

Chemistry *and*
Biochemistry *of*
Plant Pigments

Chemistry *and* Biochemistry *of* Plant Pigments

Edited by

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PREFACE

In August 1962 a colloquium on the "Biochemistry of Plant Pigments" was held by the Biochemical Society in Aberystwyth. It was successful in bringing together interested scientists from a number of countries to discuss critically the latest developments in this field. However, it also clearly demonstrated the need for collecting in one volume the scattered information discussed, and by early 1963 the Editor had planned the book and persuaded the various authors to take part in the venture and to prepare their contributions by early 1964. The coverage of the colloquium was extended to include the chemistry of the plant pigments and an analytical section was also planned. All the contributors have international reputations and the fact that they all willingly agreed to undertake the work re-emphasizes the need for this volume. A few manuscripts inevitably failed to be produced on time, but apart from this the co-operation of the contributors with the Editor has been excellent, and no Editor could have had an easier assignment. Furthermore, the expertise of the Publishers in dealing with late manuscript¹ has resulted in only a minimum delay in the appearance of the book. Owing to unforeseen circumstances it has not been possible to include a projected Chapter on plant cytochromes.

The plan of the book is simple; it is divided into four main sections, the first dealing with the chemistry and biosynthesis of the various pigments; the second and third with their function and metabolism respectively; and the fourth with analytical procedures used in dealing with plant pigments. All the contributors are still actively concerned in research in the speciality on which they write and this has resulted in critically written, forward-looking chapters in which not only is the well-established information thoroughly reviewed but the gaps in our knowledge are clearly indicated, and sufficient suggestions for future investigations are made, either implicitly or explicitly, to keep an army of research workers busy for a long time. The detailed analytical chapters, considered in association with Parts I, II and III, should be of particular value.

Biochemical investigations on higher plants have been relatively few compared with those on animals and protista. During the past few years, however, more and more biochemists have become interested in this field and, in spite of the many difficulties in dealing with higher plants, the number of investigations reported in the Journals is increasing rapidly each year. This book should be of background value to those who are contemplating entry into the field of plant biochemistry via plant pigments; it should also be useful to senior undergraduate students in biochemistry who are finding that more and more plant biochemistry is appearing in their courses every year. Their instructors may also find

useful suggestions for laboratory exercises in the analytical sections. Finally, the "Chemistry and Biochemistry of Plant Pigments" should appeal to the increasing number of botanists who are keen to specialize in the more biochemical aspects of plant physiology.

The final stages of the editing of this volume were carried out whilst the Editor was holder of a National Science Foundation Senior Foreign Scientist Fellowship at the University of California, Davis.

Dr. B. H. Davies kindly prepared the subject index.

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PART I: NATURE, DISTRIBUTION AND BIOSYNTHESIS

Chapter I

NATURE, PROPERTIES AND DISTRIBUTION OF CHLOROPHYLLS *

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I. INTRODUCTION

Chlorophyll is the general name of green pigments in organisms capable of photosynthesis. It was first used by Pelletier and Caventou (1818) to describe the pigment complex responsible for the green colouration of leaves. Years later, it was shown by Stokes (1864) to be a mixture of yellow pigments plus two different green pigments. Sorby (1873) obtained the green pigments relatively pure and published their absorption spectra; he referred to them as blue and "yellow" chlorophylls.

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Twissett (1906) separated them using column chromatography and called them chlorophylls α and β , which later became a and b . The distribution of the different chlorophylls known at present throughout the plant kingdom is given in Table I.

TABLE I
Distribution of Chlorophylls among Photosynthetic Organisms*

Organism	Pigment				
	Chlorophyll				
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>
Higher plants, ferns and mosses	+	+	—	—	—
Algae†					
Chlorophyta	+	+	—	—	—
Chrysophyta					
Xanthophyceae	+	—	—	—	+ ‡
Chrysophyceae	+	—	±	—	—
Bacillariophyceae	+	—	+	—	—
Euglenophyta	+	+	—	—	—
Pyrrophyta					
Cryptophyceae	+	—	+	—	—
Dinophyceae	+	—	+	—	—
Phaeophyta	+	—	+	—	—
Rhodophyta	+	—	—	+	—
Cyanophyta	+	—	—	—	—
	Bacterio- chlorophyll		Chlorobium Chlorophylls		
	<i>a</i>	<i>b</i>	650	660	
Bacteria					
Thio- and Athiorhodaceae	+	+	—	—	
Chlorobacteriaceae	+	—	+	+	

* See Fischer and Stern (1940), Strain (1958), Haxo and Fork (1959), Allen *et al.* (1960), Stanier and Smith (1960) and Eimhjellen *et al.* (1963).

† Taxonomic classification according to Prescott (1962).

‡ Chlorophyll *e* has been isolated from only one organism; it seems to resemble chlorophyllide *c* (Strain, 1951).

II. CHEMICAL PROPERTIES

A. STRUCTURE

Chlorophylls may be regarded as magnesium complexes of compounds derived from phorbil (Fig. 1), which, in turn, may be regarded as the dihydro derivative of porphin (Fig. 2), but with the addition of the

isocyclic ring V. Individual pyrrole nuclei are numbered I–IV; the outer (β -pyrrole) positions of the nuclei are numbered 1–8; the methine bridge carbon atoms are designated α – δ .

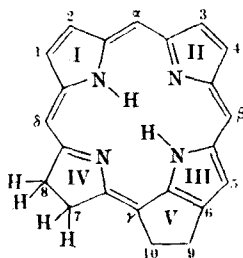


FIG. 1. Phorbin.

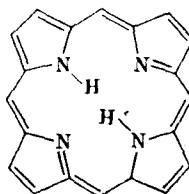


FIG. 2. Porphin.

Clarification of the structures of chlorophylls *a* and *b* (Fig. 3) involved the work of many investigators, among whom were Willstätter, Stoll, Conant and Hans Fischer. By 1940 the latter, after many years of synthetic, analytical and degradative investigations on both the blood pigments and chlorophylls, established the essential structures of chlorophylls *a*, *b* and bacteriochlorophyll *a*.

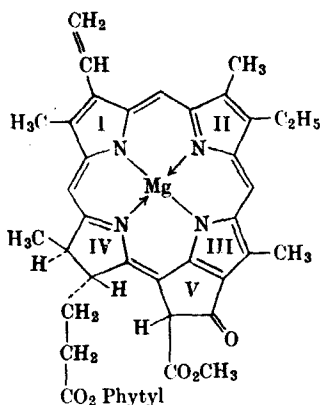


FIG. 3. Chlorophyll *a*. For chlorophyll *b*: replace $-\text{CH}_3$ at C_3 with $-\text{CHO}$. For chlorophyll *d*: replace $-\text{CH}=\text{CH}_2$ at C_2 with $-\text{CHO}$.

Confirmation of the structure of chlorophyll *a* by synthesis was advanced by Fischer *et al.* (1940) to the stage of phaeoporphyrin *a*₅ (Fig. 4), a porphyrin isomeric with phaeophorbide *a*. Recently, Strell *et al.* (1960)

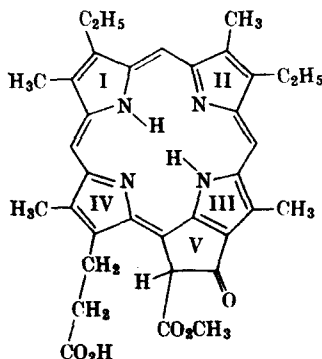


FIG. 4. Phaeoporphyrin *a*₅.

using Fischer's methods, and Woodward *et al.* (1960) using an ingenious, novel synthesis, prepared derivatives readily convertible into phaeophorbide *a*, the conversion of which to chlorophyll *a* had previously been shown to be possible.

B. NOMENCLATURE

Terms used in chlorophyll chemistry include:

Chlorophyllide. The derivative resulting from removal of the alcohol esterified to the C₇ propionic acid group.

Phytol chlorophyllide a. Chlorophyll *a*.

Phaeophorbide and Phaeophytin. The magnesium-free derivatives of chlorophyllide and chlorophyll, respectively.

Chlorins. Derivatives of phaeophytin *a* resulting from cleavage of the isocyclic ring; these compounds may possess all, some or none of the carbon atoms originally attached to C₆ or C₇.

Rhodins. Derivatives of phaeophorbide *b* corresponding to chlorins.

Purpurins. Derivatives of chlorins in which C₁₀ has been oxidized.

"Meso" compounds. Derivatives in which the C₂-vinyl group has been reduced to an ethyl group.

"Pyro" compounds. Derivatives in which the C₁₀-carbomethoxy group has been replaced by a hydrogen atom.

Porphyrin. Any derivative of the above types of compounds in which Ring IV has been oxidized by removal of the hydrogen atoms at C₇ and C₈, i.e. a substituted porphin. Porphyrins which possess a C₂-vinyl group

in place of an ethyl group are referred to as vinyl porphyrins, e.g. vinyl phaeoporphyrin a_5 (protophaeophorbide a). The subscript refers to the number of oxygen atoms in the compound.

Porphyrinogen. A hexahydro porphyrin whose methine bridges have been reduced to methylene.

Phyllin. Any chlorophyll derivative containing magnesium.

Farnesol. 3,7,11-trimethyl-2,6,10-dodecatrien-1-ol; $C_{15}H_{25}OH$ (*trans, trans*; Rapoport and Hamlow, 1961).

Phytol. 3,7,11,15-tetramethyl-2-hexadocen-1-ol; $C_{20}H_{39}OH$ (*trans*; Burrell *et al.*, 1959).

Hydrochloric acid number. The per cent hydrochloric acid (w/w) which extracts two-thirds of a metal-free derivative from an equal volume of ether.

C. REACTIONS

Below are listed some of the many reactions which have proved useful for the characterization of chlorophylls. In the main, only references not included in Willstätter and Stoll (1913), Fischer and Stern (1940), Rothemund (1944) and Aronoff (1960) are given.

1. Removal of Magnesium and the Preparation of other Metal Derivatives

Addition of dilute, aqueous mineral acid to a miscible solvent containing a phyllin causes immediate removal of magnesium with a consequent change in the visible absorption spectrum. Magnesium can be reintroduced by a Grignard reagent or its alkoxy derivative. If the phyllin is to retain the capacity to give a positive Molisch phase test, the latter reaction must be carried out in the absence of oxygen, otherwise it will be "allomerized".

Tracer experiments with ^{28}Mg *in vivo* or in aqueous acetone solution have shown that the magnesium of chlorophyll is not exchangeable (Becker and Sheline, 1955; Aronoff, 1962).

Other metals, e.g. zinc, mercury, copper, silver, iron, are introduced from acetic acid solutions, or as chlorides or acetates from methanolic solutions containing a small amount of pyridine.

2. Removal or Replacement of the C_7 esterifying alcohol

This is removed enzymatically with chlorophyllase (see Chapter 17) or hydrolytically with acid or alkali. The product(s) is either a free acid, or an ester resulting from transesterification, depending upon the conditions used. Two alcohols have been identified: phytol from chlorophylls a , b and bacteriochlorophyll a , and farnesol from *Chlorobium* chlorophylls "650" and "660" (Rapoport and Hamlow, 1961; Holt *et al.*, 1963).