Trees may aid the AIDS fight

Many medicinal substances are of plant origin: common aspirin originally came from willow trees; more effective and notorious pain-relievers are still derived from a compound in poppies; the anti-malarial drug quinine came from the bark of the tropical cinchona tree; and digitalis — the widely used drug for failing hearts — is found in that denizen of English gardens, the pretty foxglove.

With advances in organic chemistry, we can of course now synthesise many drugs in their pure form. But plants still remain a source of potentially useful new compounds, and indeed one of the reasons sometimes advanced for trying to halt the extinction of species is that we have not yet had time to appraise the pharmacological value of many of them. Nowhere is this more true than in tropical and subtropical rainforests with their enormous numbers of species (see *Ecos* 55).

Quite recently, an Australian rainforest tree has leant support to this argument. *Castanospermum australe*, also known as the Moreton Bay chestnut or black bean, has been shown to produce a compound with potential to be useful against the virus that causes AIDS. (The tree's first or genus name simply means 'chestnut seed', which the early botanists gave it because its seeds resembled those of the chestnut and horse-chestnut trees of the Northern Hemisphere.)

Dr Mervyn Hegarty of the CSIRO Division of Tropical Crops and Pastures in Brisbane provides the Australian contribution to a world-wide effort to understand the compound and make use of it in the fight against AIDS. Dr Hegarty is a chemist specialising in the study of plant toxins, particularly those that affect our grazing animals. He has been collaborating with Professor Arthur Bell of Kew Gardens in London since 1980, when the English scientist briefly worked at the CSIRO laboratories.

Professor Bell was studying the unusual compounds found in members of the plant family Leguminoseae. He wanted to use the pattern of occurrence of various substances as a possible way of detecting relations between different species, so helping in the classification of the family.

Most of the compounds are rare types of amino acid, but Professor Bell was surprised when extracts of the black bean tree (a member of the family) showed the presence of another unusual compound. Being curious about any aspects of the chemistry of the Leguminoseae, he isolated the substance, named it castanospermine after the genus of the tree, and determined its structure. Chemically, it is a plant alkaloid of the indolizidine type — so called because it contains what chemists call an indole ring (illustrated on page 21).

Like many plant alkaloids, it is toxic to mammals, and probably represents a defence mechanism against herbivores. Indeed Dr Hegarty and Professor Bell had heard of the tree's reputation for causing bad reactions in animals that had eaten it or its seeds.

Following castanospermine's extraction, Dr Linda Fellows and her co-workers at Kew Gardens discovered that it inhibits the enzyme glucosidase — important in metabolic reactions involving the sugar glucose and its polymers — which could explain some of its immediate toxicity. In this, it resembles the alkaloid swainsonine — extracted from another native legume called *Swainsona* — which interferes with mannosidase, an enzyme that deals with mannose, also a simple sugar.

In its structure, castanospermine bears some resemblance to a simple sugar. It possesses four hydroxyl groups, which protrude from the main ring of the molecule (glucose has five hydroxyls). It is likely that therein lies its ability to block the action of glucosidase. The enzyme would 'recognise' castanospermine and combine with it, because of its superficial similarity to a glucose molecule. But it is not quite the right shape for the enzyme and, once attached, could remain stuck fast, so preventing the enzyme from catalysing its proper reaction.

However, Dr Hegarty is not certain that castanospermine is necessarily responsible for all of the black bean's toxicity to mammals because other noxious substances are also present.

Virus-killer?

Learning of its effect on the enzyme glucosidase, a research group at the Fred Hutchinson Center in Seattle started to investigate the potential uses of castanospermine. Dr Larry Rohrschneider of the

What is a black bean tree?

What sort of tree is this *Castanospermum australe*, which could be about to shoot to fame?

Taxonomically speaking, the black bean is a bit of a loner. It is the only species in the genus *Castanospermum*, and its nearest relative grows in South America.

It was described scientifically, and given its English and botanical names, in the early nineteenth century by a certain Allan Cunningham, superintendent of the Botanic Gardens in Sydney.

Obviously, the Moreton Bay chestnut grows around Moreton Bay in Brisbane, but it also grows elsewhere on the Queensland and northern New South Wales coastal fringe, extending inland along waterways, and even to the Bunya Mountains in southern Queensland. It's also found in some Pacific islands.

This essentially tropical tree is striking to look at, growing to 30 metres or so and bearing large yellow–orange flowers. Consequently it has become popular in gardens and therefore its range has increased. It now grows in various frost-free spots as far south as Melbourne. (You can also grow it from seed as an indoor pot plant for a number of years.) The tree yields a fine timber: the Speaker's Chair in the British House of Commons in London is made of black bean wood, and was a gift from Australia.

The species is not endangered; it had a fairly extensive range before European settlement, and its popularity has ensured that it remains. Phytex says it is confident that it will be able to get sufficient material to meet the demand for castanospermine if the substance turns out to be effective against AIDS.

Nature to the rescue. D. Helton. *BBC Wildlife*, September 1987, 472–3.

Center, in collaboration with scientists from Harvard Medical School, reasoned that the plant alkaloid, through its effect on glucosidase, might interfere with the production of an important glycoprotein that sits on the outside of the AIDS virus.

Glycoproteins are combinations of sugars and protein and are common components in cell membranes, where they seem to function as markers, distinguishing one cell type from another, and as receptors that recognise and allow interactions with other cells or molecules.

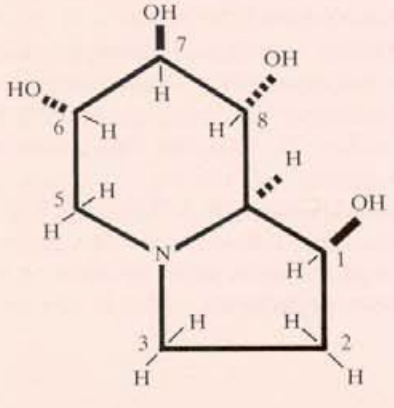
On the outside of the human immunodeficiency virus or HIV — the cause of AIDS — a glycoprotein called gp120 allows the virus to attach to its target cell type. The victims of this viral embrace are a class of lymphocytes, the white blood cells whose job it is to fight invading pathogens.

Not all lymphocytes can be infected; those that are — the helper T-cells — possess a receptor molecule called CD4 on their membranes. The virus's gp120 interacts with CD4, much like a key in a lock, following which the virus gains entry to the cell.

Knowing this, Dr Rohrschneider and his colleagues tested the anti-viral effects of castanospermine and found that the little Aussie alkaloid did indeed work by altering the nature of the glycoprotein on the outside of the virus.

Castanospermine's chemical name is 1,6,7,8-tetrahydroxyoctahydro-indolizidine. By convention the carbon atoms, occurring where lines meet, are not written. The hydroxyl (OH) groups project out of the plane of the paper either towards you (solid line) or away (broken line). Projecting hydroxyls are also found in sugar molecules and may be the recognition feature for the enzyme *alpha*-glucosidase that castanospermine inhibits. Inset: The 50¢ coin shows how big black bean tree pods and seeds are.

Castanospermine



Cattle and castanospermine

Some people, and animals, produce the enzyme glucosidase in insufficient quantity, or not at all. This unfortunate condition, caused by a defective gene, runs in families and goes by the name of type 2 glycogen-storage disease, or Pompe's disease.

Glycogen is a polymer, made up of many glucose units strung together, and it is our bodies' way of storing glucose — the basic fuel for all our cells. Immediately after a meal, much of the carbohydrate we eat is converted to glycogen and kept in the liver. After only an hour or so without further food, we would use up the glucose in the blood were it not quickly replenished from the glycogen in the liver. For this to happen, glucosidase must decompose the glycogen back into its original glucose molecules, which are then released into the blood-stream.

In Pompe's disease, the lack of the enzyme causes the stored glycogen to accumulate. Once the liver is 'full', this carbohydrate deposits itself in excessive amounts in the heart, tongue, and many other muscles. A characteristic symptom is an enormously swollen heart. Sadly, human victims with the disease in its full form rarely survive beyond the age of 2 years.

Cattle also may have this defect in their genes, and so accumulate excessive quantities of glycogen throughout their tissues. Like us, and all vertebrates, the cattle have two copies of every gene. It's therefore possible to be heterozygous for the condition — meaning that only one of the pair of genes is defective — and survive without symptoms being obvious. But a blood test, which in those cases would reveal half the normal activity of the enzyme in the white blood cells, allows us to detect such animals.

Recently, some scientists and veterinarians from the Queensland Department of Primary Industries and Queensland Agricultural College used this method to screen for Pompe's disease in a Brahman stud herd of 160 animals, and identified 18 2-year-old bulls as apparently heterozygous for the condition. Of course, with this defective gene, the animals would be considered useless for breeding. But with their known parentage, how had they acquired it anyway? Records showed that most of the affected animals had an impeccable pedigree with parents quite normal for the gene.

The researchers suspected that something else must be the cause of the low blood glucosidase levels — especially when they noticed that all of the 18 bulls had been grazing separately from the rest of the herd. In fact, the bulls were in a paddock containing *Castanospermum* trees, which they had browsed. The scientists knew of the effects of castanospermine on glucosidase, and tested their theory by removing the 18 affected beasts from the paddock.

In brief, they found that after 11 weeks away from the black bean trees, 15 animals had levels of blood glucosidase that were back to normal. The remaining three were possibly carriers of the gene.

However, the scientists needed further proof. They had to show that the seeds of the black bean, and not other species present in the paddock and grazed by the beasts, did actually affect cattle glucosidase. This they did, discovering that the consumption of only five seeds (140 g) per day for 6 successive days was enough to depress the activity of the enzyme for several weeks.

In the future, veterinarians who screen herds for heterozygote carriers of Pompe's disease must check carefully (if in an area where *Castanospermum* grows) to make sure they are not detecting a 'false positive' caused by eating of 'the forbidden tree'.

Inhibition of bovine ∝-glucosidase by *Castanospermum australe* and its effect on the biochemical identification of heterozygotes for generalised glycogenosis type 2 (Pompe's disease) in cattle. K.G. Reichmann, J.O. Twist, R.A. McKenzie, and K.J. Rowan. *Australian Veterinary Journal*, 1987, **64**, 274–6.

Viruses are simple and lazy parasites. Stripped down to essentials, they are unable to reproduce themselves, relying on the machinery of their host cell to do it all for them. So, following the entry of the AIDS virus into it, the T-cell's enzymes and foodstuffs are used to build more virus particles. This is where castanospermine comes in. It inhibits the enzyme glucosidase in mammalian cells, with the result that an infected T-cell cannot make the gp120 that sits on the virus. Although an infected cell will still die, the new virus particles that burst out of it will be defective and, lacking the crucial 'key', will be unable to infect other T-cells. Therefore no new virus particles can be produced, and the lethal cycle is broken.

At about the same time as Dr Rohrschneider's work in America, scientists at St Mary's Hospital and Queen Charlotte Hospital for Women in London also tested the antiviral activity of castanospermine on a culture of HIV-infected human cells. For this work to go ahead, Dr Hegarty supplied the black bean seeds, and gave advice on the extraction of the compound to the chemists in the Kew Garden Laboratories, who provided the purified substance to the hospitals.

To assess the effectiveness of castanospermine against the virus, the scientists used two indicators. Firstly, they measured the quantity of an enzyme only virally infected cells can produce. This enzyme, reverse transcriptase, allows copying of DNA — our cells' permanent information storage molecule — from RNA, the hereditary molecule of the retroviruses, the group to which HIV belongs.

Infected cells that received castanospermine showed but a small increase in the activity of this enzyme over an 11-day period, meaning that only slight viral multiplication was taking place. Infected cells not receiving the alkaloid showed a dramatic rise in the activity of reverse transcriptase, corresponding with the rapid production of ever-increasing numbers of virus particles.

The second means of testing the efficiency of the drug relied on microscopic assessment of a curious effect that the virus has on cells — it causes them to fuse. A collection of such fused cells is called a syncytium. The researchers noticed that, compared with untreated cells, those to which they added castanospermine at a dose of 2·5 millimoles per litre formed far fewer syncytia.

A Dutch group of researchers reported similar findings with castanospermine and other inhibitors of glucosidase.

Side-effects

The most interesting part of this work was the fact that uninfected cells continued to grow extensively when given castanospermine in a 'control' experiment. This shows that at the dosage at which it can effectively block virus multiplication the alkaloid is not toxic — at least not for the type of human cells used in the experiment.

Of course, it's a big leap from cells in a culture flask to a person dying of AIDS. We don't yet know how effective castanospermine will be at inhibiting viral growth when in the complex chemical environment of the human body, nor do we know how bad its side-effects may be. But the encouraging results in the laboratory make

A medium-sized black bean tree, popular in gardens.

clinical trials the obvious next step. Until we know the results of a series of such trials, castanospermine merely remains an interesting substance. It is a very long way from being a cure.

At the moment, a series of pre-clinical tests are being conducted by the National Cancer Institute in America. These animal trials involve studies of the metabolism and break-down of the drug in a whole body, and its rate of excretion, which, initial results indicate, is quite high. This could possibly be a problem, as an effective drug can be rendered almost useless if the kidneys excrete it before it has time to do its work.

Any such practical difficulties must first be ironed out before anybody can consider marketing the drug as a cure.

It could be that castanospermine itself will not be the answer. With this in mind, Dr Claude Culvenor of the CSIRO Division of Animal Health is extracting some Australian plant alkaloids related to it to see if any might have similar effects, or be even better.

Another approach is to consider directly modifying the castanospermine molecule. Dr George Holan of the Division of Chemicals and Polymers is using computer modelling of the castanospermine–enzyme interaction with a view to finding out if we could synthesise castanospermine-like derivatives that would remain effective for longer, be better glucosidase inhibitors, and be more lipid-soluble. This last characteristic would allow penetration into the brain (where AIDS virus may hide out), a feat that the water-soluble castanospermine may not be able to achieve.

If castanospermine or a related compound proves successful in both pre-clinical and clinical trials, the drug offers considerable economic potential. Already, a company called Phytex Australia has entered into commercial production of castanospermine in quantities sufficient for laboratory studies. Should the substance prove a 'real winner', the company is capable of increasing its production. Dr Hegarty, who has determined the distribution of the alkaloid in the various parts of the tree, will be providing advice.

Roger Beckmann

The black bean tree's showy flower, and young seed pods from a previous flower.

More about the topic

Castanospermine and other plant alkaloid inhibitors of glucosidase activity block the growth of HIV. A.S. Tyms, E.M. Berrie, T.A Ryder, R.J. Nash, M.P. Hegarty, D.L. Taylor, M.A. Mobberley, J.M. Davis, E.A. Bell, D.J. Jeffries, D. Taylor-Robinson, and L.E. Fellows. *The Lancet*, October 31 1987, 1025–6.

Interference with HIV-induced syncytium formation and viral infectivity by inhibitors of trimming glucosidase. R.A. Gruters, J.J. Neefjes, M. Tersmette, R.E.Y. de Goede, A. Tulp, H.G. Huisman, F. Miedma, and H.L. Ploegh. *Nature*, 1987, **330**, 74–7.

Inhibition of human immunodeficiency virus syncytium formation and virus replication by castanospermine. B.D. Walker, M. Kowalski, W.C. Goh, K. Kozarsky, M. Krieger, C. Rosen, L. Rohrschneider, W.A. Haseltine, and J. Sodroski. *Proceedings of the National Academy of Sciences of the U.S.A.*, 1987, **84**, 8120–4.