and antinematodal properties. In other instances, however, natural products can have cryptic (unknown) functions in their producing hosts, raising important ecological questions. Overall, natural products represent an incredibly rich reservoir of bioactive compounds that have been used since the dawn of humankind for their beneficial properties.

The 1950s and the 1960s are considered to have been the golden age of natural products drug discovery. Prompted by the previous discovery of the antibiotics penicillin and streptomycin many other life-saving

antibiotics were identified during this period (**Umezawa, 1958; Anderson *et al*., 1961**), but then a substantial slowdown followed. This was due in part to rediscovery of previously characterised natural products, as well as to an increased focus in developing semi-synthetic derivatives of already known natural products (*e.g.* ampicillin, amoxicillin, and the other semi-synthetic penicillins). In the last decade however, interest in the discovery of novel natural products has been renewed (**Baltz, 2008; Shen, 2015**). This has been prompted by the decreasing cost of genome sequencing and the advances in bioinformatics and molecular biology, which are allowing for accurate identification of 'promising' gene clusters (aggregates of genes that cooperate towards biosynthesis of a specific natural product) within microbial genomes, as well as precise genome editing. Equally, improved analytical chemistry techniques for characterisation and purification of compounds have also facilitated identification and study of natural products. The importance of natural products discovery was also recognised last year by the award of the Nobel Prize in Physiology and Medicine to William C. Campbell, Satoshi Omura, and Youyou Tu for the discoveries of avermectins (from the bacterium *Streptomyces avermitilis*) and artemisinin (from plants of *Artemisia annua* L.), respectively. These two compounds have revolutionised the treatment of two life-threatening infectious diseases: river blindness and malaria. The conferment of this prize, together with the new technologies offered by bioinformatics, synthetic biology, and genomics, acknowledge the start of a new golden age of natural product discovery (**Shen, 2015**).

The UK has emerged in this context as one of the leading countries for natural products research worldwide, with a growing number of institutions and researchers working in this field. One of the main lines of research undertaken by scientists in the UK is that of natural products discovery, which aims to find new compounds with bioactive properties that may be used as antibiotics, anticancer drugs and herbicides, to name a few. Current research in the field is focusing primarily on microorganisms, *i.e.* fungi and bacteria. These organisms exhibit several advantages compared for instance to plants, such as quicker replication times, easier genetic manipulation, and easier cultivation requirements – with the possibility of culture in large scale fermenters. The other main area of focus currently is on the biosynthesis of natural products, *i.e.* the consecutive steps that lead to the metabolite. Small molecule building blocks, which most often come from the primary metabolism, are employed in enzyme-catalysed reactions to assemble a more complex specialised metabolite. Typically, the aim of the research in this area is to find a link between the genetics and the biochemistry of the biosynthetic routes that lead to the natural products. Unsurprisingly, natural products

discovery and study of their biosynthesis were at the centre of the focus during the Chemistry and Biology of Natural Products Symposium at Warwick.

Aim and Format of the Event

On the 30th of June and 1st of July 2016, researchers working in the field of natural products gathered at the University of Warwick for the 10th edition of the Chemistry and Biology of Natural Products Symposium (CBNP10) (see Figure 1). Over the years this conference has been representing an opportunity for young researchers to present their work to an audience of experts in their field. This edition attracted 123 participants from more than 30 different institutions from the UK and beyond, with approximately three quarters of participants being PhD students and postdoctoral researchers (see Table 1). The conference had a global reach - scientists from more than 20 different nationalities were present at the event. During the meeting, 21 talks were delivered by selected participants (11 from PhD students, 8 from postdoctoral researchers, and 2 from principal investigators), alongside more than 50 poster presentations. The insights discussed here are only a sample of the work presented by the speakers during the conference, as much was unfinished and/or unpublished. In fact, half of the selected speakers were 2nd and 3rd year PhD students that were typically in the middle of their projects.

Figure 1. Pictures from the CBNP10 at Warwick.

Table 1. Breakdown of participants to the CBNP10 and their respective position.

Position	Participants
PhD students	50
Postdoctoral researchers	40
Principal Investigators/Lecturers	22
Master students	4
Undergraduate students	3
Others	4
TOTAL	**123**

Considerations emerging from the CBNP10

As mentioned above, discovery of bioactive compounds with novel chemistry is one of the main themes of research within the field of natural products. Lately, this has been prompted by the decreasing cost of next-generation sequencing technologies, which facilitates acquisition of data from microbial genomes. Bacterial and fungal genomes can therefore be sequenced in a matter of weeks for few thousand pounds. This is providing scientists with an ever increasing amount of genomic data, which can be analysed through bioinformatic techniques in the quest for potentially 'promising' gene clusters. It is relatively easy nowadays to identify such putative gene clusters using tools like antiSMASH (antibiotics and Secondary Metabolite Analysis Shell) (**Weber *et al*., 2015**) and SMURF (Secondary Metabolite Unknown Regions Finder) (**Khaldi *et al*., 2010**), which in some cases return up to 50 putative gene clusters within one microbial genome. However, it is still a difficult task to predict *a priori* from the genomic data what the exact structure of the corresponding natural product will be.

On these lines, one of the main considerations that emerged from the work presented at the CBNP10 was that efforts are being made to improve tools that allow for prediction of the chemistry of a natural product based on the DNA sequence of the biosynthetic genes it derives from. Algorithms are being developed to identify conserved DNA sequences within gene clusters (**Medema and Fischbach, 2015**), as discussed during the conference by Xiaowen Lu, from the bioinformatics group at Wageningen University (Netherlands). The increasing number of natural products that are being linked to their genetic counterparts, and the dissection of the biosynthetic gene clusters and related pathways, are allowing researchers to identify evolutionary conserved motifs within genomic sequences, which can be linked to specific conserved chemical moieties in the structure of the natural product. Predicting the structure of natural products from genomic data is therefore envisaged to become even easier in future, as conserved motifs within the biosynthetic enzymes are characterised.

Although *in silico* predictions cannot yet tell us precisely what the exact chemical structure of a natural product is, we can still use these tools to discern gene clusters and select which ones to bring forward for further experimental studies. As highlighted in the talks and posters presented at the CBNP10, a significant amount of research on natural products discovery is currently being done with the aim of identifying new antibiotics and new herbicides, which could allow the development of novel antimicrobial agents for use in human medicine, as well as

agrochemicals to provide better crop protection in agriculture. Alice Banks and Claudio Greco, both from the University of Bristol, presented work that aims to characterise novel antibiotics and herbicides, respectively, from basidiomycete fungi – a group that has been overlooked for some time for discovery of natural products (**Stadler and Hoffmeister, 2015**).

Considering that discovery of compounds with novel chemical backbones can be challenging, efforts are also being made to develop structural analogues of the already available natural products, with the aim of improving their bioactivity. Synthetic routes are being used to generate agrochemical and antibiotic precursors in sufficient amounts to then undergo structure–activity relationship (SAR) studies, where the bioactivity of the derived analogues is investigated in relation to their chemical structure. This was discussed for instance during the CBNP10 by Mairi Littleson from Allan Watson's group at the University of Stratchlyde, in regards to coronatine, a phytotoxic polyketide that has the potential to be exploited to develop new herbicide derivatives. At present however, its total synthesis remains challenging – due for instance to stereochemical requirements – and improving current approaches would likely be beneficial for the consequent generation of structural analogues (**Littleson *et al.,* 2016**).

Synthetic routes can often not be as efficient as their biosynthetic counterparts, which exploit instead the catalytic activity of naturally-occurring enzymes. The enzymes that are employed by plants or microorganisms in nature can in fact also be used in the laboratory to recreate biosynthesis of the desired natural product. Two alternative methods can be utilised for this purpose: either *in vitro* or *in vivo* studies. *In vitro* reactions are those where the enzymes involved in biosynthesis of a natural product are purified *via* overexpression in *Escherichia coli* (*E. coli*), then fed with the precursors of the natural product. Due to the low production yields normally obtained, the purpose of the reaction is often purely to characterise the catalytic activity of the enzymes. *In vivo* strategies are those where the enzymes are studied that are known to give the natural product of interest, which are then exploited either within the producing host or within another suitable and easy to culture microorganism, such as *E. coli* or the baker's yeast *Saccharomyces cerevisiae*. To this purpose, assembly of large and complex enzymes can be achieved using different technologies, such as that of the newly developed multiplexed integrating plasmids (**Fayed *et al.*, 2015**), which was presented during the CBNP10 by Hong Gao, from Maggie Smith's group at the University of York. This approach uses phage-encoded serine integrases (enzymes that can cut precisely DNA and recombine it in a

predictable way), and its efficacy was demonstrated by cloning and assembling the cluster for biosynthesis of the antibiotic erythromycin.

Conversely, in some instances, biosynthetic pathways for natural products include spontaneous reactions that are not catalysed by enzymes, but rather proceed through thermal or photochemical routes. This is the case, for example, for the polyketide heronamide C, which is a natural product with inhibitory activity against fission yeast. Thomas Booth, from Barrie Wilkinson's group at the John Innes Centre, presented work at the CBNP10 that shows how the two consecutive intramolecular reactions that lead to this natural product are a thermal and a photochemical cycloaddition, which are independent from any enzymatic catalysis (**Booth *et al*., 2016**).

It is widely recognised that understanding the biosynthetic pathway of a natural product is essential for its further manipulation. As discussed at the CBNP10, new approaches are also being developed to facilitate characterisation of biosynthetic pathways for natural products. For instance, comparative untargeted metabolomics was used to gain insights into the biosynthesis of the antibiotic bottromycin (**Crone *et al*., 2016**), as presented by Andrew Truman, from the John Innes Centre. A network analysis of mass spectrometry data was used to assess the metabolite profiles of a series of mutant strains of the bacteria that produce the antibiotic. This allowed the key steps in the biosynthesis of the natural product to be elucidated. Another innovative approach used to characterise intermediates in biosynthesis of polyketide natural products was presented during the CBNP10 by Ina Wilkening, from Manuela Tosin's group at the University of Warwick – chain termination probes (**Parascandolo *et al.,* 2016**). This strategy relies on the use of chemical probes for capturing of intermediates, which are then analysed and identified by high-resolution mass spectrometry. This enables researchers to remove the problem of polyketide intermediates being covalently tethered to their enzyme factories, and therefore prevent detection under normal conditions.

The approaches discussed here and conferred during the CBNP10 will most likely aid researchers in recreating and studying biosynthesis of other natural products from microorganisms and plants. This in turn will allow for increased yields of the compounds of interest, which can then be purified and used as an antibiotic, herbicide, or as an anticancer agent, depending on their bioactivities. At the same time, comprehensive studies on biosynthetic routes will also enable direction of biosynthesis towards different analogues with potentially enhanced bioactivities.

Conclusions and Forthcoming Meetings

Natural products are a unique source of bioactive compounds, which have been isolated from plants and microorganisms for decades and exploited in a variety of ways. Despite a slowdown in natural product drug discovery until the last decade, it seems now evident that interest in identifying novel specialised metabolites to be used as pharmaceuticals and herbicides has been renewed. Equally, the study of biosynthetic processes that lead to natural products is aiding the production of already known bioactive compounds. Conventional synthetic approaches of obtaining derivatives of natural products are now being supported (or in some cases replaced) by enzyme-catalysed reactions, whereby the use of biocatalysts is envisaged to substantially reduce the cost of industrial chemical derivatisation.

These and other recent advances in natural products research were discussed during the CBNP10, highlighting the current themes of investigation undertaken by prominent groups in the UK, as well as future perspectives on the subject. The outstanding quality of the oral presentations delivered by PhD students and postdoctoral researchers at the CBNP10 points to a brilliant and flourishing future for the study of natural products. The University of Warwick will host the Chemistry and Biology of Natural Products Symposium again at the end of June 2017, which will then be its 11th edition. Before this, another important meeting for researchers in the field of natural products will be held at Warwick at the end of March 2017. Our University will be the venue for the Directing Biosynthesis V, a meeting sponsored by the Royal Society of Chemistry, with world-leading invited speakers in the field of natural products. It now seems clear that Warwick is gaining a crucial position in the UK for natural products research, and one which will continue to thrive in the forthcoming years.

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Conference website

http://warwick.ac.uk/naturalproducts

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