PHYTOCHEMISTRY

THE PROCESS AND PRODUCTS OF PHOTOSYNTHESIS

VOLUME I

LAWRENCE P. MILLER, Editor
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Preface

Advances in plant chemistry are progressing at such a rapid pace that it is impossible for a worker in one phase of the subject to keep up with discoveries being made in fields other than his own. It is the objective of this treatise to bring together in an authoritative way the current status in all important aspects of phytochemistry. To do this, the editor has been successful in receiving the cooperation of leaders in their respective disciplines from all over the world. It is hoped that the treatise will be of value to research workers and graduate students.

In a work of this magnitude, with many authors (38) involved, there have naturally been some unavoidable delays in completing the manuscript for submission to the publisher. The editor is grateful to the authors who have been patient in accepting these delays, especially when caused by others.

The editor is indebted to Dr. George L. McNew, Managing Director of the Boyce Thompson Institute for Plant Research, who has been most cooperative in encouraging this endeavor. He has provided desk space for Mrs. Miller and myself after our official retirement from the Institute, has supplied stenographic help, and has assumed the financial responsibility for some of the typing that could not be done at the Institute. He has also critically reviewed the sections written by the editor and had the Institute Librarian, Miss Joan DeFato, check the literature citations in the editor's chapters. The editor's sections have also been reviewed by the Publications Committee of the Boyce Thompson Institute, especially Drs. Dewayne Torgeson, Alva A. App, Herman Gershon, Richard C. Staples, Mrs. Bettie Brooks, and Miss Joan DeFato. Their suggestions have been valuable.

The cooperation of many different libraries in the literature studies is gratefully acknowledged. First and foremost is the library of the Boyce Thomson Institute. In addition to checking the literature citations, mentioned above, the Librarian has been very helpful in obtaining interlibrary loans.

Other libraries used include those of the Rockefeller University, The Chemists Club, the Library of the Pharmacy Department of Columbia University, The New York Botanical Garden, The New York Academy of Medicine, the Library of the University of Florida, the Indianapolis Public Library, and the Library of Congress.

The editor is grateful to Dr. R. C. Jack who, in addition to his contribution of the Chapter on Lipids in Volume II, furnished advice and suggestions. The editor and publisher have also benefited from the comments of a reviewer who read the entire treatise and made many suggestions. Special thanks are due to Miss E. Jeanne Ross who typed most of the manuscript of the editor's sections. Not only did she not make any mistakes in her copying, but she also spotted errors in the copy submitted to her. The editor is indebted to Mr. George Narita, Editor, and Mrs. Alberta W. Gordon, Managing Editor, Professional and Reference Book Division, Van Nostrand Reinhold Company, for the excellent cooperation and help received, and to Mrs. Kathryn W. Torgeson for preparation of the subject index.

It is impossible to acknowledge in appropriate language the help given by my wife, Florence Flemion Miller, herself also a trained scientist. She has given me constant assistance throughout, with the literature and library work, with some of the writing and indexing, and in keeping me devoted to the project. In view of my interest in many different activities and my tendency to stray from one to the other, without her this treatise would have been delayed further and would perhaps not have appeared at all.

LAWRENCE P. MILLER

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INORGANIC ELEMENTS AND SPECIAL GROUPS OF COMPOUNDS

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Plants as Organic Laboratories

LAWRENCE P. MILLER

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Green plants are essential for all animal life on earth since they convert solar energy into organic carbon compounds which in turn are used to produce essential foods. Should the photosynthetic activity of green plants disappear from the earth, so would all animal life. Starting with water, CO_2, and mineral elements, plants have the remarkable capacity of elaborating carbohydrates, proteins, fats, and vitamins. Animals feeding on plants may form additional substances desirable for man's nutrition. In addition to the nutrients mentioned, all green plants also contain growth regulants and the necessary photosynthetic apparatus.

The formation of the substances mentioned above, which may be considered the primary activity of green plants, is by no means the most interesting of plant activities, since they also produce, as is well known, myriads of other products, many of which are used by man. These have been in use as drugs, as stimulants, as dyes, and for many other purposes since prehistoric times. In spite of the many new synthetic drugs available, some of our most valuable medicinal agents remain the ones discovered by primitive man.

Since these special products do not occur in all plants, they were largely ignored until several decades ago by those interested in chemical plant physiology. They have been called secondary products, for want of a better name, and have been considered as waste products of the metabolic processes taking place in plants. These compounds occur in great numbers throughout the plant kingdom. They often are the most conspicuous of the constituents of a particular species. Many of these compounds have defied the efforts of the plant chemists and biochemists to determine their structure and to duplicate them in the laboratory. The coming decade or two should prove most interesting in the developments along these lines.

Just as each of the billions of individual human beings that exists now or has existed in the past, has or has had a different set of finger prints, a characteristic mode of walking, and an individual chemistry which makes organ transplantation difficult, so has the chemistry of plants been very versatile. Thus the plant chemist faces many challenges in attempting to unravel this complicated chemistry.

The question immediately arises, why do certain species elaborate special chemicals, while others function well without them. As it became apparent to plant scientists that many of these products are tied in with the ordinary metabolic processes of the green plant, the organic chemists and the chemical plant physiologists joined forces in studies of these compounds. Examination of the secondary products has been stimulated greatly by improved methods of isolation and identification. With the use of chromatography and other modern methods and equipment, the number of new compounds isolated from plants totals about 1000 annually.

Other reasons for the greater interest and progress in knowledge of the secondary compounds include: (1) the search for chemicals from natural sources as curative agents, especially for cancer; (2) increased interest in the chemical compounds in plants from the viewpoint of pure science; (3) studies on biogenesis of essential plant constituents have begun to involve secondary constituents; and (4) interest in the use of chemical constituents as an aid to plant taxonomy, the so-called chemical taxonomy.

In spite of the great progress in the identification of the secondary compounds, the enigma of their existence continues. Problems associated with secondary compounds may be stated as follows:

1. How does a compound come into existence in an individual cell? (A biochemical, physiological, and genetic question.)

2. How did the compound come to be present in the particular species? (An evolutionary, and hence a genetic question.)

3. How does the plant control the time and place of synthesis, rate of synthesis, and final quantity of a particular compound?

4. The *raison d'être* of the secondary compounds, a basic biological question which has not received much profound analysis.

As inferred, the question as to how the sequence and time of the complicated reactions that take place are controlled has remained completely unexplained and has received scant attention in the literature. Individual cells may contain from 500–1000 different enzymes (Mitchison, 1969). Cells also have the capability of forming enzymes in response to certain conditions or the addition of compounds.

Consideration of the secondary compounds becomes more complicated as methods of detection become more and more effective. For inorganic ions, current methods (use of activation analysis) are so sensitive that it is possible to detect every known ion in every solution. Methods for the detection of organic compounds are not so sensitive, but one may suspect that every known compound is ubiquitous in its occurrence when sufficiently sensitive methods are available and may be detected everywhere. An example may be cited as to the ease with which crystallization occurs with hard to crystallize preparations once such compounds have been crystallized. Crystallization of other preparations of the same compounds often depends on the use of seed crystals which, however, in many instances need not be added directly by the experimenter. It has been reported that one worker who had obtained a syrup of a carbohydrate derivative, knowing that scientists in Washington had obtained this particular preparation in crystalline form, placed his beaker outside on a window sill facing Washington and had crystals the next morning. In another instance, a worker sent to Washington for seed crystals, and it turned out that his syrup crystallized a few days later. On investigation he found that the crystallization took place when the shipment arrived at the railroad station. Whether or not these stories are literally true, the fact remains that as methods become more sensitive, compounds of interest to the plant chemist will be found to be much more widely distributed than previously supposed.

The chemistry of plants is about as varied as the great variety of forms in which plants occur. The number of species of plants, based on morphological differences, is estimated to be in excess of 335,000 (Jones, 1941). Morphological differences which usually involve chemical differences also, may be illustrated by the range of variation in the size of seeds. Seeds vary in size from 4.9 micrograms per seed for one of the orchids, to 40–50 lbs for the seed of the double coconut palm. The small seed of the orchid does not have enough chemical components to assure the development of a seedling, and requires an association with a specific root fungus and nutrients supplied by it for the establishment of a plant, whereas the coconut palm seed contains enough nutrients to support a seedling for some years.

Not only do special compounds occur in a great variety of structures, they are often found in remarkably high concentrations, sometimes in great purity, and in some instances, a large number are found in a single species. About 3000 alkaloids and 3000 glycosides have been isolated from plants, and new ones are being reported daily. In addition to the amino acids essential for the necessary proteins, over 200 special amino acids are known. Similarly, over 100 nonessential sugars have been isolated. In general, the individual essential oils (about 9000 are known) contain very many ingredients (Hocking, 1969). In synthetic preparations, the main constituent is usually duplicated. However, these artificial essential oils do not equal the natural products in quality; for example, synthetic vanillin is much inferior to the vanilla bean, which contains many other ingredients in addition to vanillin, as a flavoring agent.

Many examples can be given both of the large numbers of related substances present in a given species and of instances in which a given compound or complex of compounds occurs in high concentration. The cardiac glycosides are very complicated in their structures, but even so, there is often a profusion of the number of different glycosides found in the same tissue (Zechner, 1966). Thus, the seeds of *Mansonia altissima* contain 30 cardenolides (Allgeier et al., 1967), the leaves of *Strophanthus boivinii* Baill. contain 24, and about 20 heart glycosides have been reported in the seeds of *Acokanthera oppositifolia* Lam. Individual heart glycosides may also occur in relatively high concentration in certain species. The seeds of *Strophanthus kombe* contain 7–10% of cardenolides, of which about half consists of K-strophanthoside. A study of the fatty acids in the peels of limes and lemons has shown the occurrence of 81 and 102 different acids, respectively (Nordby and Nagy, 1969). As methods become more sensitive, additional acids will no doubt be found.

A good example of the large number of compounds that have been reported in a single species is the 1200 compounds shown to be present in tobacco and tobacco smoke, not including the individual components of complex substances such as the brown pigments and resins which have not been resolved (Stedman, 1968). This represents an increase of 800 known compounds between 1949 and 1967. Admittedly, the number is influenced by the effects of curing; nevertheless, this is an indication of the tremendous complexity of the chemical systems in plants, the enormous number of enzymes which are present, and the very complicated set of controls which must be operating to determine when and how much of a given ingredient is to be formed.

In studying composition in relation to taxonomy, many pitfalls must be avoided. The same compound may be derived by a different biogenic route so that its occurrence in two different species may have no taxonomic significance. It seems to the writer, who has no expertise in this field, that as investigations are continued, what seem to be good correlations between certain ingredients and taxonomic divisions have a tendency to disappear as more species are examined.

Comparative studies have shown extreme differences in some groups of secondary compounds among species of similar general morphology. In other instances, plants very different in appearance have a nearly identical array of these compounds. In the latter situation, one is not able to find any common feature that is peculiar to the 2 taxa except their chemistry (Alston, 1966).

The sensitivity of methods has, in fact, become embarrassing to the workers in the field of chemical taxonomy. Chemical constituents have been an aid in verifying or calling into question classifications based on morphological characteristics. It is helpful to find certain compounds limited to tribes, families, or genera, but, as the studies continue, the compounds often turn up in unexpected places. Review articles written only a few years ago in which it is pointed out that a certain glycoside, for example, is found only in a given species, are soon out of date as new occurrences are reported.

The question as to whether the special compounds play any significant role remains basically unanswered and, in fact, has not received thoughtful consideration. An interesting guess may be possible in those instances in which individual plants of a species do not contain the secondary compound generally present. For example, some years ago it was shown that with white clover, *Trifolium repens*, some individual plants contain neither the cyanogenic glycoside(s) nor the enzyme which hydrolyzes the glucoside, others contain only the glucoside, and others only the enzyme. There is no obvious morphological difference between these individuals, but the story is complicated by the results of studies on the value of these strains as agricultural crops, considering such factors as yield, persistence in the field, and meeting of competition with other crops. When evaluated on this basis, plants containing the largest quantity of HCN producing compounds were found to be superior for agricultural purposes.

Plants will also carry nonnaturally occurring chemicals into their metabolism. That absorbed nonnaturally occurring aglycons will be converted into glycosides in various tissues was first shown by the writer about 30 years ago. Some 20 years later, other workers obtained similar results. Since then, hundreds of instances of the incorporation of added substances into plant metabolism have been reported. Procedures are in use commercially in which a desired product is obtained by adding a suitable precursor to the medium. For example, benzylpenicillin is obtained by adding a benzyl compound to the fermentation medium.

The delay in further work between 1939–1946 and 1958–1959 on the uptake of nonnatural substances and their entry into metabolic processes was partly the result of the widespread belief at that time that foreign substances do not readily pass the semipermeable membranes of cells. Actually, the whole science of therapeutics is based on the passage of various agents through the necessary membranes. An extreme example is the action of oxytocin in acting as a mammary hormone. As little as 1 microgram injected intravenously into recently parturient women will induce the release of milk within a matter of 20–30 seconds (Nickerson et al., 1954).

Plants are known to accumulate various materials from their environment which may even be detrimental to their development. The use of plants as monitors for air pollution has long been accepted (Brandt and Heck, 1968), since they often are initial indicators that something is wrong in the environment. Plants absorb many nonnaturally occurring organic compounds, including some of large molecular size. It has been amply demonstrated that fungicides are taken up by fungal spores and host plants, and enter into reactions within these tissues. The present worldwide distribution of DDT originates from the accumulation of this pesticide by phytoplankton whereby it is passed on through the food chain to fishes and birds, in which it may reach detrimental concentrations. Plants can also serve as indicators of the presence of mineral deposits in the soil in which they are growing, and sometimes accumulate many times the concentration present. Plants growing on soils containing selenium take up large quantities. Although this element is not essential for plants in general, certain species have become adapted so that they require this element for growth. These species contain selenium as constituents of amino acids (see Volume III, Chapter 1).

Some of our most challenging current problems arise from the use of the vast supply of energy from the sun stored by various plant tissues millions of years ago. Combustion of these fossil fuels (which is rarely complete) releases various chemicals which serve as primary and secondary air pollutants. Green plants also add materials to the atmosphere. As leaves become older and die, terpenes and other volatile organic compounds are released, which account for the aromaticity of mowed and drying hay in the summer and of forests especially during autumn. The blue heat or summer haze so common over vegetative land masses has its origin in a photochemical transformation of volatile organic matter from plants. Rasmussen and Went (1964) estimated the world production of plant volatiles released in the atmosphere to be 437×10^6 tons per year. Whether the atmosphere contains molecularly dispersed particles of all or many of the chemical constituents of plant origin remains to be determined as more refined techniques develop.

This serves to emphasize that plants are not exclusively the great purifiers but are making a very substantial contribution to pollution. Any activity by plants or animals, as well as by man, leads to pollution. Activities described by Whittaker and Feeny (1971) as allelochemic can also be noted as pollutant effects. These involve for the most part pollutant of soils (Tukey, 1970 p. 319). Thus, walnut trees (*Juglans nigra* and *J. regia*), through the effects of juglone, washed from leaf surfaces to the soil inhibit growth and development of other plants in the immediate vicinity (*Bode*, 1958). Leaf excretions from wormwood (*Artemisia absinthium*) reduced germination and stunted the growth of fennel (*Foeniculum vulgare*); growth was altered to a more succulent type (*Bode*, 1940). Muller and Muller (1956) showed that the desert shrubs *Franseria*