CHAPTER 1

Introduction

A garden can be a source of pleasure. The juxtaposition of different colours and floral scents together with the opportunity of eating home grown vegetables combine to provide gardeners with considerable satisfaction. This book is about the chemistry that is found in the garden and the chemical ecology involved in the interactions between the plants, micro-organisms and insects that live there.

The chemistry of the garden begins with the chemistry of the soil. Next to climatic conditions, what is present in the soil can have the biggest effect on the garden. The structure and chemistry of the soil determines the availability of nutrients, water and air to the roots. The pH of the soil and the mobility of metal ions, such as iron, affects the plants that can be grown. Part of the chemistry of gardening lies in achieving an appropriate mineral balance in the soil.

Soil and climatic conditions can have a marked impact on the chemistry of plants and the natural products that they form. For example plants that grow under arid conditions can produce a resinous or waxy covering on the leaves to reduce their water loss. The mineral content of the soil can vary the colour of flowers such as the hydrangea. Plants have the ability to take up metal ions and this can be used in the bioremediation of 'brown field' sites. It is also a warning to the vegetable gardener who may unwittingly ingest toxic metals such as cadmium.

The very act of gardening changes the chemistry of the soil. Digging, together with the distribution of fertilizer and compost, not only changes the air, mineral, water and organic content of the soil but also affects the presence of many chemical messengers within the soil. The smell of newly dug soil is an indication of the release of volatile chemicals produced by soil micro-organisms. More subtle effects involve the dispersion of insect trail substances and the redistribution of germination inhibitors produced by plants.

1.1 CHEMICAL DIVERSITY IN PLANTS

The chemistry that occurs within a plant is complex and highly organized. The organic compounds that occur in plants fall into three big groups. Firstly there are those compounds that occur in all cells and play a central role in the metabolism and reproduction of the cell. These are known as the primary metabolites and include the common sugars, the amino acids that are constituents of proteins, and the nucleic acids. The second group are the high molecular weight polymeric materials such as cellulose, lignin and the various proteins which form the structural and enzymatic components of the cell. The third group of naturally occurring compounds are those of relatively low molecular weight which are characteristic of a limited range of species. These are the secondary metabolites and they include the polyketides, the phenylpropanoids, the alkaloids and the terpenoids from which the colouring matters, the scent of flowers and the flavours of foods are derived.

Whereas many of the primary metabolites exert their biological effects within the cell in which they are produced, the secondary metabolites often exert their biological effects on other cells or other species. The range of secondary metabolites that are formed is a characteristic of particular species. Furthermore, structurally quite different secondary metabolites may play a similar role, for example, in the defence mechanisms of plants in different species. Many of the natural products that are of interest to the chemist in the garden are secondary metabolites. However the division between primary and secondary metabolites, whilst useful, is not rigid. Acids derived from primary metabolism form esters with secondary metabolites, and amino acids that are constituents of proteins are also the progenitors of alkaloids.

The typical garden displays considerable biodiversity. Even the average lawn will contain several different grasses as well as, probably, clover, daisies and moss! The variety of natural products that are found as a consequence of this biodiversity is wide. This chemical diversity is not restricted to differences between species. There is considerable infra-species variation which is reflected, for example, in the many colours exhibited by a single species such as the antirrhinum. The inheritance of colour in sweet peas laid the foundations of plant genetics. Furthermore the chemistry changes as the plant develops. This is exemplified by the changes in flavour and colour of fruit as it ripens. There is even a diurnal variation in the formation of some natural products. Flowers that are pollinated by moths are more highly scented in the evening, whilst those that rely on bees for pollination have their peak production earlier in the day.

Seedsmen have bred many cultivars of garden plants to enhance their visual properties and their resistance to disease. The organoleptic properties of vegetables have been modified, for example, to produce varieties of lettuce which do not possess the bitter taste of some sesquiterpenoid lactones. It has been reported that there are over 50 000 varieties of rose that have been produced commercially over several hundred years. In chemical terms, their natural product content has been varied. Whilst this variation may be in the finer detail of the balance between similar compounds, it can mean that some varieties do not produce a particular compound while others may even produce different natural products.

Although it is obvious in terms of plant pigments, the location of many other natural products in the plant varies widely. The constituents of the roots often differ from those of the aerial parts of the plant. Many natural products are produced as a response to a competitive stress. The nature of this competition varies between the roots and the aerial parts of the plant and this has an effect on the natural products that are produced. For example some salvias produce diterpenoid quinones in the roots with antibiotic properties and different diterpenoids (clerodanes) in the leaves as insect anti-feedants. Those natural products that are found in the flowers, or even parts of the flower, may differ from those that are found in the leaves, the stem or the fruits.

1.2 THE STRUCTURE ELUCIDATION OF NATURAL PRODUCTS

The quantities of the natural products which are produced by plants are usually small when compared to the fresh or even the dry weight of the plant. A good recovery of a natural product might be 100 mg from 1 kg dry weight and in many cases the amounts that are isolated are much smaller. These low concentrations can be a reflection of the biological activity of many natural products. However the amounts of any one compound may vary by as much as fiftyfold when, for example, it is a stress metabolite involved in the protection of a plant against microbial or insect attack.

The small quantities of natural product that are available has meant that the real diversity of structures has only become apparent over the past forty to fifty years since chromatographic methods of separation have been refined and spectroscopic methods of structure elucidation have become widespread. It is worth noting that chromatography was first introduced a hundred years ago in the context of the separation of natural products. Today many structures are established purely on the basis of spectroscopic, particularly nuclear magnetic resonance (NMR), studies.

The application of spectroscopic methods to organic chemistry has often been explored in the context of natural product structure elucidation. One of the first applications of ultraviolet (UV) spectroscopy to organic chemistry was nearly one hundred years ago in a study on berberine, the yellow alkaloid found in barberry (*Berberis* sp.). Theories relating the wavelength of the absorption maxima in the ultraviolet spectrum to the length of conjugated systems arose from the studies on the polyene carotenoid pigments of carrots and tomatoes. A useful set of correlation tables between structure and the frequencies of carbonyl absorption in the infrared (IR) spectrum, published in 1951, was produced in the context of the study of fungal metabolites including some compounds produced by *Penicillium gladioli*, an organism that is a plant pathogen found on the corms of gladioli. Many of the early studies utilizing NMR spectroscopy in the late 1950s and early 1960s involved natural products including, for example, the gibberellin plant hormones. Much of our knowledge of insect chemistry could not have been obtained without the combination of gas chromatography linked to mass spectrometry. This combination has also revolutionized the analysis of floral scents and the detection of plant hormones, which also occur in minute amounts.

A present day spectroscopic strategy for establishing the structure of a natural product involves determining the molecular formula from the high resolution mass spectrum. The functional groups and the number of double bonds are identified from the IR and NMR spectra. Consequently the number of rings can be established. At this stage the class of natural product is often clear. Most 'new' natural products are relatives of known compounds. A number of structural fragments may be apparent from the ^{1}H NMR spectrum. Further connectivities may be established by two-dimensional NMR spectra and the structure is then pieced together. The relative stereochemistry of the natural product may be established by a combination of coupling constant measurements and nuclear Overhauser effect experiments. The absolute stereochemistry may be established by NMR or circular dichroism methods. The power of modern X-ray crystallography is such that, given a suitable crystal, a structure may often be obtained in a matter of hours.

1.3 THE ECOLOGICAL ROLE OF NATURAL PRODUCTS

Many natural products, including those from garden plants such as the foxglove, were originally investigated because of their medicinal, perfumery or culinary value to man. However during the latter part of the twentieth century considerable attention was paid to the ecological role of natural products in mediating interactions between species. The chemistry of individual plants, fungi and insects in the garden cannot be considered in isolation. The chemistry of a plant is the summation of its intrinsic properties and the consequences of its interaction with its environment. A plant will produce a number of natural products in response to environmental challenges. A plant is essentially stationary. When attacked it cannot flee but it must fight. The formation of some natural products is elicited as a response to microbial or herbivore attack. These natural products can have a defensive role and may be toxic to the predator. The production of insect anti-feedants in leaves can be seasonal. Their presence in leaves prior to flowering can protect the plant against predators early in the season. After flowering and later in the year, the anti-feedants may not be present and the leaves are damaged prior to leaf fall and their decay. The damaged tissue provides a route of entry for micro-organisms involved in autumnal decay.

Some plants may exert their dominance of an area by producing natural products which inhibit the germination of seeds and prevent the growth of other seedlings. This competitive effect is known as allelopathy. Relatively few weeds will grow around mint. The constituents of the mint which inhibit the germination of seeds can be smelt by crushing the soil from around the plant.

When a fungus attacks a plant, the fungus may produce phytotoxins which damage cellular mechanisms together with enzymes which digest the plant material. The plant responds to this attack by producing natural anti-fungal agents known as phytoalexins. Attack by eelworms and other insects can also elicit the formation of natural products. Some root exudates are stimulants for the development of mycorrhizal fungi which can play an important part in mobilizing nutrients for the plant. The hatching of species:specific eelworms may also be stimulated by root exudates. Plants may contain insect anti-feedants and other toxic principles which protect the plant against attack by herbivores. In a further utilization of this activity, some butterflies can then sequester these plant toxins and use them for protection against predators such as birds. Other butterflies can overcome and metabolize the anti-feedants produced by their host plant and then, like the cabbage white butterfly, use them as oviposition stimulants in order to lay their eggs in a relatively non-competitive environment. The volatile signals released by plants to affect these aspects of insect behaviour are known as kairomones. In a further tripartite relationship some of the chemicals released by a plant infested with a grub can attract parasitic wasps to the grubs.

Many aspects of insect behaviour are regulated by chemical stimuli. The natural products that are involved are known as semiochemicals. Pheromones are semiochemicals that are involved in infra-species communication. These may involve trail substances, aggregation pheromones, and alarm and defensive substances as well as sex attractants. The effect of many of these in the garden can be easily observed. As we shall see in subsequent chapters, chemical communication and chemical warfare in the garden would put the Conventions on Chemical Warfare and the Health and Safety at Work Acts in the shade.

It is often claimed that vegetables grown under 'organic' conditions taste better than those grown under conventional conditions in which the environmental challenges are kept in check by insecticides, fungicides and herbicides. Some of these differences may be attributed to the formation of defensive substances by the plant in response to environmental challenges. However it is worth bearing in mind that these defensive compounds are produced by the plant to deter predators by their toxicity. Man in this context is a large predator.

1.4 CHANGES IN THE GARDEN

The introduction of a plant into a garden can have an impact on other plants as a consequence of the natural products which it produces. For example the root exudates of lily-of-the-valley will affect the germination and survival of neighbouring plants.

Changes in the global distribution of plants as a result of gardening can have an impact on natural products that is wider than anticipated. Rhododendrons were introduced into this country and have escaped. The toxic natural products produced by their flowers have been collected along with nectar by bees and can now present an occasional problem in honey. The escape of the giant hogweed, originally an ornamental plant (!) and the phototoxic effects of its components on the skin is a further example of an unanticipated gardening problem. Another deep-rooted alien invader which is giving cause for concern is Japanese knotweed (*Fallopia japonica,* syn. *Polygonum cuspidatum*).

The global transport of foodstuffs has a similar impact. For example it is worth remembering that the Colorado beetle was not originally a pest of potatoes but of another member of the Solanaceae. The progression of the pest from its original host plant to potatoes in the mid-west of the USA and thence to the eastern seaboard before coming to Europe has been well documented. Although other members of this family produce anti-feedants that are active against the Colorado beetle, the potato does not naturally produce them and cannot prevent an infestation. The arrival of the harlequin ladybird is a current cause of concern. The environmental perturbations brought about by transport have an impact on the ecological role of natural products.

Climatic change may not only affect the range of plants that can be grown in the garden and the yields of vegetable crops but also the metabolite production of common plants. Compare the evocative smell of a walk through a Mediterranean pine grove with a similar walk in the present day United Kingdom and then consider the impact of the increased volatility of their terpene content as the ambient temperature rises. In this context it is worth remembering that many of the semio-chemicals that affect insect behaviour are substances with a volatility which defines their zone of influence. Climate change may affect not just the plants that can be grown but also the micro-organisms that flourish in the garden. Whilst *Penicillium* species are typical of temperate climates, *Aspergilli* are more often found in warmer situations. Their characteristic metabolites are different and consequently there may be different problems arising from mycotoxin production. Climate change will have an impact on the chemistry of the garden.