Cambridge Texts in Chemistry and Biochemistry

The biochemistry of natural pigments

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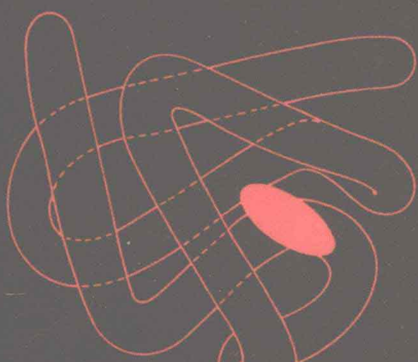

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Preface

As I write these words, the leaves on the trees in my garden are changing from their summer green to the yellow, red and brown of autumn, roses and other flowers are still blooming brightly and wading birds on the shore close by are mostly in their winter plumage. These simple observations provide good examples of the manifestations of colour and pattern in the living world, often in ways that are so familiar to us that we take them for granted. Colour and pattern are important for camouflage, to enable animals to escape the notice of predators, they are important in advertising the presence of an animal to potential mates, etc. and bright colours are important in drawing flowers and fruits to the attention of pollinating and seed-dispersing creatures. In our own everyday world, millions of gardeners grow flowers of many vivid or subtle hues to delight the eye, and brightly coloured fruits are displayed in the shops to attract customers. We should not be surprised, therefore, that the interest of scientists turned very early towards investigating the nature of these plant and animal colours and identifying the underlying mechanisms of colour production and display. It is now well known that there are two fundamentally different mechanisms for natural colour production: the physical or optical phenomena based upon the structures of the cells and tissues and giving rise to structural colours, and the presence of light-absorbing substances, pigments, responsible for the pigmentary colours. This book is concerned with the biochemistry of these natural pigments, the molecules responsible for so much of the colour in the living world. But it is not only for their colour that many of these molecules are important; the property of absorbing visible light renders them useful in many ways, for example in such vital processes as light harvesting in photosynthesis, light detection and colour discrimination in vision, and many other light-mediated responses and regulatory mechanisms. All these topics must be included in a book on natural pigments.

This book is divided into two sections. The first section describes the main features of the chemistry and biochemistry of the main groups of natural pigments; the second section is concerned with biological aspects, dealing with the main functional roles of pigments in Nature. The approach is descriptive and concentrates on the main features and principles. It cannot be comprehensive; this would lead to each chapter expanding into a several-volume series. The aim is rather to give an overall picture, to draw attention to the main points of interest, to stimulate the appetite and send the reader off in search of the key references quoted. I have had to be very selective about which topics went into the book and in how much detail. Readers may not agree with my choice or may think that I have the emphasis and balance wrong, but this is an overview of the subject as I see it. The writing and preparation of this book have been a new challenge, often enjoyable, sometimes frustrating and demanding time and attention that should really have been employed differently. During the preparation, however, I have read much and learned a lot about natural pigments. This has been very rewarding, and I hope that I have been able to pass on to the reader some of the knowledge and understanding gained and some of the great interest that the subject holds for me.

Finally, and with much pleasure, I must acknowledge the great debt of gratitude that I owe to so many people. First I wish to express my thanks publicly for the first time to my parents for their sacrifices and support during the years of my formal education which allowed me to spend these later years happily studying the world of natural pigments. My thanks are due also to Dr E. Haslam and Professor T. W. Goodwin who stimulated and encouraged my interest in the subject, and from whom I have learned so much. I acknowledge the forbearance of members of my research group over the years when I have devoted to the book time and attention which they could justly claim should have been accorded to them. I also wish to thank Dr Ernest Kirkwood, Mrs Marion Jowett and others at Cambridge University Press for their work in converting my typescript into a book.

My greatest debt of gratitude is, of course, to my family, for the many occasions when I have given in to the demands that the writing and preparation of the book made on my time and energy, when perhaps I should have put them first. My wife, Pat, has borne this with perseverance and patience and given me the added encouragement of producing a virtually perfect typescript from my imperfect and sometimes illegible handwriting. My children, Rebecca and Jonathan, have at times been deprived of the companionship and fatherly guidance to which they are entitled and which I should like to have given. It is to them that this book is dedicated, in the hope that they may derive as much pleasure as I from the world of Nature in which colour plays such a large part.

Contents

SECTION I

CHEMICAL AND BIOCHEMICAL ASPECTS

1 Light and colour

1.1 Introduction

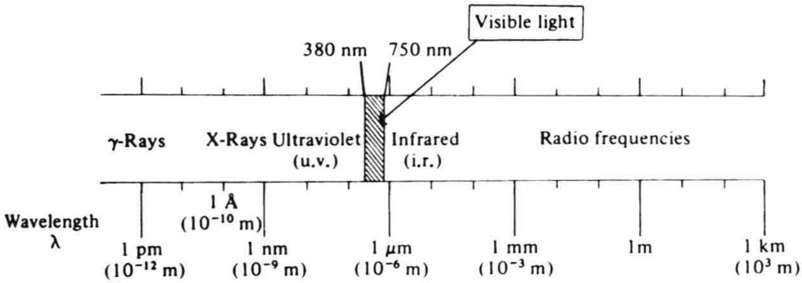
1.1.1 Solar electromagnetic radiation

All living processes on Earth ultimately depend upon that portion of the vast resources of the Sun's energy which eventually reaches the surface of our planet. The Sun emits a wide range of electromagnetic radiations, from long-wavelength infrared (i.r.) and radio frequencies to very short-wavelength ultraviolet (u.v.) and γ-rays (fig. 1.1). However, the Earth's atmosphere as it is today effectively and efficiently filters out much of this radiation, particularly the high-energy u.v., X-rays, and γ-rays that can have a disastrous effect on living tissues.

1.1.2 Visible light

Amongst the radiations that do reach the surface of the Earth, those with wavelengths between approximately 380 and 750 nanometers (nm, $\equiv 10^{-9}$ m) penetrate the atmosphere most readily, *i.e.* suffer least restriction on their passage. This wavelength range, 380–750 nm, is of fundamental importance in maintaining life. It is also the range which we recognise as 'visible light'. Animals, including ourselves, have developed very sophisticated photoreceptor systems for the detection of this light and also for accurate

Fig. 1.1. The electromagnetic spectrum.



discrimination of different wavelengths within this region in the processes of colour vision. Colour, and the property of being coloured, thus become very important in the living world.

It is the same range of light energy which is harnessed by plants and microbes in the process of photosynthesis by which atmospheric carbon dioxide is fixed into a chemical form that is not only used by the plant but also provides the primary food source for the rest of the natural world. Variations in the amount of available visible light, for example variations in length of day and night, are also monitored by various photoreceptors. This provides the basis of extremely important mechanisms for regulating growth and development.

All these properties and processes - being coloured, detecting light and colour, photosynthesis, photoregulation - require mechanisms for detecting or absorbing light from the visible range. Molecules which have the special property of absorbing light of wavelengths in the 380-750 nm range are therefore of fundamental importance. Such compounds are the **natural pigments** or **biochromes**. It is the purpose of this book to review the main features of the chemistry and biochemistry of groups of natural pigments, and to describe, as far as possible, how these pigments function at the molecular level.

1.2 Colour and colour perception

1.2.1 Colour

Simultaneous perception of radiations over the entire range, 380-750 nm, produces (in man) the sensation that we recognise as white light. Other animals are able to perceive radiations of wavelengths outside this range, *e.g.* bees can 'see' u.v. wavelengths invisible to us.

The sensation of colour is given if radiations are received from only part of the visible range. 'White light' is a continuum of electromagnetic radiations covering the wavelength range 380-750 nm. When this continuum is separated by passage (refraction) through a prism, then a series of beams is obtained, each consisting of a much narrower range of wavelengths. We see these beams as a series of colours, the familiar red, orange, yellow, green, blue (indigo), violet of the rainbow, which is produced by prismatic effects of water droplets on sunlight. The sensation of each individual colour is associated with the wavelength on which a beam is centred, *e.g.* the sensation of yellow is produced by light of wavelength around 580 nm. The sensations that individuals with 'normal' colour vision identify with particular wavelengths are shown in fig. 1.2. It is also possible to achieve the sensation of a particular colour by mixing light of wavelengths associated with other colours, *e.g.* yellow can be produced by the addition of red and green light.

Alternatively the sensation of colour may be produced by subtraction of what can be a fairly narrow band (20-30 nm wavelength range) from the white-light continuum. In this case what is 'seen' is the colour complementary to that of the missing waveband. Thus, if white light is passed through a filter or substance which absorbs blue light, i.e. 480 ± 30 nm, the emergent beam is seen as the colour complementary to blue, *i.e.* yellow. The complementary or subtraction colours observed when light of a particular colour or wavelength range is subtracted from the white-light continuum are also shown in fig. 1.2.

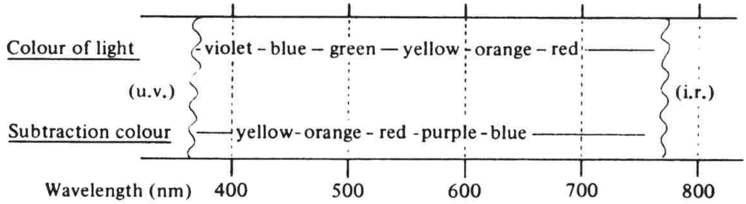
1.2.2 Light perception and colour discrimination

The above brief introduction to light and colour has frequently alluded to our ability to 'see colours'. Although the identification and description of colours is to a considerable degree subjective, there must be an underlying fundamental physiological mechanism which is not only capable of detecting electromagnetic radiations in the wavelength range 380–750 nm, but is also able to produce different sensations in response to radiations of different wavelengths within this range. Plants and microbes do not have vision as we know it, although they are able to use the energy of light of specific wavelengths in, for example, photosynthesis (chapter **10**), and they may have the ability to move or grow towards or away from a light source (chapter **11**). It is only in the animal kingdom that the mechanisms of light detection and colour discrimination have developed into the accurate and sensitive powers of vision that we know and enjoy.

1.2.3 The eye and colour vision

The processes of light detection and colour recognition by animals take place in the eye. In man and many other animals there are specific rod and cone cells in the retina of the eye; these cells contain the photoreceptors, or visual pigments. Mammalian retinal rod cells are responsible for the detection of low-intensity light. They contain pigments, **scotopsins**, which are sensitive to very low levels of light. The sensitivity maximum of the human scotopsin, **rhodopsin**, is at about 520 nm, although light of quite a wide

Fig. 1.2. The visible spectrum, showing the colours which individuals with 'normal' colour vision identify with particular wavelengths and also the complementary or subtraction colours observed when light of a particular colour or wavelength range is subtracted from the white light continuum.



range of wavelengths around this value can be detected. In the visual process, light of the appropriate wavelength is absorbed by the visual pigment. This leads in turn to the generation of an electrical stimulus and a neural impulse. The same response is produced by light of all wavelengths that can be absorbed by the scotopsin, *i.e.* there is no differential response to light of different wavelengths.

The retinal cone cells, on the other hand, contain a set of visual pigments, **photopsins**, that are sensitive to different wavelength ranges. In man there are three such pigments sensitive to blue, green and red light, respectively. These three photoreceptors cover almost the entire range of the visible spectrum and provide a colour-discrimination mechanism sensitive enough to distinguish very subtle variations in colour, shade or hue. This trichromatic system and the pigments involved are described in more detail in chapter 9. Modern colour television also employs a trichromatic system to produce any colour, shade or hue by mixing red, green and blue light.

1.3 Colour in living organisms

When most living organisms or tissues receive white light, *e.g.* from the sun, they pass on to the eye of the observer light of only part of the visible range. In other words they appear to possess colour. This may be **structural colour**, produced as a consequence of the physical nature of the surface of the tissue. Alternatively the colour may be due to the presence of chemical compounds (**pigments** or **biochromes**) which absorb specifically some of the wavelengths of visible light.

1.3.1 Structural colours

In the animal kingdom there are many examples in which the observed colour is the result of optical phenomena such as light scattering, interference or diffraction by microscopic structures present in the tissues. Colours produced in this way are known as structural colours. The subject of structural colours is a large and important one, but detailed descriptions of the characteristics of structural colours and of the optical phenomena that produce them are not really within the scope of this book. Only a very brief account will be given.

1.3.2 Light scattering – Tyndall blue

Very small particles, smaller in diameter than the wavelength of red or yellow light, will reflect or scatter more of the short-wave than of the long-wave components of white light. The most familiar example of this effect is the blue of the sky. Minute particles of dust, *etc.*, in the atmosphere scatter incident white light so that the light reflected to the surface of the Earth contains a greater proportion of short-wave (blue and violet) than of longer-wave (red-yellow) light, and is thus seen as the familiar sky-blue colour. This process is often called Rayleigh or Tyndall scattering, and the colour produced is known as Tyndall blue.

Most non-iridescent blue colours in animals are Tyndall blues. Thus the blue colour of human eyes is due to the scattering of white light by minute protein particles in the iris. In the blue feathers of many birds, *e.g.* blue tit, budgerigar, parrot, light-scattering particles in the form of minute air-filled lamellae are present within the keratin of the feather barbs.

Tyndall blues are identified as structural colours by virtue of the fact that no blue pigment can be isolated from the tissues and because the blue colour is not evident when the tissues are viewed by transmitted white light. They are characterised by a matt, non-iridescent appearance, and by exhibiting the same colour when viewed from almost all angles.

Green colour, especially in feathers, is often due to superimposition of structural blue and a yellow pigment.

1.3.3 Iridescent colours

Among the most striking visual effects produced in Nature are the glittering, iridescent structural colours frequently encountered in the animal kingdom, particularly in birds, insects and fishes. It is a characteristic of iridescent colours that the observed hues change according to the angle of viewing. Two optical phenomena are involved, interference and diffraction.

Interference. The property of interference is perhaps known best from the example of a thin film of oil on the surface of water. Light reflected from the lower surface (oil-water interface) of the film travels a small but finite distance farther than light reflected from the upper, oil–air, surface. When the difference in distance travelled is equivalent to half the wavelength of the light, then the two light rays reflected from the upper and lower surfaces will effectively be 'out of phase' and will cancel each other out. Thus light of this particular wavelength will not be present in the reflected light observed. The reflected beam will therefore appear coloured. With a more acute viewing angle the travelling distance between the upper and lower surfaces is greater. The interference will therefore occur in a different part of the spectrum (longer wavelength) and hence a different colour will be seen.

There are many examples of interference colours in animals. The transparent wing structure of many insects serves as a thin film producing a range of interference colours when seen from different angles. Many butterflies have in the surface of their wing scales laminae with minute air spaces between them. The intralamellar distance is approximately constant so that an almost constant colour may be given over a reasonably wide range of viewing angles.

Interference colours are commonly found in birds, *e.g.* the peacock. The flattened feather barbules that contain the laminar structures which constitute