BIOSYNTHESIS OF NATURAL PRODUCTS

PAOLO MANITTO

BIOSYNTHESIS OF NATURAL PRODUCTS

PAOLO MANITTO
Professor of Organic Chemistry
University of Milan

Translation Editor:
P. G. SAMMES
Professor of Organic Chemistry
University of Leeds

ELLIS HORWOOD LIMITED
Publishers · Chichester
Halsted Press: a division of
JOHN WILEY & SONS
New York · Chichester · Brisbane · Toronto

First published in 1981 by

ELLIS HORWOOD LIMITED
Market Cross House, Cooper Street, Chichester, West Sussex, PO19 1EB, England

The publisher's colophon is reproduced from James Gillison's drawing of the ancient Market Cross, Chichester.

Distributors:

Australia, New Zealand, South-east Asia:
Jacaranda-Wiley Ltd., Jacaranda Press,
JOHN WILEY & SONS INC.,
G.P.O. Box 859, Brisbane, Queensland 40001, Australia

Canada:
JOHN WILEY & SONS CANADA LIMITED
22 Worcester Road, Rexdale, Ontario, Canada.

Europe, Africa:
JOHN WILEY & SONS LIMITED
Baffins Lane, Chichester, West Sussex, England.

North and South America and the rest of the world:
Halsted Press: a division of
JOHN WILEY & SONS
605 Third Avenue, New York, N.Y. 10016, U.S.A.

© 1981 P. Manitto/Ellis Horwood Ltd., Publishers

British Library Cataloguing in Publication Data
Manitto, Paolo
Biosynthesis of natural products.
1. Biosynthesis
2. Natural products
I. Title
574.1'929 HQ345 80–41739
ISBN 0–85312–062–5 (Ellis Horwood Limited, Publishers)
ISBN 0–470–27100–0 (Halsted Press)

Typeset in Press Roman by Ellis Horwood Limited.
Printed in the U.S.A. by Eastern Graphics Inc., Old Saybrook, Connecticut

COPYRIGHT NOTICE – © Ellis Horwood Limited 1980
All Rights Reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the permission of Ellis Horwood Limited, Market Cross House, Cooper Street, Chichester, West Sussex, England.

BIOSYNTHESIS OF NATURAL PRODUCTS

PAOLO MANITTO
Professor of Organic Chemistry
University of Milan

Translation Editor:
P. G. SAMMES
Professor of Organic Chemistry
University of Leeds

ELLIS HORWOOD LIMITED
Publishers · Chichester
Halsted Press: a division of
JOHN WILEY & SONS
New York · Chichester · Brisbane · Toronto

First published in 1981 by

ELLIS HORWOOD LIMITED
Market Cross House, Cooper Street, Chichester, West Sussex, PO19 1EB, England

The publisher's colophon is reproduced from James Gillison's drawing of the ancient Market Cross, Chichester.

Distributors:

Australia, New Zealand, South-east Asia:
Jacaranda-Wiley Ltd., Jacaranda Press,
JOHN WILEY & SONS INC.,
G.P.O. Box 859, Brisbane, Queensland 40001, Australia

Canada:
JOHN WILEY & SONS CANADA LIMITED
22 Worcester Road, Rexdale, Ontario, Canada.

Europe, Africa:
JOHN WILEY & SONS LIMITED
Baffins Lane, Chichester, West Sussex, England.

North and South America and the rest of the world:
Halsted Press: a division of
JOHN WILEY & SONS
605 Third Avenue, New York, N.Y. 10016, U.S.A.

© 1981 P. Manitto/Ellis Horwood Ltd., Publishers

British Library Cataloguing in Publication Data
Manitto, Paolo
Biosynthesis of natural products.
1. Biosynthesis
2. Natural products
I. Title
574.1'929 HQ345 80–41739
ISBN 0–85312–062–5 (Ellis Horwood Limited, Publishers)
ISBN 0–470–27100–0 (Halsted Press)

Typeset in Press Roman by Ellis Horwood Limited.
Printed in the U.S.A. by Eastern Graphics Inc., Old Saybrook, Connecticut

COPYRIGHT NOTICE – © Ellis Horwood Limited 1980
All Rights Reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the permission of Ellis Horwood Limited, Market Cross House, Cooper Street, Chichester, West Sussex, England.

此为试读，需要完整PDF请访问：www.ertongbook.com

Author's Preface

Natural products have always attracted chemists and biologists. Many of them challenge the organic chemist's analytical, synthetic and speculative capacities. The structures of terpenes, alkaloids, polyketides, plant pigments, etc., are extremely varied and often very complex. They show a wide reactivity range and sometimes physiological properties. The organic chemist considers these secondary metabolites as the *par excellence* natural substances.

Since the beginning of this century research chemists have increasingly been pointing out the peculiarity of natural organic substances, that is their formation by living organisms. This 'biosynthesis' can be regarded as their 'fourth dimension'!

The technological development of the last two decades has made the detection of isotopic nuclides (radioactive or not) easier and easier; in addition, isotopically labelled compounds are increasingly more available. As a consequence, biosynthetic studies based on tracers have expanded enormously. The large amount of experimental data so far collected makes it possible to form a picture of the synthetic capabilities of plant and animal organisms. Such a picture, although incomplete and with many speculative points, clearly indicates a biochemical evolution. The secondary metabolites, which reflect the differentiation of the species at a chemical level, appear to originate from general biosynthetic schemes common to all or nearly all species populating our planet.

In my opinion a university course in Chemistry of Natural Products should largely deal with biosynthesis. In fact, most *in vivo* chemical reactions have extremely interesting mechanisms and stereochemistry. The student should be able to recognise the origin of natural compounds from their structures: he should therefore know the fundamental biogenetic principles, the reaction mechanisms most frequently involved in enzyme-catalysed processes, the key roles played by coenzymes, and the most important pathways to secondary metabolites.

It has been my intention to write a textbook covering basic biosynthetic topics and to be used by: (a) chemistry students interested in biological-organic trends, (b) graduate chemists wishing to widen their vision of current biosynthetic

problems and methodologies, and (c) biologists and pharmacologists, with a limited knowledge of organic chemistry, yet interested in chemical aspects of secondary metabolism.

Thus, from my teaching experience and taking into account the different background knowledge of potential readers, I have emphasised some biological concepts in Chapters 1-3 for chemists lacking the essential rudiments of biology and biochemistry, and I have included Appendices 1-4 containing basic notions of reaction mechanisms and stereochemistry to assist biologists.

The text is largely based on my lecture notes used at Milan University since 1970 in the course of Chemistry of Organic Natural Substances for chemistry and biology students. Many paragraphs could certainly have been more detailed, but obvious reasons of brevity have limited them. For the same reasons alkaloid biosynthesis has been omitted. The references given at the end of each Chapter should offer useful sources for the reader wishing to go beyond this book. In order to achieve this aim, the most important texts, reviews and articles of the last fifteen years have been chosen.

I wish to thank Dr. Guido Serra-Errante, who translated the Italian manuscript; Prof. P. G. Sammes, who critically read the English version; Prof. A. Guerritore for his useful biological suggestions; Prof. L. Canonica for stimulating my interest in the field of natural product biosynthesis; and my wife, who helped me in preparing this book with her patience and constant encouragement.

Milan,
December, 1980

Paolo Manitto

Table of Contents

Chapter 1 Primary and Secondary Metabolism

1.1 Introduction 9
1.2 Primary and Secondary Metabolism 10
1.3 The Biogenetic Path 12
1.4 Plant and Animal Organisms 14
1.5 The Cell 19
1.6 Research Methods and Techniques 26
1.6.1 Isotopic Tracer Methods 32
1.6.2 Administration of Labelled Substrates to Living Organisms or Cells 42

Chapter 2 Enzyme Reactions

2.1 Introduction 51
2.2 Protein Structures of Enzymes 51
2.3 The Active Site 54
2.4 Specificity of Enzymes 57
2.5 Cofactors, Coenzymes and Vitamins 61
2.6 Enzyme kinetics 68
2.7 Control of Metabolic Processes 77
2.8 Classification of Enzymes 80
2.9 Enzyme Reaction Mechanisms 81
2.9.1 Oxidations 81
2.9.2 Arene Oxides and the N.I.H. Shift 94
2.9.3 Oxidative Coupling of Phenols 98
2.9.4 Reductions 100
2.9.5 Carbocations and Carbanions 100
2.9.6 Fates of Carbocations 103
2.9.7 Alkylations 105
2.9.8 Decarboxylations and Carboxylations 106
2.9.9 Activation Reactions 110
2.9.10 Reactions dependent upon B_{12} coenzymes 114

Chapter 3 Primary and Intermediate Metabolism
3.1 Chlorophyll Photosynthesis 125
3.1.1 Absorption of Light Quanta. The 'Light' Phase 127
3.1.2 'Dark' Reactions. The Calvin-Bassham Cycle 130
3.2 Glycolysis 139
3.3 The Pentose Phosphate Cycle 141
3.4 The Entner-Doudoroff Pathway 144
3.5 D-Glucuronic and L-Gulonic Acids, Glucosides, Glucuronides and Ascorbic Acid 145
3.6 Fermentation 150
3.7 The Tricarboxylic Acid Cycle (TCA Cycle) 150
3.8 Anaplerotic Processes 158
3.9 Metabolism of Fatty Acids 160
3.10 One-carbon Metabolism 163
3.11 The C_3 Unit 165

Chapter 4 The Polyketides
4.1 Introduction 169
4.2 Addition of C_2 Units 175
4.3 Modifications of Polyketide Chains 179
4.4 Reactions of the Polyketomethylene Chain 183
4.5 Secondary Modifications of Polyketides 186
4.6 Quinones 191
4.6.1 Benzoquinones 192
4.6.2 Naphthoquinones 193
4.6.3 Anthraquinones and Anthrones 195
4.6.4 Other Quinones 199
4.7 Benzophenones and Xanthones 200
4.8 Depsides and Depsidones 202
4.9 Aflatoxins 204
4.10 The Tetracyclines 207
4.11 Macrolide Antibiotics 208

apter 5 The Isoprenoids
5.1 Biosynthesis of the C_5 Unit 215
5.2 Combination of C_5 Units 219
5.3 Monoterpenes 222
5.3.1 Open Chain Monoterpenes 222
5.3.2 Cyclohexane Monoterpenes 225
5.3.3 Iridoids and Seco-Iridoids 228
5.3.4 Irregular, Apparent and False Monoterpenes 233

5.4 Sesquiterpenes 237
5.4.1 Sesquiterpenes from Fungi 250
5.4.2 Bicyclofarnesol Sesquiterpenes. 252
5.4.3 Sesquiterpenes of particular Biological Importance 253
5.5 Diterpenes 255
5.5.1 Monocylic Diterpenes. 256
5.5.2 Bicyclic Diterpenes 257
5.5.3 Tri-, Tetra- and Pentacyclic Diterpenes 260
5.5.4 Diterpenes of fungal origin 262
5.6 Sesterterpenes. 265
5.7 Triterpenes. 266
5.7.1 The Biosynthesis of Squalene and its 2,3-epoxide 270
5.7.2 Oxidative cyclisation of Squalene; Lanosterol and the Amyrins 275
5.7.3 Non-oxidative cyclisations. 286
5.7.4 Cyclisations at both ends of the Squalene molecule 286
5.8 Carotenoids 287
5.9 Polyisoprenes 295
5.10 Meroterpenes and Prenylation Reactions 297

Chap. 6 Biosynthesis of Steroids
6.1 Introduction. 314
6.2 Sterols. 316
6.3 Vitamin D 324
6.4 Transformation of Sterols in plants. 326
6.4.1 Cardiotonic Glycosides. 326
6.4.2 Steroidal Saponins. 330
6.5 Ecdysones 332
6.6 Bile Acids and Alcohols 336
6.7 Steroidal Hormones 338
6.7.1 Cortico-Steroids 340
6.7.2 Gestogens 340
6.7.3 Androgens 340
6.7.4 Estrogens. 343

Chapter 7 The Shikimate Pathway
7.1 Biosynthesis of Chorismic Acid 349
7.2 Protocatechuic and Gallic Acids-Hydrolysable Tannins 349
7.3 *p*-Hydroxy and *p*-Aminobenzoic acids; Salicyclic and Anthranilic acids; Folic acid 355
7.4 Biosynthesis of Tryptophan. 357
7.5 Biosynthesis of Phenylalanine and Tyrosine 359
7.6 Napthoquinones 360

Chapter 8 Phenyl-Propanoids

8.1 Derivatives of Phenylpyruvic Acid 365
8.2 Cinnamic Acids 368
8.3 Coumarins and Neoflavanoids 372
8.4 Transformations of the Side chain of Cinnamic Acids 380
8.4.1 C_6-C_3 Compounds: Allyl and Propenyl Phenols 381
8.4.2 Lignans and Neolignans 384
8.4.3 Lignins 388
8.4.4 C_6-C_2 Compounds, Styrenes 393
8.4.5 C_6-C_1 Compounds, Benzoic Acids 395
8.4.6 C_6 Compounds, Simple polyphenols 397
8.4.7 C_6-C_2-$(C_2)_n$ Compounds, 2-Pyrones and Stilbenes 398
8.5 Flavonoids 400
8.6 Isoflavonoids 424

Appendix 1 Reaction Mechanisms 430
Appendix 2 Principles of Stereochemistry 444
Appendix 3 Non-Classical Carbocations 497
Appendix 4 Isotopic Effects 512
Appendix 5 Key to Numbering and Classification of Enzymes 527
Appendix 6 Arrangements of Order and Families in Spermatophyta and Alphabetical List of Orders and Families 533

Index 539

此为试读,需要完整PDF请访问: www.ertongbook.com

Chapter 1

Primary and Secondary Metabolism

1.1 INTRODUCTION

Even before the end of the Eighteenth Century, organic materials had been isolated from living organisms and their products. The German chemist, Karl Wilhelm Scheele (1742-1786) was particularly distinguished in this art: he was able to extract some simple compounds, including glycerol and oxalic, lactic, tartaric, and citric acids from various organic sources, both vegetable and animal. Friederich W. Sertürner (1783-1841) obtained morphine from opium in 1806 and Pelletier and Caventou isolated strychnine, brucine, quinine, cinchonine, and caffeine in the next fifteen years.

Because of their chemical complexity and important physiological properties, these alkaloids should be considered as the first of the typical, natural organic substances isolated by man as pure compounds. The isolation of many thousands of other compounds from natural sources rapidly followed and continues to this day.

Organic chemistry developed in parallel with the isolation and study of natural substances. Because of their large structural variety, the molecules produced by living organisms are valuable to the organic chemist: he can work on them to widen and deepen his knowledge of organic reactions and, in particular, they can be used to verify certain hypotheses, such as on mechanisms. Some established examples are, steroids – conformational analysis; cyclic terpenes – molecular rearrangements; tropolones – ring chemistry; pigments – electronic absorption spectroscopy, etc.

Besides giving useful substrates for mechanistic and stereochemical studies, living organisms also stimulate the organic chemist in two other ways. On one hand is the challenge to elucidate certain extraordinarily complicated structures (such as that of vitamin B_{12}) and to synthesize them *in vitro*; on the other hand is the unravelling of the secrets of enzymatic reactions (biocatalysis) and the 'building up' processes by which the cells make organic molecules (biosynthesis

or biogenesis)†. The information available from such investigations, together with various theories and hypotheses, form a well-defined and largely autonomous branch of chemistry called the biogenesis of natural products.

The biogenesis of natural products, although connected with traditional organic chemistry and biochemistry, differs from both by its different aims. Organic chemistry mainly deals with structural studies, the physical and chemical properties of compounds, and the synthesis of compounds, natural or otherwise, using *in vitro* methods, but tends to ignore the characteristic peculiarity of natural compounds, that is to say, their mode of formation and their biological role. Biochemistry, on the other hand, tries to answer the most general questions about life on our planet, preferentially dealing with primary metabolism (see later), and neglecting secondary processes such as the formation of alkaloids, terpenes, etc.

In Table 1.1 some fundamental dates in the history of natural products are listed. It illustrates how the greatest progress in biogenetic research has taken place in the last twenty five years. This is principally due to the more extensive use of compounds labelled with either stable or radioactive isotopes. Since 1950 substances containing ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, and ^{18}O have been commercially available and sophisticated instruments for detecting such isotopes have been developed: examples of this are scintillation counters, gas chromatographs coupled to mass spectrometers, nuclear magnetic resonance (n.m.r.) spectrometers with Fourier transform facilities, and so on.

1.2 PRIMARY AND SECONDARY METABOLISM

Polysaccharides, proteins, fats, and nucleic acids are the fundamental building blocks of living matter and are thus considered to be **primary metabolites.** The whole range of processes by which organisms synthesise and demolish these substances, in order to survive, constitute the *primary metabolism processes.* The primary metabolism of all organisms, even those which are genetically very distant, is similar.

Other chemical processes take place only in certain species or else give different products according to the type of species. Such reactions do not appear to be essential for the existence of the organism and hence are called *secondary metabolic processes.* Products from secondary metabolism tend to coincide with the traditional natural products of the organic chemist, such as terpenes, alkaloids, pigments. Although not essential for the existence of the

† The terms **biosynthesis** and **biogenesis** both mean the formation of natural substances by living organisms. Although they are often used synonymously, there is a slight difference in their meanings; the first one refers to the acquisition of experimental data, whilst the second one emphasises the speculative aspect of certain facts. Current practice further restricts the word biosynthesis to the elaboration of natural molecules from *less* complex structures by endoergonic reactions. Such reactions are typical of anabolic processes in secondary metabolism.

individual, secondary metabolites often play a key role in the survival of the species over others. Included here are the defence chemicals, sex attractants, and the pheromones. The reason why many secondary products are produced remains a mystery. Some authors believe they are detoxification products of poisonous or overabundant metabolites, which cannot be otherwise eliminated by the organism. This explanation would account for the greater production of secondary metabolites in plants rather than in animals. Whilst animals have sophisticated processes for removing secondary products through their liver and kidneys, plants are obliged to transform their unwanted materials into compounds which can be stored in the cellular vacuoles, in cell walls, etc. Some scientists consider such metabolites to be a store of energy and food in plants, which can be utilised in times of necessity. Whatever the purpose of secondary metabolites, a lot is known about their chemistry and how they are formed.

Table 1.1 Some important dates in the study of natural products

Isolation of some important natural products		
1806	morphine	Sertürner
1818	strychnine	Pelletier and Caventou
1845	camphor	Bouchardat
1859	cocaine	Niemann
1864	bilirubin	Städeler
1909	cholesterol	Windaus
1934	progesterone	Butenandt
1938	penicillin	Florey and Chain
1948	aureomycin	Lederle Laboratories
Structure elucidation and synthesis		
1932	cholesterol	Wieland
1946	strychnine	Robinson
1954	strychnine synthesis	Woodward
1955	vitamin B_{12}	Hodgkin
1959	colchicine synthesis	Eschenmoser
1960	chlorophyll synthesis	Woodward
1968	β-amyrin synthesis	Barton
1971	erythronolide B	Corey
Biogenetic researches		
1953	acetate hypothesis	Birch
1955	shikimic acid pathway	Davis
1956	mevalonic acid discovered	Merck Laboratories
1959	isoprene rule	Ruzicka
1964	oxidative coupling	Barton

Intermediate metabolism usually indicates certain reactions which allow exchange of material between different metabolic pools and producing energy required by both the single cells and the whole organism. Intermediate metabolism corresponds, approximately, to the central area of Fig. 1.1 and usually involves small molecules (acetic acid, etc). These molecules are partly burnt to CO_2 and H_2O with production of energy. Because of its vital importance the intermediate metabolism is generally discussed with primary metabolism.

Chapters 4, 5, 6, 7, and 8 deal with chemical aspects of secondary metabolism: Chapter 2 surveys enzymic aspects of metabolism, whilst Chapter 3 describes some of the more important primary metabolic processes. A good knowledge of primary metabolism is essential for an understanding of the biogenesis of natural products, since a close connection exists between the various chemical processes occurring inside a cell. In fact, the distinction between primary and secondary metabolism is merely artificial; the concept is a useful simplification of the complex phenomena which occur *in vivo* (Fig. 1.1).

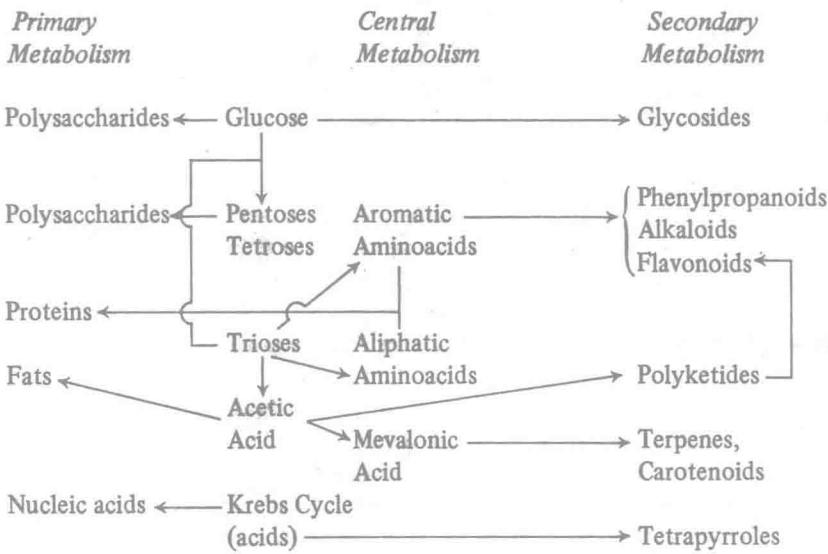


Fig. 1.1 Relationships of primary and secondary metabolism.

1.3 THE BIOGENETIC PATH

The *biogenetic path* is the sequence of reactions through which an organism synthesises a substance X starting with a molecule A. The products B, C, D . . . , into which A is transformed during this progression towards X are called *natural*

intermediates and usually increase in chemical complexity during the biosynthetic process (Fig. 1.2(a)). Substance X can, in turn, be changed to another compound, Y, thus becoming an intermediate of the latter product.

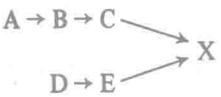
A *natural product* is not always the result of one unique biogenetic path. It can well occur at the end of several pathways and, under these circumstance, one of the convergent paths usually predominates over others (Fig. 1.2(b)). The natural intermediates of a unique or overwhelmingly predominant pathway are called *obligatory*; blocking the synthesis of such intermediates causes an almost total interruption of formation of the final compound.

A *biogenetic pathway* can be ramified at one or several points (Fig. 1.2(c)). The combination of convergent and ramified paths leads to a *metabolic grid* (Fig. 1.2(d)). Quite frequently, a substance is formed and used at about the same rate and thus its concentration in the organism is virtually constant. A stationary state then exists (an equilibrium between anabolic and catabolic phases): the amount of substance either formed or transformed per unit of time is called the *turnover rate*.

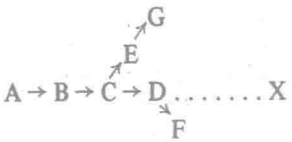
(a) Linear Process

$$A \rightarrow B \rightarrow C \rightarrow D \ldots\ldots X \rightarrow Y$$

(b) Convergent Process



(c) Ramified Process



(d) Metabolic Grid

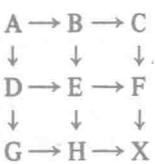


Fig. 1.2 Various types of metabolic pathways.

1.4 PLANT AND ANIMAL ORGANISMS

The chemist studying natural products depends heavily on the type of living organism chosen for his biogenetic research. It is important in such studies to understand the essential biological and taxonomic features of the selected organism. It is also important to collaborate with experts from other scientific disciplines such as biology, pharmacology, microbiology and so on, in order to maximise the value of such studies.

Living organisms are traditionally divided into the two large *animal* and *plant* kingdoms. The universal unit of organisation is the *cell*. On the basis of fundamental structural, cellular features living organisms are divided into:

(i) *Procaryotes*, in which the genetic material (DNA) is *not* enclosed in a well-defined nucleus;

(ii) *Eucaryotes*, which contain a membrane-surrounded nucleus and various cytoplasmic organelles, each delineated by membranes (mitochondria, chloroplasts, etc).

Despite their ability to reproduce, *viruses* are not included by most biologists amongst living organisms. These nucleoprotein particles are devoid of any metabolic processes of their own and can only multiply within living cells.

Animals are typical encaryotic organisms and they utilise the oxidation of organic substances as the only source of chemical and physical energy. The animal kingdom has ten major *phyla*: Protozoa (protozoans), Porifera (sponges), Coelenterata (coelenterates), Aschelminthes (aschelminthes), Platyhelminths (flatworms), Echinodermata (echinoderms), Mollusca (molluscs), Anellida (anellid worms), Arthropoda (arthropods), and Chordata (chordates). The plant kingdom, which includes all other forms of life is more heterogeneous and is divided into *divisions*. The four main groups in the plant kingdom are:

1. **The Schizophytes** (procaryotes), which are further subdivided into the Cyanophytes (blue algae) and Bacteriophytes (bacteria).
2. **The Algae** (other than the Cyanophytes). These are eucaryotes and are divided into the Phytoflagellates, Chlorophytes (green algae), Phaeophytes (brown algae), and Rhodophytes (red algae).
3. **Fungi and Lichens** (eucaryotes).
4. **Euphytes** (eucaryotes), which includes all the common green plants.

These four divisions need to be discussed in more detail, as follows.

Schizophytes (from the Greek words $\Sigma\chi\iota\zeta\omega$ = ro divide, and $\phi\upsilon\tau o\nu$ = plant) are inferior vegetables, usually unicellular, which reproduces by cell division. In some cases the formation of *spores* or *cysts* is noted. These are durable cells with a thick membrane and dense protoplasm. The pigmentation of the Schizophytes is closely connected with their metabolic activity. Colourless organisms, and the nearly yellow and red pigmented cells, for example bacteria, are *etherotrophic* (they use organic matter for energy and food). They can be classified as follows:

此为试读,需要完整PDF请访问: www.ertongbook.com