Article

Misassigned Natural Products

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Richard attended Timaru Boys High School before moving to Dunedin to study at the University of Otago, graduating with a BSc(Hons) in 2014. Richard plans to begin his PhD studies under the supervision of Dr Bill Hawkins this year working on the total synthesis of bioactive natural products.

Throughout history, before drug discovery and modern chemistry, natural products have been used to treat illness. Cocktails composed of nature's ingredients such as herbs, animal products or inorganic materials, perhaps intertwined with witchcraft, mysticism, astrology or religion, were used to effect these treatments. Early treatments were recorded and documented, eventually leading to a disciplined scientific description of natural materials that could be used in medicine. As scientific knowledge grew and improved, the active constituents from these remedies were isolated, characterised and subsequently synthesised in the laboratory.[1]

The more active and selective of these natural products, derivatives of, or compounds inspired by natural products, have become many of the drugs used by people every day. In fact, natural product-derived pharmaceuticals constitute about half of those used in the clinic.[2] As such, considerable research and interest is dedicated to the investigation of these architecturally diverse and structurally complex molecules, which have been shaped by evolution and nature over the course of millions of years. These compounds are often decorated with multiple ring systems, stereocentres and unique and imaginative structural motifs. Not only do these compounds meet an unfulfilled need for new drugs, but techniques and methodologies associated with detecting biological activity, isolation and purification, characterisation, synthesis and biological evaluation for these compounds can also be discovered and improved.

A classic example of such a natural product is morphine (**1**, Fig. 1), which was extracted from the opium poppy plant by Friedrich Serturner and the first alkaloid ever extracted from a plant source.[3] Of course, the discovery of this powerful analgesic that is used in the clinic every day all around the world was of huge benefit to mankind, but the 122 year delay between its isolation and structural elucidation saw equally important advancements in chemical synthesis and characterisation, as well as an improved understanding of reactivity and the three-dimensional nature of organic compounds.[4] To borrow words from Doering: "In the beginning, the isolation of chemicals from natural sources provided an unceasing stimulus to the creation and development of science".[5,6]

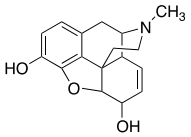

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Fig. 1. Morphine

In the 19th century and the early half of the 20th century, before the arsenal of spectroscopic and other characterisation techniques of today, chemical synthesis was the only way in which the structural determination of natural products could be carried out. Molecular architecture was revealed through meticulous and laborious derivatisation and degradation, assuming large enough quantities of the compound in question could be obtained. Depending nearly solely on chemical synthesis as a means of carrying out structural elucidation was fraught with errors and limitations. A classic example of one of these early structural misassignments was made in the 1920s by two researchers in Germany, Weiland and Windaus, who proposed the structural motifs of a number of steroids including cholesterol (**2**, Fig. 2). Although they were awarded the Nobel Prize for this work, the inaccuracies associated with their work can instantly be recognised today and their mistakes were corrected in 1932 through the use of X-ray crystallography and thus establishing the correct steroid core structure (**3**, Fig. 2).

Nowadays, with a whole host of more powerful, accurate and less time-consuming techniques such as multi-dimensional NMR and X-ray crystallography, structural assignments of natural products have become much more accurate, practical and relatively rapid. However, in a review published by Nicolaou *et al.* in 2005,[5] the case is made that chemical synthesis still has an important role to play in the structural elucidation of natural products. Between January 1990 and April 2004, there have been well over 300 cases of structural misassignments reported in the scientific literature. These structural revisions cover virtually every compound class and include not only stereochemical misassignments, but also extend to include complete constitutional changes. Nicolaou *et al.* point out that these mistakes can be attributed to the fact that every structural elucidation technique has its own inherent weaknesses, some of which cannot be resolved even when every other tool is applied.

For example, while X-ray crystallography is generally seen as an infallible technique, complications can arise when investigating functional groups lacking hydrogen atoms. As X-ray crystallography uses electron density to map out the position of atoms, it is unable to reliably reveal the location of hydrogen atoms. This can make differentiating between functional groups devoid of hydrogen atoms difficult, for example, between N-H groups and O atoms. NMR spectroscopy, despite being an extremely powerful tool for structure elucidation, can demonstrate weaknesses especially when there are insufficient hydrogen atoms to correlate ^{13}C and ^{1}H resonances.

One example of a misassigned natural product is kinamycin C. It was originally isolated from *Streptomyces murayamaensis* in 1973, and was shown to possess antibacterial activity against mainly gram-positive bacteria. The structure of this compound was originally assigned using a whole battery of techniques including mass spectrometry, X-ray crystallography, NMR, UV-vis, and IR spectroscopies, as well as chemical derivatisation and degradation.[6] It was not until 21 years later that it was realised the cyano group on **4** (Fig. 3) was actually a diazo group as in **5** (Fig. 3), which was confirmed by 2D NMR spectroscopy[7] and chemical synthesis.[8]

Another example of a structural misassignment was diazonamide A, an unusual halogenated cyclic peptide with potent *in vitro* cytotoxicity.[9] The compound was isolated from the ascidian (sea squirt) *Diazonia chilensis* and was originally assigned as **6** (Fig. 4) using a combination of NMR spectroscopy experiments as well as X-ray crystallography. It was not until a decade later that the compound identical to the structure proposed in 1991 was synthesised.[10] Upon comparing the analytical data of the synthetic compound **6** to the naturally occurring compound it was found they were not identical and upon further analysis the structure was revised to **7** (Fig. 4).

Since 2005, there have been plenty more cases where the structure of natural products have been revised following synthetic studies. Azaspiracid-1 is a natural product isolated from *Mytilus edulis* (a species of mussel) and was discovered after at least eight people fell ill following its consumption.[11] The marine toxin was originally assigned using multi-dimensional NMR experiments and mass spectrometry, and featured two spiro ring domains, a cyclic amine and a carboxylic acid (**8**, Fig. 5). The challenge of synthesising azaspiracid-1 was taken up by the Nicolaou group and its completion revealed the original spectra of the natural product did not match that of the synthetic target.[12] Following degradation of the neurotoxin into three different compounds, the analytical data was matched with synthetically derived fragments. A new structure **9** (Fig. 5) was subsequently proposed, which was ultimately confirmed through its total synthesis.[13]

More recently, the structure of cinbotolide, a natural product isolated from the phytopathogen *Botrytis cincera* has also been revised following synthetic studies.[14] The originally proposed structure **10a** is shown in Fig. 6. Again, the spectra of a synthetic analogue (**10b**) having significant differences to that of the natural product prompted further investigation into the correct structure of the compound. Quantities of the natural product were obtained from a *B. cinera* mutant that overproduced the polyketide. Following a series of chemical transformations, multi-dimensional NMR and IR spectroscopy experiments, a revised structure (**11**, Fig. 6) was proposed and ultimately confirmed through an asymmetric total synthesis.

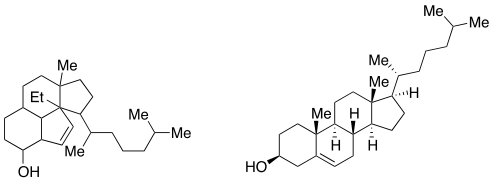

Fig. 2. Originally proposed (**2**) and correct (**3**) structure of cholesterol

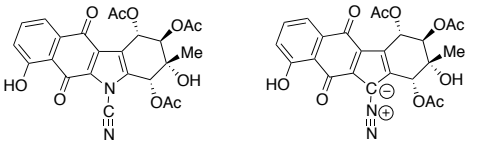

Fig. 3. Original (**4**) and revised (**5**) structure of kinamycin C

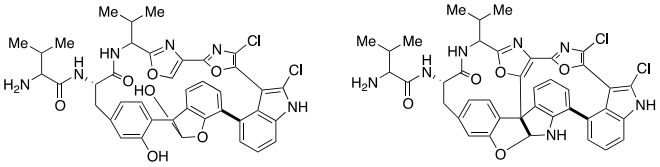

Fig. 4. Original (**6**) and revised (**7**) structure of diazonamide A.

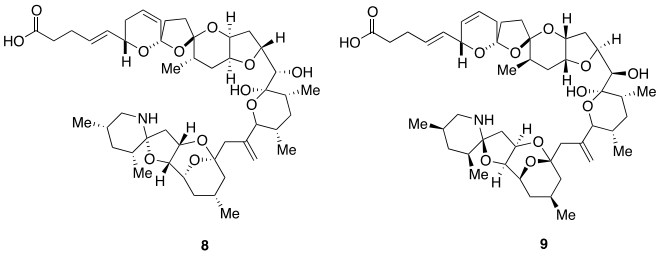

Fig. 5. Original (**8**) and revised (**9**) structure of azaspiracid-1

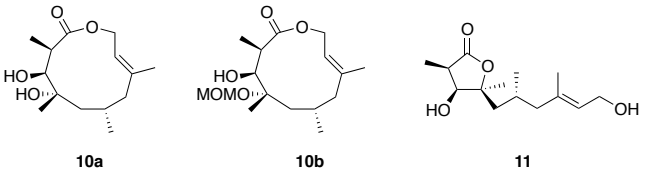

Fig. 6. Originally proposed structure (**10a**), synthetic methoxymethyl (MOM) protected ether analogue (**10b**) and revised (**11**) structure of cinbotolide

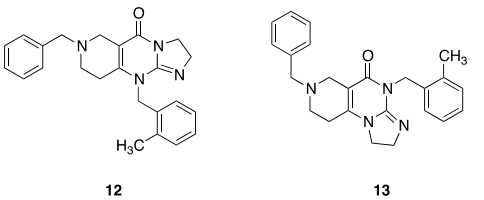

Fig. 7. Original (**12**) and revised (**13**) structure of TIC10

Another example of a structural misassignment was uncovered in 2014 following the synthesis of TIC10. This compound is not a natural product, but the story illustrates the potential financial and health risks that structural misassignments could incur. TIC10 was originally discovered by a group from Pennsylvania State University through a search of a free National Cancer Institute (NCI) database. It was found to induce apoptosis by promoting the expression of a tumour suppressor protein called TRAIL and to be efficacious *in vivo* and *in vitro* against glioblastomas, prostate cancer, sarcomas, melanoma and lymphomas.[15]

After the compound had been patented and licensed to a pharmaceutical company and clinical trials initiated, another research group from the Scripps Research Institute in California became interested in the same compound in the context of anticancer-combination therapy. After synthesising the compound they found the previously patented structure **12** (Fig. 7) to be biologically inactive.[16] In order to address this disparity, the second group obtained the repository compound from the NCI and found it to be biologically active. Following 2D NMR spectroscopy and X-ray crystallography experiments, they showed that the patented structure had been misassigned and the correct, biologically active compound (**13**, Fig. 7) was actually a constitutional isomer of the originally patented structure. As a result, the patent and clinical trials have been called into question and the two research groups and pharmaceutical companies could be led into an unprecedented legal case.

The invention and continual improvement of characterisation and isolation techniques has seen a marked improvement in the accuracy and speed with which new natural products can be discovered. However, based on the examples above, as well as hundreds of other cases, mistakes can still occur with far reaching consequences as seen with TIC10. The value of organic synthesis as a means of obtaining these scarce and valuable compounds for biological evaluation and drug discovery, often accompanied by important advances in the field of organic chemistry, is unparalleled. These examples underscore the idea that the structural intricacy, connectivity and reactivity of these interesting and complex compounds can only fully be appreciated through the act of physically making them.

Acknowledgements

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