Hot new chemistry from a kiwi pepper tree

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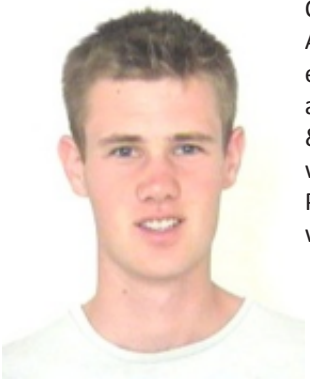
Keywords: *kawakawa*, *natural products*, *alkaloids*



Originally from Christchurch, where he attended the Christchurch Rudolf Steiner School, Alistair moved to Dunedin for undergraduate study. Having recently completed a Bachelor of Science majoring in both chemistry and food science, he will be studying towards an Honours degree in chemistry throughout 2015. He has also worked closely with Plant & Food Research, having participated in their summer studentship programme working with the Chemistry Team at the Palmerston North site. This has led to working with the Plant Extracts Group, Plant & Food Research at the University of Otago, with whom he will complete the research component of his Honours degree.

Introduction

Prior to the colonisation of New Zealand by European immigrants, Māori had developed an extensive knowledge of the native plants and their uses.[1] While plants such as mānuka (*Leptospermum scoparium*) and rimu (*Dacrydium cupressinum*) were primarily used for crafting weapons and canoes, many other native species were valued for their medicinal properties.[1] Harakeke (*Phormium tenax*) and koromiko (*Hebe stricta* and *H. salicifolia*) have a host of uses in treating the sick or injured.[1,2] Diarrhoea, sore throats and open wounds can all be cured using different parts of these plants prepared in a variety of different ways.[2] Harakeke has an added bonus, as it may also be used to stitch together the skin when treating more serious cuts.[1] However, perhaps one of the most prominent native plants in Māori medicine is kawakawa (*Macropiper excelsum*).

Kawakawa is most commonly found growing in shady areas along the coastline of the North Island and upper South Island.[1] The small shrub-like tree can be identified by its shiny green leaves and small yellow fruit (Fig. 1), both of which were exploited by Māori for their significant healing properties.[1] Steaming or boiling the leaves was a common treatment for stomach pain and, after the Europeans arrived, gonorrhoea.[1] Poultices made from the leaves and bark could be applied to cuts and wounds as well as inflamed areas, to reduce swelling and prevent infection.[2] As well as being a crucial part of Māori medicine, kawakawa has significant spiritual meaning, generally associated with the cycle of life and death.[1] A sprig of kawakawa is seen as a good luck charm and as such is present at many traditional Māori ceremonies from birth and naming to funerals.[1]

More recent uses of kawakawa have been in the food industry.[3] Leaves of the plant have been used to give the characteristic peppery flavour and aroma to Taakawa beer (Fig. 2).[3] The fact that kawakawa provides a hint of pepper is not unexpected given that it is sometimes referred to as 'pepper tree'.[1] Other species belonging to the *Piperaceae* family are responsible for providing the original pepper flavours.[4] These include *Piper nigrum,* which is more commonly known as black pepper.[4] The fruits of kawakawa and *Piper nigrum* even exhibit some similarities in their appearance. The fact that kawakawa has such a plethora of beneficial properties to both pharmaceutical and food applications, provides a basis to investigate the chemistry of this species and determine the source of its biological activity.

Fig. 1. Leaves (left) and fruit (right) of kawakawa *(Macropiper excelsum)* (from https://www.flickr.com/photos/fostert/5385589463/in/photostream/ with permission).

Fig. 2. Taakawa Indigenous Ale of Aotearoa, flavoured using kawakawa leaves and fruit of *Piper nigrum*.

Chemistry of kawakawa leaves

Research into the chemical composition and biological activity of kawakawa is limited. The majority of this research has focused on the leaves of the plant and little is known regarding the fruit or roots.[5] The most significant findings have been the isolation and identification of two known bioactive molecules, myristicin and diayangambin (Fig. 3), from the leaves.[5]

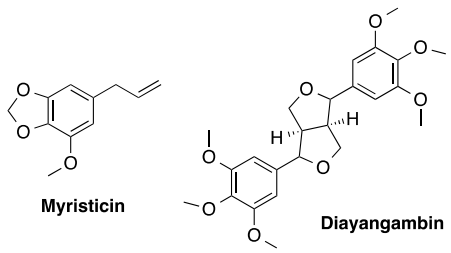


Fig. 3. Chemical structures of myristicin and diayangambin

Myristicin is the more common of the two and can also be found in other plant sources including nutmeg, parsley and dill.[6] The biological activity of myristicin has been well studied and helps to explain the medicinal properties of kawakawa. Not only is myristicin an anti-inflammatory and an anti-microbial agent but is also hepatoprotective, helping to prevent damage to liver cells.[6,7] Furthermore, myristicin is a known psychoactive drug and precursor to the psychedelic drug MMDA, a transformation which some have suggested may even occur during metabolism in the body.[8] This may well explain the symptoms described by those who have overindulged in beverages made from kawakawa leaves or root.[1] As if myristicin did not already have sufficient bioactivity it has also been found to act as an effective insecticide.[1] This activity has been utilised to keep away insects by burning kawakawa branches around food storage pits.[1]

Conversely, diayangambin is a much more rare natural product, but it also has significant bioactivity.[9] Not only does diayangambin exhibit anti-inflammatory activity, but it is also an immunosuppressive agent.[9] Drugs that utilise immunosuppressive agents such as diayangambin are used to treat several conditions in which the body's immune system requires down-regulation. This includes patients whose body rejects an organ transplant as well as those who suffer autoimmune diseases such as rheumatoid arthritis.[9]

The unknown fruit: recent research

The presence of myristicin and diayangambin accounts for the medicinal properties of kawakawa leaves. However, the chemical composition and activity of the fruit is largely unknown, as are the compounds responsible for its characteristic flavour profile. A research team from Plant & Food Research at the University of Otago investigated the chemistry of kawakawa fruits and discovered a complex series of alkaloids.

The first significant finding was made by Otago undergraduate student Jeremy Lei who isolated a rare bioactive alkaloid, known as piperchabamide A (Fig. 4), from kawakawa fruits. Piperchabamide A was originally isolated from another member of the Piperaceae family, *Piper chaba,* and since then has only been identified in a handful of other natural sources.[10] Extracts of the *P. chaba* have been shown to have gastroprotective properties, but the specific activity of piperchabamide A is largely unexplored.[10]

Further investigation into the chemistry of the fruits by Elaine Burgess of the Plant & Food Research team revealed that piperchabamide A was far from being the only alkaloid present. Amongst the compounds isolated were two new alkaloids (Fig. 4), referred to as compounds **1** and **2**. Since both compounds are previously unreported there is no information available regarding their properties, including any potential biological activity.

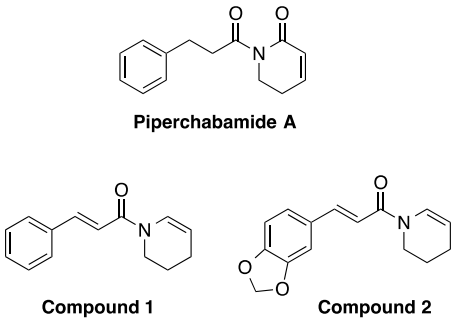


Fig. 4. Chemical structures of specific alkaloids isolated from the fruits of kawakawa (*Macropiper excelsum*)

One of the few things known about the new compounds is the presence of a restricted conformational exchange phenomenon (Fig. 5). This interesting property was identified when characterising the molecules and was investigated further as part of an undergraduate research project undertaken by the author. The phenomenon was first observed when using NMR spectroscopy to determine the structure of the molecule. It was noticed that the hydrogen atoms of the nitrogen-containing ring (Fig. 5) were represented by two peaks, rather than the expected single peak. This suggests that these hydrogen atoms experience two different environments, something that could be explained by the presence of two different conformations.

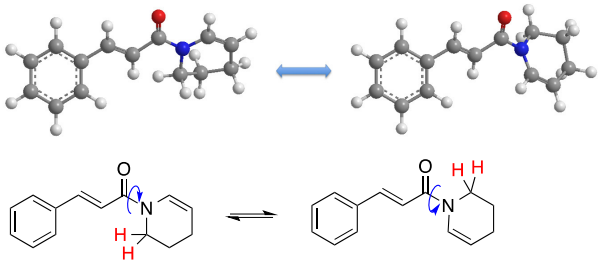


Fig. 5. Conformational exchange phenomenon exhibited by new alkaloids from kawakawa: 3D depiction (top) and schematic representation (bottom)

By using more advanced NMR techniques it was found that two conformations were possible due to rotation about the amide bond (Fig. 5). Due to the partial double bond character of the amide linkage, the rotation is restricted at low temperatures as an energy input is required for the rotation to occur. At high temperatures,

rotation, and therefore exchange between conformations, occurs much more rapidly. Again this effect may be observed using variable temperature NMR. At low temperatures two distinct signals are seen, one for each conformation as interconversion is very slow, whereas at higher temperatures the conversion rate is more rapid and the signals become broad and less defined (Fig. 6). This is not only interesting from a purely academic point of view as it may also have implications regarding the biological activity. For example, one conformation may be able to bind to a receptor in the body, while the other may not bind due to the relative orientation of groups.

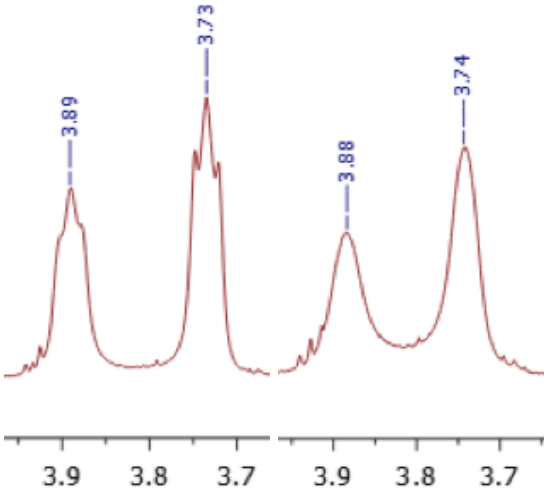


Fig. 6. ^{1}H NMR signals for protons highlighted in Fig. 5 at room temperature (left) and at 40 °C (right)

The newly discovered alkaloids are also of great interest due to the fact that they display structural similarities to several biologically significant alkaloids. One such compound is piperine (Fig. 7), an alkaloid responsible for the pungency of black pepper.[4] As well as providing flavour, piperine has been the focus of studies investigating its efficacy as an anxiolytic (anti-anxiety) and sleep-inducing agent.[4] Currently in the spotlight for its ability to selectively kill cancer cells, piperlongumine (Fig. 7) is another alkaloid with many similarities to the newly discovered kawakawa compounds.[11] It has been proposed that the activity of piperlongumine is caused by a combination of two mechanisms.[11] The first is an elevation of reactive oxygen species (ROS) in the cell, putting the cell under an oxidative stress.[11] The second involves the binding of piperlongumine to the cells' glutathione, an antioxidant which prevents cell damage by ROS.[11] Binding of piperlongumine to glutathione renders it incapable of performing its function in the cell.[11] The combination of these effects is enough to cause irreversible damage and induces cell death.[11] The most significant feature of piperlongumine's activity is that it is selective for cancer cells, leaving healthy cells unaffected.[11] While it is easy to postulate on the potential activity of the new alkaloids based on structural similarities to highly bioactive molecules, any reported activity must be based on thorough testing.

The University of Otago Plant & Food Research team is working in conjunction with Sarah Baird from the Department of Pharmacology and Toxicology to determine

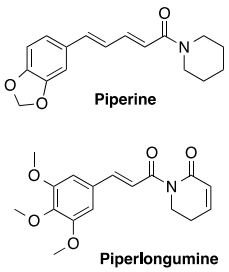


Fig. 7. Chemical structures of biologically significant alkaloids, piperine (top) and piperlongumine (bottom)

whether the new compounds are in fact bioactive. Cytotoxicity assays on the alkaloids isolated from kawakawa fruits will provide information as to whether there is biological activity present, and a series of related synthetic alkaloids will also be studied alongside the natural samples. The preparation of the synthetic analogues continues to be investigated with Bill Hawkins (Department of Chemistry, University of Otago) and aims to provide more insight into which parts of the alkaloid are responsible for their activity. Furthermore, the synthesis of the kawakawa compounds affords larger quantities of the compounds as they have a tendency to degrade over time, leaving little or no material for the relevant bioassays.

Conclusions

Kawakawa fruits have proven to be an interesting research topic with much more depth than was initially anticipated. The identification of new compounds provides exciting potential for use in pharmaceutical, perfume or food applications.

Acknowledgements

Thanks to Nigel Perry, Elaine Burgess and Bill Hawkins for their help and contributions to this research.

References

1. Riley, M. *Maori Healing and Herbal*, Viking Sevenseas N.Z. Ltd: Paraparaumu, 1994.
2. Nga Tipu Whakaorange database, http://maoriplantuse.landcareresearch.co.nz, Records 1119, 1080 and 1068 (accessed 13/01/2015).
3. Indigenous Ale of Aotearoa (2011) http://www.taakawa.com/ (accessed 5/10/2011).
4. Wimmer, L.; Schönbauer, D.; Pakfeifer, P.; Schöffmann, A.; Khom, S.; Hering, S.; Mihovilovic, M. D. *Org. Biomol. Chem*. **2015**, *13*, 990-994.
5. Russell, G. B.; Fenemore, P. G. *Phytochemistry* **1973**, *12*, 1799-1803.
6. Morita, T.; Jinno, K.; Kawagishi, H.; Arimoto, Y.; Suganuma, H.; Inakuma, T.; Sugiyama, K. *J. Agric. Food Chem.* **2003**, *51,* 1560-1565.
7. Kuete, V.; Krusche, B.; Youns, M.; Voukeng, I.; Fankam, A. G.; Tankeo, S.; Lacmata, S.; Efferth, T. *J. Ethnopharmacol.* **2011**, *134*, 803-812.
8. Shulgin, A. T. *J. Psychedel. Drug.* **1976**, *8*, 167-169.
9. De Leon, E. J.; Olmedo, D. A.; Solis, P. N.; Gupta, M. P.; Terencio, M. C. *Planta Med.* **2002**, *68*, 1128.
10. Matsuda, H.; Ninomiya, K.; Morikawa, T.; Yasuda, D.; Yamaguchi, I.; Yoshikawa, M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2038-2042.
11. Adams, D. J.; Dai, M. J.; Pellegrino, G.; Wagner, B. K.; Stern, A. M.; Shamji, A. F.; Schreiber, S. L. *P. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 15115.