Introduction to Ecological Biochemistry

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PREFACE

The last decade has witnessed the growth of a new interdisciplinary subject, variously termed ecological biochemistry, chemical ecology or phytochemical ecology, which is concerned with the biochemistry of plant and animal interactions. Its development has been due in no small measure to the increasingly successful identifications of organic molecules in microquantities, following the application of modern chemical techniques to biological systems. It has also been due to the awareness of ecologists that chemical substances—and particularly secondary metabolites such as alkaloids, flavonoids and terpenoids—have a significant role in the complex interactions occurring between animal and animal, animal and plant or plant and plant in the natural environment. A further stimulation has been the possible applications of such new information in the control of insect pests and of microbial diseases in crop plants and in the conservation of natural communities. The present text is intended as an introduction to these new developments in biochemistry that have so enormously expanded our knowledge of plant and animal ecology.

Much has been written in symposia proceedings on plant-animal interactions and some of the present material has been adapted from such works. Two publications which have been drawn on extensively are "Chemical Ecology" (1972) edited by the late E. Sondheimer and J. B. Simeone and "Phytochemical Ecology" (1972) edited by the author. Two other valuable books which should be consulted by all interested in the topics discussed here are "Insect-Plant Relationships" (1973) edited by H. F. van Emden and "Co-evolution of Animals and Plants" (1975) edited by L. E. Gilbert and P. H. Raven. The choice of selection of examples to illustrate various interactions has been difficult and many interesting studies have had to be omitted for reasons of space. Other topics such as the biochemistry of plant parasitism have been omitted because present knowledge is still fragmentary. However, no doubt, a more coherent story will be available in this and other areas of present research by the time a new edition may be called for.

The present text is based on a course taught by the author over a number of years. It has been planned so that it is suitable for second or third year University teaching in departments of botany, biochemistry and biological sciences. Because of its interdisciplinary content, it should be an especially useful course in those Universities which offer combined Honours degrees in Botany and Zoology. It is also hoped that the book will be of more general value as a simple introduction to a new subject area.

The author is grateful to Dr Miriam Rothschild for her introductory Foreword. By her own pioneering experiments with aposematic insects and equally her encouragement of other scientists, Dr Rothschild has contributed more than anyone else to this new subject and this book owes much to her example. The author is also particularly indebted to Professor Tony Swain for his many valuable comments on the first draft of this book. Other colleagues have helped in many matters, including Drs Roy Snaydon and Christine Williams and Mrs Audrey Wooldridge. Finally, he is grateful to the staff of Academic Press for their interest and encouragement and for organizing such rapid publication.

July, 1977

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FOREWORD

Science, says François Jacob, attempts to confront the possible with the actual, and by so doing must inevitably renounce a unified world concept. Jeffrey Harborne puts this to rights in one corner of the biological and biochemical fields, and that is perhaps the main reason why one is so wholeheartedly delighted with his book. We have all been waiting impatiently for a synthesis fusing plant/insect relationships with their ecological biochemistry, but in addition to the welcome unification of the underlying theme, the subject matter is presented with felicitous directness and simplicity. We are given a masterly *überblick*, so that with a sigh of appreciation and relief we know for the first time not only where we are, but also the lines along which we should now proceed. Although the complicated and tangled subject matter is presented in a straightforward fashion, with perspicacious sifting and appraisal of the evidence, without frills and in a logical and scientific manner, there is still a romantic undertone which reveals the author as a fine and enthusiastic naturalist as well as a laboratory investigator.

Curiously enough, one of the first attempts to link the fields of entomology and plant chemistry in the modern sense was initiated by field naturalists (British Empire builders in fact)—their imagination fired by the evolutionary implications of the theory of warning coloration and mimicry among butterflies. It was their infectious enthusiasm and ebullient writing, now often dismissed as "anecdotal", which first encouraged me to investigate the chemistry of Lepidoptera/plant relationships. The observations of C. F. M. Swynnerton were particularly enthralling and it was tragic that the vast piles of notes he had accumulated by virtue of his drive and tireless energy, could not be satisfactorily decoded when he died suddenly in the prime of life. In 1915 he published an account of feeding experiments (in Africa) with butterflies as prey and woodhoopoes, hornbills and babblers as predators, which showed without doubt that Danaids, unlike their mimics, were toxic species. He also demonstrated unequivocally that taste and odour, as well as the chemical ingredients producing emesis in his captive birds, were deterrent qualities possessed by these butterflies. His description of the manner in which a parent bird, who had swallowed the nauseating insect, tried to dissuade its young from eating it was wholly delightful and telling. We are awaiting further research into bird vision and behaviour in this field.

Simultaneously, E. B. Poulton, who encouraged Swynnerton in his experimentation and astute observations, and had an encyclopaedic knowledge of the field, came to the conclusion after evaluating the theories of Haase, Wallace, Müller, Meldola and Slater that these authors' speculations were justified: aposematic butterflies and moths, the larvae of which feed on poisonous plants, can derive protective toxins from their foliage and serve as models for innocuous species. He began to agitate for chemical proof of these theories. It was necessary, perhaps, that such proof should be provided by a combined biochemist and botanist, but no such individual materialized until 50 years later when T. Reichstein, whose knowledge of the chemistry of Asclepiad and Aristolochic plants was enormous, turned his attention to the Danaids and Papilios which fed upon them. In the meantime, however, Jane van Z. Brower had made a great step forward, with an impeccable series of laboratory experiments proving that mimicry really worked. Further important observations of hers (later emphasized and elaborated in a series of joint papers with Lincoln Brower) were that mimics as well as models are somewhat distasteful to predators, and that the disagreeable experience produced by the plant toxins stored by a butterfly model leave a lasting impression on the predators. However it was T. Reichstein's matchless and trustworthy chemistry which suddenly floodlit the scene and linked the herbivores and their host plants in a biochemical synthesis.

In a sense this scene had already been set by Gottfried Frankel's intuitive interpretation of the protective role of secondary plant substances, which in turn inspired Ehrlich and Raven to produce their memorable paper on the co-evolution of plants and butterflies. The field naturalists who provided the basic observations which made their synthesis possible, in particular Sevastopulo, van Someren and Carcasson, must not be forgotten. It is a curious facet of modern science that these authors actually experienced difficulty in publishing their invaluable lists of food-plant records. Also all of those interested in Danaid ecological chemistry were fortunate in having to hand the knowledge accumulated and published by F. A. Urquhart on the general biology, life-style and migrating habits of the Monarch.

We are now entering on a period when undreamed of subtle adjustments between plant and insect become apparent—thus the ovipositing female Monarch selects for preference those species of *Asclepias* as food plants, from which the larvae can best assimilate and store the toxic secondary plant substances; the mimics of certain Danaids not only resemble the models in appearance but secrete chemicals which mimic the cardioactivity of cardenolides.

Reichstein's identification of ten cardiac glycosides in both *D. plexippus* and its food plant seemed to be the spark which ignited a small conflagration, for up to 1966 there was really relatively little interest in the co-evolution of plant and insect biochemistry. Such an explosion of interest—*embarras de richesse*—leaves the scene in some sort of confusion, and we can now be grateful to Jeffrey Harborne for drawing the scattered pieces of the jigsaw together, thereby achieving a synthesis, richly flavoured with his own original ideas and lucid interpretations.

One of the most stimulating aspects of this book is the many doors he deliberately leaves ajar. It is all too easy when reviewing an intricate field to give a student new to the area the feeling that everything is now known about the subject. This book has exactly the reverse effect on the reader: a dozen new ideas spring to mind at the end of each chapter. Although grateful for an integrated and unified survey, particularly because of its intricacies and scattered literature, one is stimulated to take the next step forward and push hard against the nearest door.

Ashton Wold
Peterborough, England
July, 1977

MIRIAM ROTHSCHILD

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3. Plant Toxins and Their Effects on Animals

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1 The Plant and Its Biochemical Adaptation to the Environment

I. INTRODUCTION

The marriage between such diverse disciplines as ecology and biochemistry may seem at first a curious alliance. Ecology is largely observational, is concerned with interactions between living organisms in their natural habitats and is carried out in the field. By contrast, biochemistry is experimental, is concerned with interactions at the molecular level and is carried out at the laboratory bench. Nevertheless, these two distinctive disciplines have cross-fertilized in recent years with astonishing success and a whole new area of scientific endeavour has opened up as a result. Ecological biochemistry is only one of a variety of phrases that have been employed to describe these exciting developments.

To the ecologist, knowledge of biochemistry has illuminated to a remarkable degree the complex interactions and co-evolutionary adaptations that occur between plant and plant, plant and animal and animal and animal. It has led to the realization, for example, that plants are functionally interdependent with respect to their animal herbivores and form what are termed "plant defense guilds" (Atsatt and O'Dowd, 1976). Similarly, to the biochemist, studies in ecology have provided for the first time a rational and satisfying explanation for at least a part of the enormous proliferation of secondary metabolism that is observed in plants. Much of the purpose of the synthesis of complex molecules of terpenoids, alkaloids and phenolics lies in their use as defense agents in the plant's fight for survival against animal depredation.

The aim, therefore, of the present text is to provide an account for the student reader of the explosive development in ecological biochemistry that has occurred in the last decade. The various chapters deal in turn with the plant and its interactions with animals and with other plants, while animal–animal interactions are considered in some detail in Chapter 7. It should be emphasized at this point that the biochemistry of many interactions has been deliberately simplified here in order to present a coherent story. It must be recognized that a given interaction between a plant host species and its animal predator species can be very subtle and complex and certain aspects of such an interaction may require many years of study before all is revealed.

The term plant is generally used throughout this book to refer to higher plants and mainly to angiosperms, gymnosperms and ferns. Fungi, bacteria and viruses will usually be referred to as micro-organisms; other groups of plants will rarely be mentioned—i.e. algae, mosses and liverworts—largely because their ecological biochemistry has not yet been studied in much detail.

The selection of animals mentioned in this text is restricted to those taxa that have been studied experimentally and is certainly very unrepresentative of the Animal Kingdom as a whole. This is because plant–animal interactions in terms of feeding and defense have largely centred on the insect kingdom and only more recently have biochemical aspects of mammalian ecology been explored to any extent.

The emphasis here on the plant is due, at least in part, to the fact that plants are richer than animals in their biochemical diversity. Although secondary metabolism occurs in animals (Luckner, 1972), nevertheless, over four-fifths of all presently known natural products are of plant origin (Robinson, 1975; Swain, 1974). Some idea of the range of secondary compounds found in plants can be obtained from Table 1.1, which lists some of the major classes, together with an indication of numbers of known compounds, distribution patterns and biological activities. Many of these substances will be mentioned in more detail in subsequent chapters. The richness in secondary chemistry in plants is at least partly explicable in the simple fact that plants are rooted in the soil and cannot move; they cannot respond to the environment in ways open to animals.

Table 1.1 Major classes of secondary plant compounds involved in plant–animal interactions

Class	Approx. number of structures	Distribution	Physiological activity
NITROGEN COMPOUNDS			
Alkaloids	5,500	Widely in angiosperms, especially in root, leaf and fruit	Many toxic and bitter-tasting
Amines	100	Widely in angiosperms, often in flowers	Many repellent smelling; some hallucinogenic
Amino acids (non-protein)	400	Especially in seeds of legumes but relatively widespread	Many toxic
Cyanogenic glycosides	30	Sporadic, especially in fruit and leaf	Poisonous (as HCN)
Glucosinolates	75	Cruciferae and ten other families	Acrid and bitter (as isothiocyanates)
TERPENOIDS			
Monoterpenes	1,000	Widely, in essential oils	Pleasant smells
Sesquiterpene lactones	600	Mainly in Compositae, but increasingly in other angiosperms	Some bitter and toxic, also allergenic
Diterpenoids	1,000	Widely, especially in latex and plant resins	Some toxic
Saponins	500	In over 70 plant families	Haemolyse blood cells
Limonoids	100	Mainly in Rutaceae, Meliaceae and Simaroubaceae	Bitter tasting
Cucurbaticins	50	Mainly in Cucurbitaceae	Bitter tasting and toxic
Cardenolides	150	Especially common in Apocynaceae, Asclepiadaceae and Scrophulariaceae	Toxic and bitter
Carotenoids	350	Universal in leaf, often in flower and fruit	Coloured
PHENOLICS			
Simple phenols	200	Universal in leaf, often in other tissues as well	Anti-microbial
Flavonoids	1,000	Universal in angiosperms, gymnosperms and ferns	Often coloured
Quinones	500	Widely, especially Rhamnaceae	Coloured
OTHER			
Polyacetylenes	650	Mainly in Compositae and Umbelliferae	Some toxic

In this first chapter, attention is focused on biochemical adaptation. In its widest sense, this topic continues in later chapters, but here, it is taken in the narrower sense as adaptation to the physical environment. Attention is deliberately restricted to the plant kingdom, where information on biochemical adaptation is only of recent origin. Much is known about biochemical adaptation in animals and the subject is well documented in textbooks on comparative biochemistry (e.g. Baldwin, 1937; Florkin and Mason, 1960–1964). A useful reference to recent developments in the subject of biochemical adaptation of animals to environmental change is that of Smellie and Pennock (1976).

Adaptation represents the flexibility of a living organism to fit into a changing environment, at the same time improving its chances of survival and ultimately of reproducing itself. The extensive diversity of life forms on this Planet (i.e. several million species) and their presence in every type of habitat are witness to the view that living organisms indeed are morphologically and anatomically adapted to their environments. Such ideas are fundamental to the Darwinian view of nature and have been supported by much experimentation during the last century. Ideas of physiological and biochemical adaptation came later, during the 1920s and 1930s, with the experimental development of these two subjects. It is only, however, very recently that biochemical aspects have been developed sufficiently with plants to warrant their separate consideration, as in this present chapter.

Adaptation is generally considered as occurring on an extensive time-scale, involving many generations, but it can also take place during the lifetime of an individual, when it is sometimes termed acclimatization. The term adaptation is used here largely in the evolutionary sense. Biochemical adaptation is particularly closely connected to physiological adaptation and indeed it is sometimes difficult to distinguish the two. Physiological adaptation in plants will be considered here where appropriate; for a comprehensive account, see Levitt (1972).

Biochemical adaptation can operate at different levels in metabolism. It may affect the enzymes and produce amino acid substitutions in the primary sequence of protein or else alter the balance of isozymes. It may affect intermediary metabolism; an example in the case of the carbon pathway in photosynthesis is mentioned later. Finally, it may affect secondary metabolism; this is especially true of the plant's adaptation to animal feeding.

The environmental factors that plants are subject to can be broadly divided into five types:

(1) *Climatic factors*. These include temperature, light intensity, daylength, moisture and seasonal effects.
(2) *Edaphic factors*. All plants, except epiphytes and parasites, obtain their mineral nutrition through the soil. The soil is also the source of symbiotic

microbes, e.g. those required by legumes and other nitrogen-fixing plant species. Through contact with the soil, plants may have to cope with toxic heavy metals or with excess salinity. Equally, they may be subject to biochemical stress due to mineral deficiency in the soil.

(3) *Unnatural pollutants*. These are distributed through the upper atmosphere (ozone, industrial gases, gasoline fumes) or through the environment (organic pesticides) and may be potentially toxic to many plants.

(4) *Animals*. Although there is an element of symbiosis in animal feeding, herbivores are primarily hostile to plants, since they depend on them for their very existence. Many different defense adaptations are known in plants. An element of symbiosis is also present in the case of those animals which visit plants for the purpose of pollination (see Chapter 2).

(5) *Competition from other plants*. This can be either competition between different higher plants or between different forms of plant life, e.g. higher plants and micro-organisms.

In this chapter, we are concerned only with the first three factors: the climate, the soil, and unnatural pollutants. Biochemical adaptation to animal predation and biochemical interactions between plants will be the subject matter of later chapters in this book.

II. THE BIOCHEMICAL BASES OF ADAPTATION TO CLIMATE

A. General

Anatomical and morphological adaptation of plants to different climatic factors is well known and indeed its study is a major part of the science of plant ecology. Everyone is familiar with the ways that desert cacti and succulents are adapted to their parched habitats and are able to reduce moisture loss under the scorching desert sun. This is done by extending the area of soil for water uptake, by reduction of water loss through the leaf or by increased water storage in the tissues. Situations where biochemical features are involved in climatic adaptation are less often considered or discussed. Nevertheless, there is a growing awareness of the need to explore biochemical aspects, from the practical viewpoint.

In recent years, there has been much study of the hormonal control, through the sesquiterpenoid abscisic acid, of moisture loss from plants by stomatal closure. There is practical incentive here in the need to develop drought-resistant crop varieties for growing in marginal desert areas of the world. In Israel, for example, plant scientists are working on the development of agricultural crops which will grow successfully in areas of the Negev desert. Conversely, plants may have to adapt to excess moisture and something is now known of the adaptation of intermediary metabolism to the flooding of plant