Evaluation and Licensing Opportunities

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Patent Literature

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Crop insect resistance by engineering limonoid synthesis

Identification of key enzymes responsible for protolimonoid biosynthesis in plants:
a) providing *in planta* insect resistance
b) enabling biosynthesis of limonoids

Professor Anne Osbourn and co-workers at the John Innes Centre have now achieved the first characterisation of protolimonoid biosynthetic enzymes. They identified three enzymes in the limonoid biosynthetic pathway that are conserved in the citrus and mahogany families, enabling to engineer plants with **insect resistance** and to produce in plants to **new to nature crop protection or medicinal limonoids**.

Limonoids are a diverse class of plant natural products classified as tetranor-triterpenes with a tetracyclic triterpene scaffold (C30). The immediate precursors to limonoids are known as protolimonoids. Limonoid production is largely confined to specific families within the *Sapindales* order which include the mahogany (*Meliaceae*) and citrus (*Rutaceae*) families. *Rutaceae* limonoids are partially responsible for the bitterness in citrus fruits and also have **medicinal** activities. While so far about 50 limonoids have been found in the citrus family the mahogany family is known to produce around 1500 structurally diverse limonoids, of which the seco-C-ring limonoids (e.g. salannin and azadirachtin) are the most dominant and of particular interest because of their **anti-insect activity**. Azadirachtin (isolated from *Azadirachta indica*, the Neem tree) is particularly renowned because of its potent insect anti-feedant activity. Ground up neem seeds are used as a natural crop protection agent; application is somewhat cumbersome, as it has to be applied repeatedly on a daily basis. Neem acts as an anti-feedant, repellent, and egg-laying deterrent, leading to insect starvation and death within a few days.

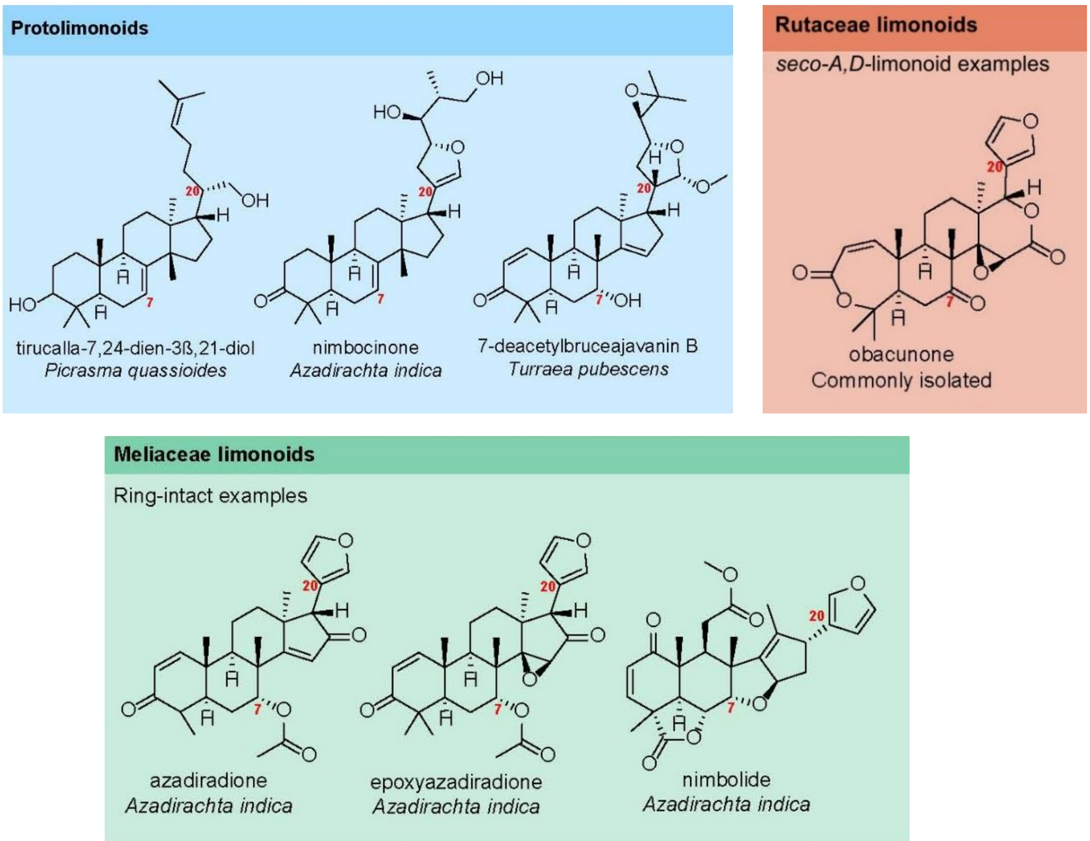

Figure 1: Structures of some protolimonoids and family-specific limonoids

Chemical synthesis of limonoids is very challenging and economically not viable, synthesis of azadirachtin involves 71 steps with 0.00015% total yield and limonin synthesis from geraniol requires 35 steps. In addition to extraction of *A. indica* seeds other approaches are needed to harness the potential benefits of limonoids both for agriculture and medicine.

Professor Anne Osbourn and co-workers identified the early steps in limonoid biosynthesis; an oxidosqualene cyclase able to produce the potential 30-carbon triterpene scaffold precursor tirucalla-7,24-dien-3ß-ol and two cytochrome P450 enzymes that are co-expressed with the tirucalla-7,24-dien-3ß-ol synthase in *M. azedarach*. This pathway results in hemiacetal ring formation and the production of the protolimonoid melianol, as indicated in Figure 2 A below. This pathway for the biosynthesis of the protolimonoid melianol is conserved in the mahogany and citrus families.

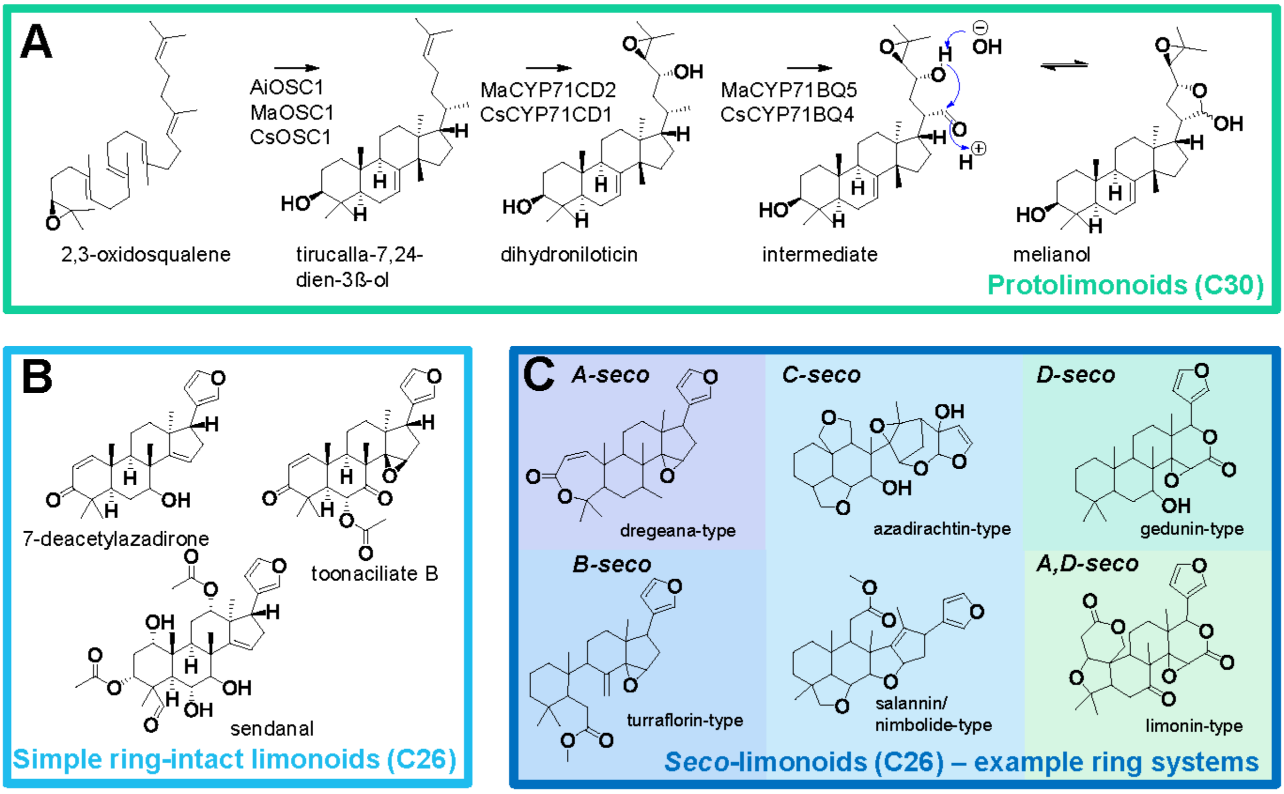

Figure 2: Overview of characterised and predicted biosynthetic routes to protolimonoids and limonoids. (A) Characterised pathway to the protolimonoid melianol; (B) Examples of simple ring-intact limonoids; (C) Example ring structure systems (excluding decoration) of ring-seco limonoids

The elucidation of the pathway and cloning of the three genes paves the way for:

i) engineering crop plants with **insect resistance,**
ii) producing **novel crop protection chemicals** and
iii) producing high value limonoids for **pharmaceutical** and other applications by expression in heterologous hosts

The group has already demonstrated that:

- All three enzymes can be simultaneously expressed in *Nicotiana* leaves when agroinfiltrated with HT CPMV vectors (to generate up to gram-scale quantities of purified triterpene in the lab) containing the three genes, which leads to
- *In planta* production of melianol, with leaves tested
- in feeding studies resulting in a significant decrease in *Manduca sexta* (tobacco hornworm) larvae feeding relative to control leaves

In addition the group is currently using the system to co-express other enzymes in the HT CPMV expression system and thereby produce novel limonoid or protolimonoid compounds that are tested for insect control or medicinal properties.

References:

Hannah Hodgson *et al.* (2019) Identification of key enzymes responsible for protolimonoid biosynthesis in plants: Opening the door to azadirachtin production *PNAS* **116** (34), 17096–17104