CHAPTER 10

Natural products

Chapter outline

This chapter considers various specific examples of naturally occuring toxins.

- Plant toxins
  - pyrrolizidine alkaloids
  - pennyroyal oil
  - ricin
  - bracken
  - fluoroacetate
- Animal toxins
  - snake venoms
  - tetrodotoxin
- Fungal toxins
  - Death Cap mushroom
  - aflatoxins
- Microbial toxins
  - botulism and botulinum toxin

Although many of the toxic chemicals in the environment that worry the general public are man-made, there are also many hundreds of natural poisons of animal, plant, fungal and microbial origin. Indeed, the *most toxic* substances known to man are natural poisons such as **botulinum toxin** (Table 1.1) and it is certainly not reasonable to imply, as do some of the advertisements for health foods and herbal medicines, that natural substances are intrinsically harmless and safe. For example, allergies to natural constituents of food are known to occur just as they do to synthetic additives. Some of these natural, toxic substances have been known about for centuries and have been used for murder or suicide, or even sometimes misguided medical treatment (see Chapter 1).

Natural substances also still occasionally feature in accidental poisoning cases although this is relatively rare compared with poisoning by drug overdose. Natural toxins are of diverse structure and mode of action, and there are far too many categories to consider each individually in this book. Consequently we will simply examine a few interesting and important

examples of toxic substances derived from plants, animals, fungi and micro-organisms.

Plant toxins

There are many well known plant toxins ranging from the irritant **formic acid** found in nettles (and ants) to more poisonous compounds such as **atropine** in deadly nightshade berries (*Atropa belladonna*), **cytisine** in laburnam and **coniine** in hemlock. Let us consider a few less well-known plant toxins which have been studied recently.

PYRROLIZIDINE ALKALOIDS

Pyrrolizidine alkaloids are a large family of structurally related compounds found in over 6000 plants in the Leguminosae, Compositae and Boraginacae families. Many of these occur as weeds throughout the world. About half of the pyrrolizidine alkaloids have been identified as toxic and these plant constituents are probably the most common cause of poisoning in the world for humans and animals, both livestock and domestic. This may occur as a result of contamination of cereal crops or as a result of use of plants in herbal remedies.

Case study *In Austria an 18-month-old boy presented with veno-occlusive disease. He had been given herbal tea since he was 3 months old. The herbal tea had been intended to have been made with Coltsfoot but was in fact made with Alpendost. The boy had congestion of the sinusoids of the liver and necrosis and bleeding from the small veins.*

A study in South Africa in two hospitals identified 20 children suffering from veno-occlusive disease which was thought to be due to the use of traditional remedies. Most of the children had fluid in the abdominal cavity and an enlarged liver. There was high morbidity and mortality and in those that survived the disease progressed to liver cirrhosis. In four cases pyrrolizidine alkaloids were detected in the urine.

Poisoning has occured in various parts of the world, especially where agricultural conditions are poor and the indigenous population may be forced to use the contaminated crops. For example, in South Africa during the 1930s poor whites suffered the toxic effects of these alkaloids because their staple diet was wheat which became contaminated, whereas their Bantu neighbours, who ate maize which was not contaminated, were not affected. More recently, poisonings have occurred in Tashkent, Central India and Northern Afghanistan. In one incident where 1600 poisoning cases were reported, the threshed wheat was found to be contaminated with ***Heliotropium popovii*** seeds giving an alkaloid concentration of at *least 0.5 per cent.* In the West Indies especially, these plants may also be used in traditional medicine to make herbal teas.

The toxicity depends on the particular alkaloid. One of the most studied pyrrolizidine alkaloids is **monocrotaline**. This is found in *Heliotropium*, *Senecio* and *Crotolaria* species and it causes liver injury in humans, such as after acute exposure to herbal teas for example. The liver injury is of an unusual kind causing damage to the sinusoids in the liver, known as **veno-occlusive disease**.

Monocrotaline will consistently produce veno-occlusive disease in rats and also damage the lungs. The effect of chronic exposure to low doses is **liver cirrhosis** which can be seen in some members of the West Indian population, estimated to account for one third of the cirrhosis seen at autopsy in Jamaica. The constituent

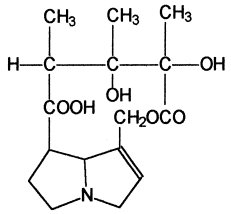

FIGURE 10.1 *The structure of the pyrrolizidine alkaloid monocrotaline.*

alkaloids, such as **monocrotaline** (Figure 10.1), undergo metabolic activation to a reactive metabolite which damages the cells lining the liver sinusoids as well as the hepatocytes, leading to haemorrhagic necrosis and finally to the **veno-occlusive disease**. This blockage of the blood vessels in the liver eventually gives rise to alteration of the vasculature such that the liver blood supply is diverted and new blood vessels grow.

Animals may also be exposed and suffer the toxic effects. Where there is abundant vegetation for grazing, animals will ignore plants such as **ragwort (*Senecio jacoboea*)** which contain the alkaloids but in some countries, such as Australia, widespread losses of horses, cattle and sheep have occurred from *Heliotropium* poisoning. This may also be another route of human exposure as the alkaloids can be detected in the milk of cows grazing on such plants.

PENNYROYAL OIL

The Pennyroyal plant and the oil prepared from it have been used to induce abortions in the USA where it is possible to buy the oil 'over the counter'. The plant may be used to make a tea or the oil may be taken directly. Both may cause toxic effects, especially liver damage as well as inducing abortion. The oil contains a number of terpenoid compounds and metabolic activation is believed to be required for the toxicity.

RICIN

Ricin is a highly toxic plant product found in the seeds of the castor oil plant. It achieved some notoriety when it was claimed that it had been used by the Bulgarian secret police to kill the Bulgarian journalist **Georgi Markov** in London in 1978. Although no trace of any poison was found in the victim's body clearly an extremely potent poison had been used and the symptoms were consistent with those of ricin poisoning. A tiny metal pellet was recovered from a wound on the victim's leg, seemingly inflicted accidentally by an umbrella. The pellet almost certainly was a reservoir for a toxic substance, but it could only contain a few nanograms of the substance.

Ricin is a small **protein** consisting of **two polypeptides**, a short A chain and a longer B chain. The A and B chains are linked via a disulphide bridge. The B chain attaches the ricin molecule to the outside of the mammalian cell by binding to the galactose part of a glycoprotein. The cell membrane invaginates and the ricin is taken into the cell inside a vacuole. The ricin molecule is released from the glycoprotein and the A and B chains then break at the disulphide bridge. The B chain makes a channel through the vacuole cell wall, allowing the A chain to enter the cytoplasm and reach the ribosomes where it blocks protein synthesis and kills the cell. One molecule of ricin is sufficient to kill one cell.

BRACKEN

The bracken fern contains a substance, **ptaquiloside**, which degrades into a compound which

is carcinogenic. In Japan, the shoots of the bracken fern are eaten and this may explain the high incidence of throat cancer among the Japanese. Animals which eat the fern as fodder suffer from bladder and intestinal cancer. The breakdown product of ptaquiloside reacts with DNA, specifically the base adenine, and this is lost with the result that the DNA chain breaks.

FLUOROACETATE

For a description of the toxicity of this naturally occurring plant toxin see Chapter 8.

There are many more well-known substances derived from plants such as the drugs **heroin**, **morphine**, **cannabis**, **nicotine** and **digitalis**.

Animal toxins

As with plant toxins, animal toxins comprise a diverse range of structures and modes of action (Figure 10.2). A simple and well-known example is formic acid which is found in ants (the name is derived from the Latin word, *formica*, for an ant). Other examples are **tetrodotoxin** found in the **Puffer Fish** and **saxitoxin** found in shellfish and fish which have consumed certan dinoflagellates. Animal toxins are often mixtures of **complex proteins**. Most of us suffer

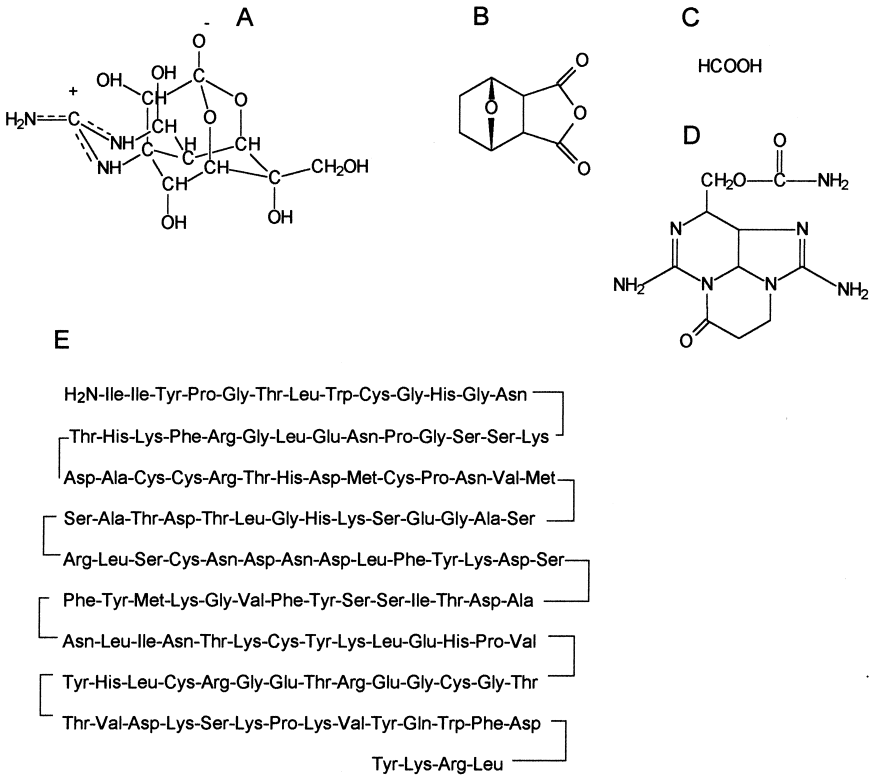

FIGURE 10.2 *The structures of various animal toxins. A: tetrodotoxin; B: cantharidin; C: formic acid; D: saxitoxin; E: amino acid sequence from honey bee venom phospholipase A.*

from animal toxins at some time in our lives even if it is only a wasp or bee sting. However, in some countries death and illness due to animal poisons represents an important proportion of poisoning cases and a significant cause of illness and death.

SNAKE VENOMS

Snake bites are one of the most common forms of poisoning by natural toxins worldwide. Many snake venoms are similar in their mode of action and constituents, being mixtures of **proteins or polypeptides**. The proteins may be enzymes, especially **hydrolytic enzymes**. Some of the more important are **proteinases**, **phospholipases**, **ribonucleases**, **deoxyribonucleases**, **phosphomonoesterases** and **phosphodiesterases**, and **ATPases**. The toxicity of some snake venoms is shown in Table 10.1. The venoms may be mixtures and consequently cause a variety of effects. For example, the presence of foreign proteins may cause an **anaphylactic reaction**, although this is rare, and such allergic reactions may cause death in minutes. The enzyme components can *digest* various tissue constituents either at the site of action, causing local necrosis, or elsewhere causing systemic effects. For example, the bite of the diamondback rattlesnake, the most poisonous snake in the USA, produces a very painful swelling within minutes. Nausea, vomiting and diarrhoea may occur and cardiac effects, such as a *fall* in systemic arterial blood pressure and a weak, rapid pulse, may be seen. The central nervous system can be affected, leading to respiratory paralysis. Haemolytic anaemia and haemoglobinuria sometimes occur, and there may be thrombosis and haemorrhage. Vascular permeability and nerve conduction can change, and cerebral anoxia, pulmonary oedema and heart failure also develop. The **phospholipases** found in snake venom sometimes cause *intravascular haemolysis* by direct action on the red cell membrane.

Most snake venoms contain a **phosphodiesterase** which *attacks* polynucleotides.

TETRODOTOXIN

This toxin is found in the **Puffer Fish** and also in the **Californian newt** and bacteria and has been studied extensively. The fish is eaten as a delicacy in Japan and provided it is properly prepared is edible and safe. However, fatalities have occurred which resulted from incorrect preparation of the fish and about *60 per cent of poisoning cases are fatal.* The tetrodotoxin and another

TABLE 10.1 *Comparative toxicity of snake venoms*

Snake venom	Yield (g)	LD_{50} i.v. mg kg^{-1}
Copperhead	40–72	10.92
African puff adder	130–200	3.68
Mojave rattler	50–90	0.21
Russell's viper	130–250	0.08
Sea snake	7–20	0.01

Source: F. W. Oehme, J. F. Brown and M. E. Fowler (1980), Toxins of animal origin, in *Casarett and Doull's Toxicology*, J. Doull, C. D. Klaassen and M. O. Amdur (Eds), 2nd edition, New York: Macmillan.

toxin **ichthyocrinotoxin** are found in the roe, liver and skin of the fish. Tetrodotoxin is a very potent nerve poison, lethal at doses of around 10 μg kg^{-1} body weight. Initial effects are a tingling in the mouth followed within 10–45 minutes by muscular incoordination, salivation, skin numbness, vomiting, diarrhoea and convulsions. Death results from skeletal muscle paralysis. Sensory as well as motor nerves are affected and it is believed that tetrodotoxin *selectively blocks* the **sodium channels** along the axon, preventing the inward action potential current.

Fungal toxins

Many fungi produce toxins of a variety of chemical types and these can cause acute or chronic poisoning. Poisonous mushrooms may be confused with the edible varieties and hence accidental acute poisoning may occur. Poisoning may also occur through the intentional eating of fungi believed to contain psychoactive substances. Several fungal toxins have been fully identified and characterized. The toxic effects vary from relatively mild gastrointestinal disturbances to severe organ damage. Some, such as the psychoactive constituents **mescaline** and **psilocin**, affect the central nervous system. Certain fungal products, such as the **aflatoxins** which are discussed in Chapter 7, are potent **carcinogens**.

DEATH CAP MUSHROOM

The Death Cap mushroom, ***Amanita phalloides*** is probably the most poisonous mushroom in Britain. It is occasionally eaten by mistake, but poisoning with this mushroom is rare in the UK. The mushroom contains a number of toxins: the phallotoxins, including **phalloidin**, **phalloin** and **phallolysin**, and the **amatoxins** (α, β and γ amanitin). The phallotoxins cause violent **gastroenteritis** which occurs rapidly (4–8 hours) after the mushroom is eaten.

The amatoxins have a delayed toxic effect, with the liver and kidney as target organs; **liver necrosis** and **destruction of renal tubular cells** may result. Both the phallotoxins and the amanitins are strongly bound to plasma proteins yet are toxic in this form. Consequently, treatment involves *displacement* from the proteins by a drug such as a **sulphonamide** or **benzylpenicillin** which will reduce toxicity. This is probably due to increased excretion of the unbound form as protein binding of foreign compounds slows excretion (see Chapter 2).

After the mushroom is eaten sometimes there may not be any symptoms for up to a day then vomiting and diarrhoea occur possibly followed by jaundice, hypoglycaemia, acidosis and other effects on blood chemistry. In severe cases hepatic failure can result.

AFLATOXINS

These fungal toxins have already been discussed under food contaminants (Chapter 8). **Aflatoxin B_1** causes **liver damage** after high doses but in humans chronic exposure to lower doses via the diet is a more likely occurrence which can cause **liver tumours**. The toxin is most likely to occur in food infected with the mould ***Aspergillus flavus*** or prepared from infected food or ingredients.

Microbial toxins

There are many toxins produced by bacteria. As with other natural toxins, they are of a variety of chemical types and consequently they cause a

variety of different toxic effects, ranging from gastrointestinal effects to severe and fatal effects on the nervous system. We will consider just one well known toxic syndrome, botulism.

BOTULISM AND BOTULINUM TOXIN

Botulinum toxin is a toxicant derived from a spore forming anaerobic bacterium, *Clostridium botulinum*. The bacterium produces a mixture of six **neurotoxic proteins** and poisoning with these toxins causes a syndrome, botulism, which has a high mortality rate. The toxin is one of the most toxic substances known to man with an LD_{50} of around 0.01 μg kg^{-1} and so less than a microgram would be lethal for a human. The spores are quite resistant but the toxin is destroyed by heat (80°C). The symptoms of poisoning normally appear 18–36 hours after ingestion although the appearance may be as short as 4 hours or as long as 4 days.

The mechanism underlying the effect of botulinum toxin is irreversible blockade of the motor nerve terminal at the **myoneural junction**, preventing the release of acetylcholine. The result is that the muscle behaves as if it is denervated and the victim suffers paralysis. This leads to weakness of limbs and difficulty in breathing which may be fatal if severe. There is fortunately an anti-toxin available.

Although cases of botulism are thankfully rare, they still occur from time to time, sometimes affecting several victims at the same time as a result of contaminated food. This is typically home-canned food such as seafood, vegetables, sausages and other meat products, which have not been adequately preserved or refrigerated.

Case study *In October and November 1987 eight cases of botulism occurred, two in New York and six in Israel. All of the victims had eaten Kapchunka, air-dried, salted whitefish, which had been prepared in New York and some transported by individuals to Israel. All the patients developed the symptoms of botulism within 36 hours and one died. Some were treated with anti-toxin and two received breathing assistance.*

Summary and learning objectives

There are thousands of chemicals found naturally in the environment in plants, fungi, bacteria and animals and a significant number are toxic. For example, ***pyrrolizidine alkaloids*** such as monocrotaline, found in plants such as *Senecio* and *Heliotropium*, cause poisoning (liver damage) in animals and humans as a result of contamination of crops or use as herbal remedies. ***Pennyroyal oil***, from the Pennyroyal plant and used to induce abortions in the USA, causes liver damage. ***Ricin*** from the castor oil plant, is a protein and the most toxic substance known. ***Ptaquiloside*** found in the ***bracken*** fern is a carcinogen in both humans and animals. ***Animal toxins*** such as ***snake venoms*** generally consist of a variety of hydrolytic enzyme toxins (e.g. proteinases, phosphoesterases) which may cause anaphylactic shock as well as tissue necrosis. ***Tetrodotoxin***, found in Puffer Fish and bacteria, is a potent nerve poison. Phalloidin from the ***Death Cap mushroom***, contains several toxins that damage liver and kidneys. Another fungal toxin is ***aflatoxin*** from *Aspergillus flavus*. ***Botulinum toxin*** from the bacterium *Clostridium botulinum*, consists of neurotoxic proteins which are extremely potent, causing irreversible blockade of the myoneural junction. Poisoning may often result in paralysis and fatal respiratory failure.

Questions

Q1. Indicate which of the following are true:

a pyrrolizidine alkaloids are found in mushrooms
b *Amanita phalloides* is a poisonous snake
c Botulinum toxin is produced by bacteria
d ricin is the toxin in puffer fish
e fluoroacetate is a plant toxin.

Q2. Which of the following statements about tetrodoxin are true:

a it is a toxin found in blue green algae
b it causes paralysis of muscles in humans
c it affects calcium channels
d it blocks nervous transmission.

SHORT ANSWER QUESTION

Q3. What is the basic mechanism underlying the toxicity of botulinum toxin?

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