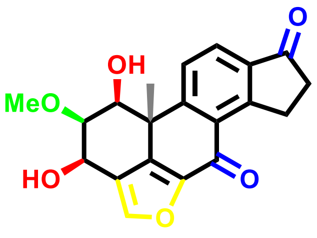
DEPARTMENT OF CHEMISTRY

UNIVERSITY OF OXFORD

Stealing Ideas from Nature

As chemists seek to solve new problems, turning to Biology might just be the answer.

The natural world is an abundant source of useful compounds and chemists are always looking to nature for fresh inspiration. Viridiol is a potent herbicidal compound, extracted from the fungus *Gliocladium virens* and belonging to a class of compounds known as furanosteroid antibiotics (**Figure 1**). In addition to this herbicidal behaviour, the other members of this family have demonstrated some useful biological properties in humans, most notably anti-cancer activity.



viridiol

Figure 1: *Structure of viridiol. Black: steroidal core, blue: carbonyl group, red: hydroxyl group, green: methyl ether, yellow: furan ring.*

Related compound wortmannin is able to bind irreversibly to a protein involved in cell division, therefore preventing cells from replicating (**Figure 2**). As viridiol contains the same structural unit used to bind this protein, it is expected to share wortmannin's anti-cancer activity. Unfortunately, limited quantities of viridiol are available naturally (extraction from the fungus is highly inefficient), so a synthetic approach to produce viridiol, and related compounds, is highly desirable.

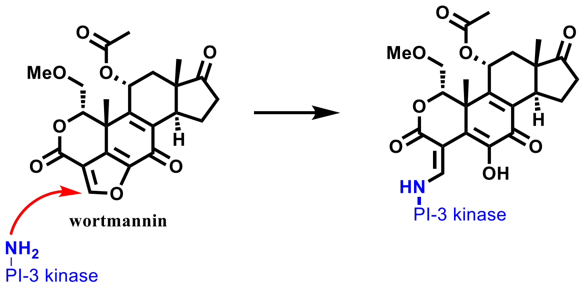


Figure 2: *Demonstrated anti-cancer activity of wortmannin. Cancer protein indicated in blue becomes inactive after binding.*

The Chemistry community has lots of strategies for assembling the four-ring 'steroidal core' (**Figure 1**, black), a structural motif that is common across many natural products. The major challenge in the synthesis of viridiol is the installation of all of the oxidative functionality: two carbonyl groups (blue), two hydroxyl groups (red), a methyl ether (green), and a furan ring (yellow) onto this carbon skeleton.

Whilst the Chemistry toolkit contains a raft of reagents to achieve specific transformations, there are still some reactions which are not synthetically possible and a key aim of organic Chemistry is to fill these gaps. One of the most significant examples of this is an area of chemistry called C-H functionalisation (**Figure 3**). This refers to the process in which a C-H bond is converted into another, more reactive functional group (X), usually *via* some 'activating species' (A) such as a metal catalyst or a high energy reagent.

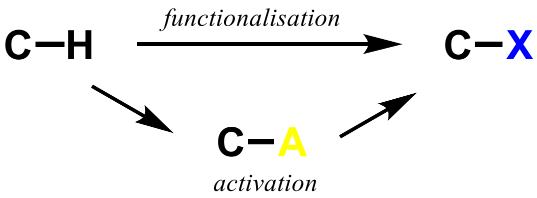


Figure 3: *C-H functionalisation.*

But as C-H bonds are ubiquitous in organic molecules, selectivity becomes a real challenge as a single reagent has to differentiate between many similar bonds. Current understanding within chemistry is able to target the most reactive (i.e. weakest) C-H bonds (**Figure 4a**). These are generally next to electron withdrawing groups (EWGs) such as aromatics, carbonyls and double bonds which pull electron density away from the carbon atom, weakening the C-H bond. Steric activating systems (i.e. physically big molecules) may also be used to differentiate between primary, secondary, and tertiary C-H bonds (**Figure 4b**). The large activating group is only able to get close enough to react with the uncrowded primary C-H bonds, leaving the secondary and tertiary bonds unreacted. However, to achieve the specific arrangement of oxidised bonds on viridiol, both of these reaction preferences must be overcome.

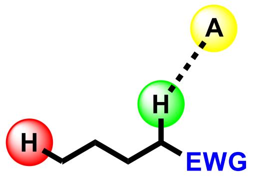


Figure 4a: *C-H functionalisation of most reactive C-H bond. EWG = electron withdrawing group such as a carbonyl, aromatic or double bond which weakens the proximal C-H bond by pulling the electrons towards itself. Green: successful C-H activation, red: no C-H activation.*

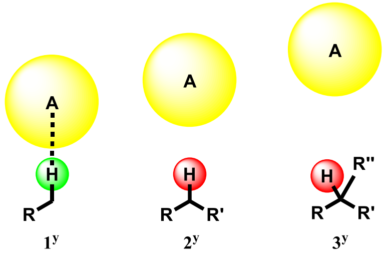


Figure 4b: *C-H functionalisation of most accessible C-H bond using a large activating group. Green: successful C-H activation, red: no C-H activation.*

Here an interdisciplinary approach is valuable, stealing a few more ideas from nature to fill the gaps in Chemistry. Enzymes are nature's catalysts; large, specifically-shaped proteins controlling the life-sustaining reactions in all living things. The critical region of the enzyme, the active site, has a unique shape, perfectly fitting the reaction substrate and enabling it to bind and form the enzyme-substrate complex (**Figure 5**). In this configuration reaction is able to occur very efficiently, resulting in an enzyme-product complex. But as the product is a slightly different shape from the substrate, it no longer fits in the active site and diffuses out, releasing the free enzyme to catalyse another reaction.

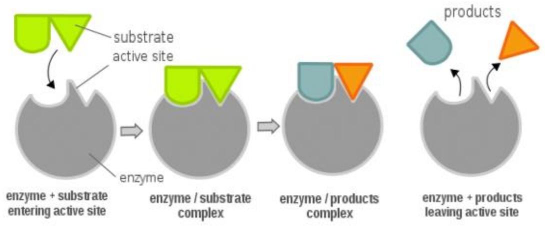


Figure 5: *General mechanism for an enzyme-catalysed reaction.*

Cytochrome P450 is an enzyme of recent interest to chemists, famed for inserting atmospheric oxygen into C-H bonds. Found in almost all forms of life, its major roles in humans are toxin metabolism in the liver and the synthesis of steroid hormones such as oestrogen and testosterone. Recombinant DNA technology has enabled biologists to develop a means of farming this enzyme, meaning large amounts are readily available.

But crucially, the structure of this enzyme may be mutated. As a protein, at its simplest level an enzyme is a specific sequence of amino acids (primary structure, **Figure 6**) which are able to interact with each other, depending on their position, to build the complex 3D shape of the active site (tertiary structure). When an enzyme is mutated, this sequence of amino acids is changed, in turn changing the interactions which build the 3D shape and hence changing the shape of the active site. This is known as an evolved enzyme. A differently shaped active site is able to accept different substrates and therefore achieve reactions which have never been done before.

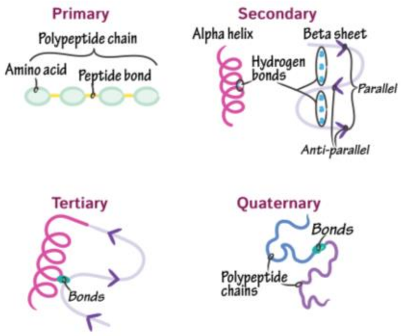


Figure 6: *Structure of proteins.*

For the cytochrome P450 enzymes, this means that they are able to target previously inaccessible C-H bonds, overcoming the innate preference to interact with the most reactive or least hindered bond. Applying this C-H activation strategy to the carbon skeleton of viridiol enables oxidation in all of the required positions and not simply those accessible by conventional chemical means. Enzymes are therefore an incredibly powerful tool for chemists and by recruiting a little bit of Biology, the synthesis of viridiol has truly been inspired by nature.

By Dr Victoria A M Atkinson