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# SPECTROSCOPIC STUDIES ON REVERSIBLE REACTIONS IN COMPOUNDS OF THE PORPHIN SERIES

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## INTRODUCTION

One has merely to list only a few examples of compounds of the porphin series to show clearly, without further comment, the urgent need for a comprehensive study of the compounds of this series, from the simplest onwards. Compounds of this series include chlorophyll — the green pigment of the photosynthesising mechanism in plants, haem — the red pigment of blood, responsible in haemoglobin for the transport of oxygen and carbon dioxide in the circulatory system, the prosthetic groups of cytochromes — biological catalysts controlling the processes of tissue respiration, and phthalocyanines — synthetic compounds which have found extensive application as dyestuffs.

The structure and physicochemical properties of chlorophyll were first studied at the beginning of last century, but it was only in the nineteen-twenties and -thirties that the work of several generations of Russian and other scientists led to the first synthesis of the simplest porphin derivatives and the determination of the structural formulae of chlorophyll and haem†. In the post-war years the attention of many research workers was drawn to the specific ability of compounds of the porphin series to undergo reversible reactions. These reactions are undoubtedly of particular scientific interest, since they characterise or even determine the biological activity of the pigments. Considerable progress was made in this field as a result of the application of new physical methods, particularly spectroscopic methods: electronic and vibrational absorption spectra, luminescence spectra, electron spin resonance (ESR) spectra, and nuclear magnetic resonance (NMR) spectra. Valuable experimental material has now accumulated, but is is difficult to see one's way through the vast quantity of unrelated data. Thus at the present time, in addition to a continuation of experimental work, there is a need for systematisation and summary of published results, and this is the purpose of the present review. We shall examine the interactions of molecules of the porphin series with the medium and also some of their photochemical reactions. The reversible reactions involved in the reduction and oxidation of chlorophyll, and its photosensitising action, have been studied in detail in reviews by Krasnovskii[8], Evstigneev[9], and Livingston[10] and in a monograph by Rabinowitch[1], so that we shall not deal with these topics. In view of their specific character and direct relationship to the function of haemoglobin, the properties of the iron-containing complexes form a separate chapter of science and will be discussed only in the examination of the general properties of metal derivatives of the porphin series. The purely spectroscopic characteristics of compounds of the porphin series have been described in a review by Gurinovich et al.[11] and their physicochemical properties have been described in papers by Phillips[12,13] and Falk[14].

† The history of the discovery and study of chlorophyll, haem, and other porphin derivatives is described in more detail in a number of monographs[1-7].

## I. NOMENCLATURE AND MOLECULAR STRUCTURE OF COMPOUNDS OF THE PORPHIN SERIES

The skeleton of the porphin molecule (I, II) is made up of four pyrrole rings joined to one another by methine bridges to form a closed cyclic system with conjugation throughout the entire ring. Depending on the arrangement of the two central H atoms, two isomers of porphin are possible in principle, with *trans*- or *cis*-arrangement of the imine NH bonds.

Porphin derivatives with substituents in positions 1–8 are called porphyrins. For example, etioporphyrin I is 2,4,6,8-tetraethyl-1,3,5,7-tetramethyl-porphin, and protoporphyrin IX is 1,3,5,8-tetramethyl-2,4-divinylporphin-6,7-dipropionic acid. It is possible to distinguish four isomers of etioporphyrin and 15 isomers of protoporphyrin, depending on the mutual arrangement of the substituents. The isomers are denoted by Roman numerals (etioporphyrin I, etioporphyrin II, etc.). The nomenclature of the porphyrins, methods for their preparation, and properties have been described in detail in a monograph by Fischer[6] and in a review by Phillips[13].

The H atoms of the methine bridges of the porphin molecule, like the H atoms of the pyrrole rings, can also be replaced. Thus for example $\alpha\beta\gamma\delta$-tetraphenylporphin and $\alpha\beta\gamma\delta$-tetramethylporphin (or simply tetraphenylporphin and tetramethylporphin) are known.

The porphin derivative in which a benzene ring is fused to each pyrrole ring is called tetrabenzoporphin (III).

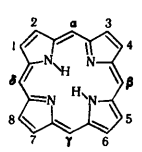
Hydrogenation of one or two of the pyrrole rings of the porphin molecule gives dihydroporphin or chlorin (IV) and tetrahydroporphin or bacteriochlorin (V) respectively. Chlorin and bacteriochlorin also form derivatives corresponding to those of porphin. It is suggested that the hydrogenated pyrrole rings in the molecules of tetraphenylbacteriochlorin[15] and octaethylbacteriochlorin[16] may be situated in either the *trans*-position or the *cis*-position relative to one another (V, VI). It may be assumed that the same possibility exists for other bacteriochlorin derivatives‡.

A unique position among porphin derivatives is occupied by the azaporphins — compounds in which some or all of the methine bridges are replaced by nitrogen atoms. Derivatives of monoaza-, diaza, triaza, and tetra-azaporphin(VII) are known. Tetrabenzotetraazaporphin is usually called phthalocyanine (VIII).

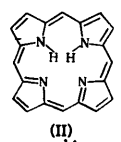
Porphin and all its derivatives can form complexes with various metals, by replacement of the H atoms of the imine groups in the centre of the molecule. The complex

‡ The term "bacteriochlorin" is obtained by analogy with the term "bacteriochlorophyll" (the tetrahydro-derivative of porphin with the *trans*-arrangement of the hydrogenated pyrrole rings). Up to the present, therefore, this term has been applied in the literature only to the *trans*-isomers of tetrahydroporphin and its derivatives. Since a terminology for the hydrogenated derivatives of porphin has not yet been established, however, it will be convenient to extend this term to the *cis*-isomers.

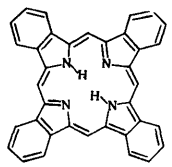
formed by porphin with a bivalent metal (M-porphin) is represented by formula (IX). When porphin derivatives form complexes with tervalent or quadrivalent metals, one or two anions respectively, usually $OH^-$, $HSO_4^-$, $Br^-$, or $Cl^-$, are attached to the metal atom at ring angles to the plane of the molecule.



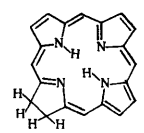
(I) porphin, *trans*-isomer



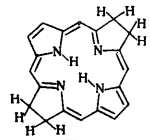
(II) porphin, *cis*-isomer



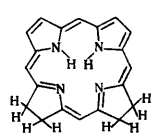
(III) tetrabenzoporphin



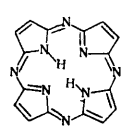
(IV) chlorin



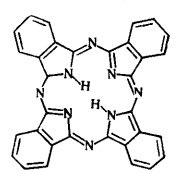
(V) bacteriochlorin *trans*-isomer



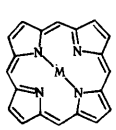
(VI) bacteriochlorin *cis*-isomer



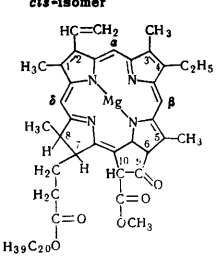
(VII) tetra-azaporphin



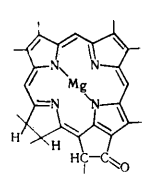
(VIII) phthalocyanine



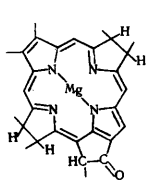
(IX) M-porphin



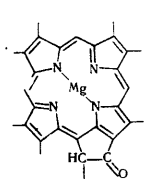
(X, *a*) chlorophyll *a*



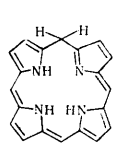
(X) chlorophyll



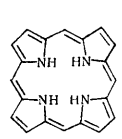
(XI) bacteriochlorophyll



(XII) protochlorophyll



(XIII) phlorin



(XIV) isophlorin

Most of the biologically important pigments among the porphin derivatives are complexes with metals. Thus haem is $Fe^{II}$-protoporphyrin IX; haemin (chlorohaemin) is $Fe^{III}Cl$-protoporphyrin IX; and haematin is $Fe^{III}OH$-protoporphyrin IX. Chlorophyll, bacteriochlorophyll, and protochlorophyll are magnesium-containing derivatives of chlorin, bacteriochlorin, and porphyrin respectively. The skeleton of the molecular structure of these pigments is given by formulae (X), (XI), and (XII); the substituents in all are the same [see formula (X,*a*)]. An important characteristic of the molecular structure of chlorophyll, bacteriochlorophyll, and protochlorophyll is the presence of the so-called cyclopentanone ring joined to the tetrapyrrole skeleton of the molecule via the C atoms in positions 6 and $\gamma$. There exist analogues of chlorophyll without a metal atom in the centre of the molecule (pheophytin) and analogues in which the Mg atom is replaced by an atom of another metal (Cu-pheophytin, Ni-pheophytin, etc.). Methylchlorophyllide is a chlorophyll analogue with a methyl group in place of the $C_{20}H_{39}$ group in position 7.

Woodward [17] recently suggested that porphyrins may be hydrogenated not only in the pyrrole rings but also at the methine bridges and central nitrogen atoms with the formation of the so-called phlorins (XIII) and isophlorins (XIV). Strictly speaking, phlorin cannot be included among the compounds of the porphin series, since these are characterised by the presence of a closed ring of conjugated bonds.

The presence of the cyclic system of conjugated bonds in the molecules of porphin and its derivatives is the factor which determines the characteristic features and properties of these molecules. Measurements of the limiting luminescence polarisation of symmetrically substituted M-porphin derivatives (where M is a bivalent metal) have led to the conclusion that the molecules of these compounds belong to a point group with symmetry $D_{4h}$. This means that, as a result of the delocalisation of the $\pi$ electrons in the system of conjugated bonds, all four pyrrole rings and all four M–N bonds and other symmetrically arranged atoms and bonds become equivalent [11]. In particular, the concept of "semi-isolated" double bonds [1] in the porphyrin molecules becomes meaningless. In the case of non-symmetrical derivatives of porphin (chlorins, bacteriochlorins, and unsymmetrically-substituted porphyrins) the equivalence of the atoms and bonds breaks down to some extent, leading to a change in some of the properties of the compounds. In this case also, however, the degree of delocalisation of the $\pi$ electrons in the conjugated system is fairly high and it is possible to speak of the relative equivalence of the M–N bonds and non-hydrogenated pyrrole rings.

## II. ISOMERISM AND TAUTOMERIC CHANGES

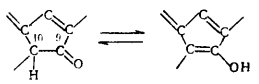
Let us examine first the problem of the isomerism of the imino-groups in the porphin molecule. As noted above, isomers of porphin with *cis*- and *trans*-arrangement of the imino-groups (I) and (II) are possible in principle. The point group symmetry of the molecule of the *trans*-isomer is $D_{2h}$, and that of the molecule of the *cis*-isomer is $C_{2v}$. According to the selection rules, for molecules with symmetry $D_{2h}$ the range of the stretching vibrations of the NH bonds should contain only one absorption band, but for symmetry $C_{2v}$ two bands are possible [18]. Spectroscopic measurements have shown that porphin [18] and all its derivatives, including chlorin, bacteriochlorin [19], and phthalocyanine [20], have only one band in this range, corresponding to *trans*-arrangement of the imino-groups. Measurements of the limiting luminescence polarisation of porphin and its symmetrically substituted derivatives [11] confirmed that the molecules of these compounds belong to the point group with symmetry $D_{2h}$.

It would, however, be premature to conclude that the possibility of *cis*-arrangement of the imino-groups in molecules of the porphin series is excluded. Spectroscopic measurements in the infrared range have up to the present been mainly qualitative; they do not exclude the possibility that the absorption bands are superimposed and hence that the interpretation of the results is ambiguous. This possibility is confirmed by the infrared spectrum of pheophytin *a*. In this spectrum the range of stretching vibrations of the NH bonds contains an absorption band with three maxima, which can be assigned to isomers of the pigment with different arrangements of the imino-groups [21].

The conclusion that tautomeric transformations of the *trans*- and *cis*-isomers of porphin compounds into one another are possible was reached by Dorough and Shen [22], who observed reversible changes in the fluorescence and

absorption spectra of solutions of tetraphenylporphin and tetraphenylchlorin with decrease in temperature from 20° to -196°. The spectra of metal derivatives of these pigments did not show similar changes. This phenomenon was attributed to displacement of the dynamic equilibrium between tautomers with change in temperature. This explanation requires verification, however, since it is held that the change in the spectroscopic characteristics of tetrapyrrole pigments with decrease in temperature is due not to tautomeric changes in the molecules but to resolution of the vibrational structure of electronic transitions [11]. Further progress towards a solution of the problem of isomerism and tautomeric changes in porphin compounds requires new studies, particularly in infrared and NMR spectroscopy.

According to data obtained by many workers [1], the Molisch phase-test reaction, which involves the production of a brown colour in an ethereal solution of chlorophyll when alcoholic alkali is added, begins by alkali-induced enolisation of the carbonyl group in position 9:


(XV).

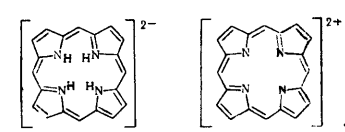
It is not excluded that keto–enol tautomeric changes also take place in chlorophyll molecules in the absence of alkali. In the determination of the proportion of enolised chlorophyll molecules under different conditions, considerable reliance is placed on infrared spectroscopy. It is suggested that the intensity of the absorption bands corresponding to the vibrations of the OH, C=C, and C=O bonds may provide a reliable measure of the quantity of keto and enol forms of chlorophyll molecules. The difficulties of interpretation of the infrared spectra, however, have led to contradictory views on the data obtained [23–28]. It is found that the presence of absorption bands in the spectrum of chlorophyll in the range corresponding to the stretching vibrations of the OH groups does not provide adequate proof of the existence of enolised molecules, since in this range radiation is also absorbed by molecules of water, which is associated with the chlorophyll and is difficult to remove [29]. The absorption bands of chlorophyll in the range of vibrations of the C=C and C=O bonds (1500–1750 $cm^{-1}$) can be interpreted in different ways [23–28,30] and therefore cannot provide a sufficient basis for concluding that keto–enol tautomerism occurs in chlorophyll. In this connection it must be admitted that conclusions regarding the presence of an appreciable quantity of enolised chlorophyll molecules in various solvents, reached merely on the basis of spectro-analytical measurements in the infrared range [23–25,30,31], have insufficient foundation. Recently, study of isotope exchange between chlorophyll and $CD_3OD$ in chloroform has shown that the rate of replacement of the H atom in position 10 by deuterium is relatively low. Without excluding the possibility, in principle, of the existence of enolised chlorophyll molecules, this experiment shows that the proportion of such molecules under the conditions of the experiment is small and cannot determine to any significant extent the absorption spectrum of the pigment in the infrared range [26,32,33].

## III. MOLECULAR COMPOUNDS

Let us examine the reaction of molecules of porphin compounds with one another or with the molecules of the medium, leading to the formation of association and molecular complexes with a significant change in the properties of the pigments, including their spectroscopic characteristics. The ability of molecules of the porphin series to form complexes with the molecules of the medium is by no means a distinctive feature of this series but is of exceptional biochemical importance. This property is decisive in enabling haemoglobin to perform its function as oxygen carrier. The reaction of chlorophyll molecules with the molecules of the medium ensures that the interacting components are prepared for the fundamental photochemical step.

1. Compounds with an ionic structure. In the extreme case of strong interactions ionisation of the pigment molecule takes place. Porphyrins are amphoteric compounds [12,13]. All four nitrogen atoms of the porphyrin molecule are basic centres and may act as proton acceptors. The imino-groups (NH) are capable of losing protons under certain conditions, that is, of acting as acidic centres. If the neutral porphin molecule is denoted by $H_2\Pi$, six ionic forms are possible in principle: reaction with strong bases may lead to the formation of $(H\Pi)^-$ and $\Pi^{2-}$ ions and reaction with acids to the formation of the ions $(H_3\Pi)^+$, $(H_4\Pi)^{2+}$, $(H_5\Pi)^{3+}$, and $(H_6\Pi)^{4+}$. Divalent anionic and cationic forms of some porphyrins have been isolated in the form [12] of salts $Na_2^+\Pi^{2-}$ and $(H_4\Pi)^{2+}Cl_2^-$. In particular, tetraphenylporphin and tetraphenylchlorin dihydrochlorides are formed when the original pigments are kept in gaseous HCl [34,35]. This method can apparently also be used to prepare the hydrochlorides of other porphin derivatives.

Measurements of the luminescence polarisation show that the divalent porphyrin cation $(H_4\Pi)^{2+}$, like the metal–porphin molecule, has the point group symmetry $D_{4h}$. From this it follows that the excess of charge in the divalent ion is distributed uniformly throughout the cyclic system of conjugated bonds and that all four pyrrole rings are equivalent [11]. The position and width of the absorption bands in the region of the stretching vibrations of the NH bonds in the infrared spectra of tetraphenylporphin and tetraphenylchlorin dihydrochlorides [34,35] show [36] that these bonds have ionic character $(NH)^+$. This means that the excess of charge in the structure of the divalent porphyrin and chlorin cations, although included in the cyclic system of conjugated bonds, is chiefly concentrated close to the NH bonds and is distributed uniformly between them. On the basis of these ideas, the structure of the cation can be represented by formula (XVI). The analogous formula (XVII) can be used to describe the structure of the divalent anion:


(XVI) porphin divalent cation


(XVII) porphin divalent anion

Singly-charged porphyrin ions have as yet been observed only in exceptional cases. Thus it is suggested that the absorption spectrum of the monovalent protoporphyrin cation can be observed in the presence of certain aqueous detergents [37]. The singly-charged porphyrin ions are evidently short-lived intermediate products which are rapidly converted into doubly-charged ions. This is apparently due to the instability of structures in which one of the central N atoms is not equivalent to the others (in the case of the monovalent anion it form an imino-group, unlike

the other three N atoms, and in the case of the monovalent cation it has no H atom whereas the other N atoms form imino-groups). In the reaction between acids and chlorins in which the equivalence of the pyrrole rings has been broken down by hydrogenation of one of the lateral double bonds both divalent and monovalent cations can be observed spectroscopically [11].

The ionic forms of metal derivatives of the porphin series have been less extensively studied. On contact with metallic sodium in absolute dry tetrahydrofuran in a vacuum, Mg-phthalocyanine is converted into the anionic form [38,39]. The number of electrons attached to the pigment molecule in the process is still unknown. On contact with oxygen, the Mg-phthalocyanine anions are reversibly converted into the original pigment. It was recently found that the reaction of Zn-tetraphenylporphin with certain organosodium compounds leads to the formation of salts of the monovalent and divalent anions of the pigment. The salts $Na^+(ZnTPP)^-$ and $Na_2^+(ZnTPP)^{2-}$ were isolated in pure form [40]. As in the case of porphyrins containing no metals, the excess of charge in the Mg-phthalocyanine and Zn--tetraphenylporphin ions is apparently distributed uniformly throughout the cyclic system of conjugated bonds.

The metal derivatives of compounds of the porphin series can be divided into two groups according to the nature of their reaction with acids. The first group consists of those derivatives in which this reaction leads to replacement of the central metal atom by two H atoms, that is to irreversible conversion of the metal-containing complex into pigment containing no metal. The second group consists of metal derivatives which are fairly stable towards acids but which react to form cations in which the central metal atom is preserved. The stability towards acids depends chiefly on the stability of the bond formed by the metal atom with the ligand. This stability decreases in the order [12]:

$$Pt^{II} > Pd^{II} > Ni^{II} > Co^{II} > Ag^{II} > Cu^{II} > Zn^{II} > Mg^{II} > Cd^{II} > Sn^{II} > Li > Na > K > Ag.$$

The stability towards acids also depends on the structure of the pigment molecule and on the conditions under which the reaction with acids takes place. Thus when sublimed layers of Zn-phthalocyanine and Zn-tetraphenylporphin are brought into contact with gaseous HCl, the former is stable [41] whereas the latter is converted into the hydrochloride of tetraphenylporphin containing no metal. In the same way, a sublimed film of Mg-phthalocyanine is stable both towards gaseous HCl and towards acetic acid, whereas in acetone solution Mg-phthalocyanine is gradually converted into metal-free pigment when HCl is added [42], and a film of chlorophyll deposited from solution is converted into pheophytin in the presence of acetic acid vapour [43].

Let us turn to the question of the structure of the reaction products formed by phthalocyanine and its stable M--derivatives with acids. Unlike other porphin derivatives, the phthalocyanine molecule has not four but eight nitrogen atoms (VIII), each of which is capable of acting as a proton acceptor. Evstigneev and Krasnovskii observed a marked displacement towards longer wavelengths of the red absorption band of metal-free phthalocyanine and Cu--phthalocyanine when these compounds were dissolved in concentrated $H_2SO_4$. Since this displacement was not observed in the case of chlorophyll, it was suggested that in acid medium the protons are attached not to the pyrrole nitrogens but to the peripheral N atoms of the phthalocyanine [44]. In a study of ionic equilibrium in solutions of phthalocyanines in $H_2SO_4$, Berezin [45,46] concluded that the solutions of both the metal-free phthalocyanine and the M-phthalocyanines contain only the cations $H_2PcH^+$ and $MPcH^+$, where Pc is the ligand in the M-phthalocyanine molecule; he also suggests that the proton is attached to the peripheral N atom. Comment on these conclusions is necessary.

Firstly, it is known that, when Cu-tetraphenylporphin, which is stable in this solution and has no peripheral N atoms, is dissolved in $H_2SO_4$, the displacement of the absorption towards longer wavelengths, characteristic of the reactions of compounds of the porphin series with acids, is observed [47]. In this case there is apparently no reason to doubt that the protons can be attached only to the pyrrole N atoms, although they are also joined to a metal atom, which creates steric hindrance for this attachment. The possibility of attachment of protons to the pyrrole N atoms in metal-free phthalocyanine is also confirmed by the possibility of H $\rightarrow$ D isotope exchange in the imino-groups in the presence of DBr.[41]

Secondly, in the reaction with gaseous HCl or HBr the M-phthalocyanine molecule may add one or more protons. Grounds for this statement are provided by the stepwise nature of the desorption of HCl from M-phthalocyanine, revealed in the associated sequence of changes in the infrared spectra of the specimens [41].

It must evidently be assumed that both the pyrrole nitrogen atoms and the peripheral nitrogen atoms of the phthalocyanine molecules may react with protic acids, depending on the conditions.

Important evidence confirming the ionic structure of the products of the reaction of phthalocyanines with gaseous HCl, HBr, and acetic acid is provided by the infrared spectra of these products [41]; absorption bands corresponding to the vibrations of the ionic $NH^+$ and $CH_3COO^-$ groups can be readily identified. The ionic structure of tetraphenylporphin and tetraphenylchlorin hydrochlorides is confirmed by the presence of absorption bands [34,35] due to the $NH^+$ groups in their spectra.

The influence of the central metal atom in a phthalocyanine molecule on the ability of this molecule to form hydrochlorides and hydrobromides [41] is remarkable. Thus Mg-, Zn-, and Fe-phthalocyanines react with HCl to form the corresponding hydrochlorides, whereas Cu-phthalocyanine and the metal-free phthalocyanine do not react. All the above phthalocyanines react with the more active HBr molecules. We shall see later that this ability of the central metal atom to facilitate intermolecular interaction between the pigment and the medium is shown not only for HCl and HBr but also for a number of other compounds.

The salts formed by the cations of porphin derivatives are generally readily decomposed with formation of neutral molecules. Thus phthalocyanine hydrochlorides and hydrobromides are converted into neutral phthalocyanines when the specimens are heated in a vacuum to 280–300° or kept in pyridine vapour (an organic base). In concentrated sulphuric acid phthalocyanine cations are converted into neutral molecules when the solutions are diluted with water. Tetraphenylporphin and tetraphenylchlorin are also regenerated from their hydrochlorides with increase in temperature or by treatment with pyridine.

2. Complexes. Let us examine the complexes formed by compounds of the porphin series with the medium, where

the reaction of the components is not so clearly ionic as in the reaction of the pigments with strong acids and bases. Such complexes may be formed in the reaction with comparatively weak bases (aniline, pyridine, or hydrazine), NO, CO, water, and other compounds. The formation of complexes may take place in solutions or when the pigments in the solid state are brought into contact with various gaseous compounds. The process of complex formation is generally reversible.

The ability of metal derivatives of the porphin series to form complexes is much greater than that of the corresponding metal-free pigments. This is apparently due to the ability of certain metal atoms to form additional coordinate bonds as well as valence bonds and to the ability of these atoms to activate the tetrapyrrole ligand with respect to various reactions, including complex formation. The metal atoms in M-derivatives of the porphin series can be divided into three classes [12] according to their coordinating power:

1. Fe, Co, and Mn have a coordination number of 6. In the pigment molecule these atoms are combined with four nitrogen atoms and are able to combine with a further two molecules of a ligand by means of coordinate bonds. The resulting complex has the structure of an octahedron whose centre is occupied by the metal atom and whose apices are occupied by the four nitrogen atoms, joined to the metal atom, and two molecules of a ligand at right angles to the plane of the pigment molecule.

2. Mg, Zn, and Cd have a coordination number of 5 and combine with one additional molecule of a ligand. The complexes have the shape of a four-sided pyramid. The metal atom lies at the centre of the base of the pyramid, the N atoms lie at the corners of the base, and the ligand molecule occupies the fifth apex of the pyramid above the plane of the pigment molecule.

3. Cu and Ni are very weak complex-forming atoms and generally retain the coordination number 4. All free main and coordinate valencies are satisfied by the nitrogen atoms of the tetrapyrrole ring of the pigment molecule. This classification is not rigorous. It is suggested, for example, that in some cases Mg-, Zn-, and even Cu-porphyrins form complexes with an octahedral structure [26,27,48].

As expected, the stability of the complexes decreases with increase in temperature [48,51]. Moreover, it depends to a marked extent on the nature of the central metal atom. Thus direct measurements of the constants for the equilibrium between the monopyridine complexes and free molecules of M-tetraphenylporphin in benzene solution in the presence of small quantities of added pyridine showed that the stability of the monopyridine complexes decreases in the order: Zn, Cd, Hg, and Cu. It is suggested that Mg-tetraphenylporphin and Mg-tetraphenylchlorin, unlike the other metal derivatives studied, form bispyridine complexes under these conditions. On going from porphin to chlorin, there is a slight increase (compared with the change on going from one metal atom to another) in the stability of the pyridine complexes [49].

The formation of complexes by the pigment with a ligand generally leads to changes in the spectroscopic characteristics of both reacting components. These changes are observed not only in the electronic spectra but also in the vibrational spectra [52-55], in the fluorescence spectra [58], and in the photoelectric conductivity [57] of the pigments. The spectroscopic changes on complex formation are more marked, the more stable the resulting complex. They are very marked in the absorption spectra of the Fe-porphyrins, which give particularly stable complexes in their reactions with CO, pyridine, and water [12]. In these cases the appearance of the spectrum of the pigment changes completely. When less stable complexes are formed, the absorption bands are displaced with a more or less marked change in intensity [49,51,52]. Weak interaction between the components in the complex may not produce changes in the electronic spectra of the pigments capable of being recorded at the present stage of development of spectrometric technique. In this case evidence for complex formation is obtained indirectly. For example, in a study of the quenching of the fluorescence of chlorophyll and pheophytin by various oxidising agents and reducing agents it was found that in some cases quenching is observed at concentrations of the quenching agent equal to the concentration of the pigment. Further increase in the concentration of the quenching agent did not lead to an increase in quenching. This phenomenon can apparently be attributed to the formation of complexes between the molecules of the pigment and the quenching agent, although the existence of these complexes is not revealed by the electronic spectra [59].

The electronic absorption spectra of the ligands in complexes with tetrapyrrole pigments have unfortunately not yet been observed, since the pigments and solvents themselves absorb in this range. In the infrared spectra of solid films of M-phthalocyanines it has been possible to observe the absorption bands of indole, aniline, benzylamine, water [52-54], and nitric oxide [55], attached to the pigments from the gaseous phase.

There is apparently no reason to doubt that the central metal atom in molecules of the porphin type is the most probable centre at which a ligand molecule exhibiting electron-donating properties is attached in the formation of a complex. The bond formed between the central metal atom and the ligand molecule is a donor–acceptor bond. This structure for the complex is confirmed by the following evidence: (1) the complexes formed by metal-free pigments with electron-donating molecules are not so stable as those formed by metal derivatives; (2) the frequencies of the absorption bands of aniline, benzylamine, and indole in their complexes with M-phthalocyanines depend to a marked extent on the nature of the metal atom [52,53]; and (3) the magnetic susceptibility of $Fe^{II}$-phthalocyanine decreases on complex formation with NO, indicating pairing of the *d* electron of the Fe atom with the unshared electrons of the N atom of the nitric oxide [55].

The possibility that the ligand molecules are localised at other active centres in the structure of the pigment molecule cannot, however, be denied. These centres include particularly the pyrrole N atoms, which carry unshared electron pairs and which can therefore take part in the formation of donor–acceptor bonds, including the formation of hydrogen bonds of the type N...H–X. In the spectra of the complexes formed by indole with Mg- and Zn-phthalocyanines, in the range of the stretching vibrations of the NH bonds, it is possible to observe two absorption bands instead of the single band characteristic of free indole (solution or solid layer) [52,53]. These bands apparently correspond to complexes of different type: the comparitively narrow high-frequency band corresponds to indole molecules attached to the metal atom and the broad low-frequency band corresponds to indole molecules joined to

the pyrrole nitrogen atom by a hydrogen bond. An analogous explanation can also be given for the spectra of aniline and benzylamine, which in their complexes with certain M-phthalocyanines give more absorption bands in the range corresponding to the NH bonds than in the spectra for the liquid state[52,53]. The fact that the metal-free phthalocyanine is unable to form complexes with indole, aniline, and benzylamine does not mean that the molecules of these compounds in the complexes with M-phthalocyanine are necessarily attached to the metal atom. As already noted, the inclusion of a metal atom in the phthalocyanine molecule leads to a significant change in its properties, and groups which were previously inactive become active. Thus the metal atom activates the phthalocyanine molecule towards HCl molecules, which are undoubtedly attached not to the metal atom but to the nitrogen atom[42].

In organic solvents in the absence of moisture chlorophyll forms stable 1 : 1 complexes with the metal chlorides $FeCl_3$, $HgCl_2$, $BeCl_2$, $AlCl_3$, $BiCl_3$, $NbCl_5$, and $SnCl_2$, and also with $AgClO_4$. These complexes, whose formation leads to marked spectroscopic changes in the pigment, can be isolated from solutions in the solid form[60-62]. With the passage of time, the chlorophyll in complexes with metal chlorides is gradually converted into the corresponding M-pheophytin. This leads to the suggestion that, when the metal ion of the halide forms a complex with chlorophyll, it establishes a bond with the nitrogen atoms of the pyrrole rings, leading to the possibility of conversion of the chlorophyll into M-pheophytin[60].

Let us consider separately the reaction of phthalocyanines and chlorophyll with water. In air Mg-phthalocyanine forms a stable dihydrate[63]. A dihydrate, slightly less stable than the dihydrate of Mg-phthalocyanine, is also formed by Be-phthalocyanine[64]. The infrared spectrum of an Mg-phthalocyanine specimen kept in water vapour, in the range of the stretching vibrations of hydroxy-groups, shows an absorption band consisting of several components[54]. The characteristic isotopic displacement of this band when $H_2O$ is replaced by $D_2O$ and its disappearance on treatment in a vacuum at 50–100° show unambiguously that this band is due to molecules of water in a complex with Mg-phthalocyanine. Since on treatment of the specimen in a vacuum the components of this band do not disappear simultaneously from the spectrum[54], it may be assumed that the two water molecules in the Mg-phthalocyanine dihydrate are joined to the pigment in different ways. This difference is possibly due to a difference in the localisation of the water molecules in the complex. It is possible to picture a scheme in which one water molecule is coordinated to the magnesium atom via its oxygen atom. The resulting complex has a pyramidal structure in which the magnesium atom has a coordination number of 5. The second water molecule is joined to the nitrogen atoms of the Mg-phthalocyanine molecule by a hydrogen bond on the other side of the pigment molecule.

The stepwise character of the desorption of the water molecules from Mg-phthalocyanine may be given a different explanation, according to which the two water molecules are joined to the central magnesium atom to form a complex with an octahedral structure. As the water is desorbed, the Mg-phthalocyanine dihydrate is converted first into a monohydrate with a pyramidal structure and then into the anhydrous pigment. The states of the water molecules in the Mg-phthalocyanine dihydrate and monohydrate should naturally be different, leading to spectroscopic differences in the infrared range. In this interpretation, however, it is difficult to explain the extremely complex structure of the absorption band of the water joined to Mg-phthalocyanine, so that the first explanation (a difference in the localisation of the two water molecules in Mg-phthalocyanine dihydrate) is to be preferred.

Like Mg-phthalocyanine, chlorophyll adds on water molecules[1]. In this case, however, we cannot speak of the existence of hydrates with definite composition: the decisive feature is the conditions under which the reaction between chlorophyll and water takes place[29]. The infrared absorption spectrum of the water in the complex with chlorophyll depends to a marked extent on the state of the pigment. In the spectra of chloroform solutions and amorphous chlorophyll film the structureless band at 3400 $cm^{-1}$ is similar to the absorption band for liquid water[24]. In the spectrum of a crystalline film of chlorophyll *a* the absorption band for the water, like the absorption band for water joined to Mg-phthalocyanine, consists of several components[31]. This suggests that the localisation of the water molecules is approximately the same in the complexes with chlorophyll and Mg-phthalocyanine. The absence of structure in the absorption band for water joined to chlorophyll in solutions and in the amorphous film can be attributed to the interaction with one another of the water molecules in neighbouring complex molecules. This interaction is reduced to a considerable extent when the complexes are arranged in ordered fashion in the crystalline specimens.

It is suggested that, on contact with chlorophyll, the water molecules may react with the carbonyl group of the cyclopentanone ring[65,66]. This possibility cannot be denied, but the reaction of a water molecule with the keto-group of the cyclopentanone ring is apparently insufficiently strong and does not determine the location of the molecule in the complex with chlorophyll. As in the complex with Mg-phthalocyanine, the water molecules are apparently localised at the N and Mg atoms of the chlorophyll molecule. This suggestion is confirmed by the following evidence: (1) complexes with water are formed not only by chlorophyll, but also by Mg-phthalocyanine, which has no cyclopentanone ring; and (2) although pheophytin and Cu-pheophytin, like chlorophyll, have a cyclopentanone ring, they form only comparatively weak complexes with water[24]. This cannot be attributed to the influence of the central magnesium atom on the cyclopentanone ring, since the similarity in the absorption bands of the keto-groups of chlorophyll, pheophytin, and Cu-pheophytin in the infrared range[24] shows that the influence of the metal atom on the keto-group of the cyclopentanone ring is slight. In the complexes with pheophytin and Cu-pheophytin water molecules are apparently localised at the pyrrole nitrogen atoms.

The structure of the absorption bands for water in complexes with Mg-phthalocyanine[54] and crystalline chlorophyll[31] in the range of its stretching vibrations indicates that strong distortion of the $H_2O$ molecule takes place when it is attached to the pigment. In particular, the presence of broad components extensively displaced towards lower frequencies in the structure of these bands indicates a considerable decrease in the strength of the bonds between the H atoms and O atoms in the water molecules, that is, it indicates a partial positive charge on the H atoms in these molecules. In the green leaf this "dark" partial dissociation of the water molecule possibly precedes the fundamental photochemical step leading to complete splitting of the $H_2O$ molecule followed by the formation of the primary products of photosynthesis.

3. Complexes with charge transfer. In some cases derivatives of the porphin series form complexes, illumination of which leads to transfer of an electron from one of the components of the complex to another. In the process the neutral complex acquires an ionic structure, whose existence, however, cannot be prolonged, since the structure is energetically unstable, and the electron is returned with the liberation of a quantum of energy in the form of heat[67]. A charge-transfer complex is detected spectroscopically by the appearance of a specific absorption band which corresponds to the transition between the energy levels characterising the complex in the neutral ground state and in the excited state with ionic structure.

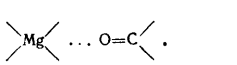
The study of charge-transfer complexes of the porphin series is extremely important, since these complexes may be intermediates in various photochemical reactions of the pigments. Only preliminary work has as yet been carried out in this field, however. It has been found that etioporphyrin, tetraphenylporphin, and Zn-tetraphenylporphin in solution form 1 : 1 complexes with trinitrobenzene. The system absorbs in the region of 500 mμm, which has been attributed to charge transfer in the complex[68]. 1 : 1 charge-transfer complexes are also formed by Zn-phthalocyanine with trinitrobenzene, trinitrotoluene, and other compounds[69]. In all these cases the partners of the porphin derivatives in the complexes are typical electron acceptors, so that in the excited state the pigment molecule forms a positive ion. Since porphin derivatives are amphoteric, we may expect the existence of complexes in which electron transfer takes place from the ligand molecule to the pigment molecule. Electron-donor ligands include triethylamine and ammonia, in the presence of which the spectra of solutions of Mg-etioporphyrin II show a new absorption band, attributed to charge transfer in the complex[70]. The correctness of this interpretation is doubtful, however, since the position of this band in the spectrum (640 mμm) is the same for the complexes formed by the pigment with ammonia and triethylamine, which have quite different ionisation potentials.

4. Association. Association of the molecules of tetrapyrrole pigments with one another is observed in the solid state and in solutions. It is detected in the vibrational and electronic emission and absorption spectra.

In the solid phase phthalocyanines have a crystalline structure; two crystalline modifications, referred to as the α- and β-modifications, are known. The α-modification is less stable and on heating is converted into the β-form. The crystalline structure of phthalocyanine is clearly revealed in the vibrational[20,71,72] and electronic absorption spectra and in the photoconductivity and photoelectromotive force spectra for layers of the pigment[73]. Comparison shows that the infrared spectrum of the β-modification of phthalocyanines is richer than the spectrum of the α-modification with respect to the number of absorption bands and their relative intensity. This is apparently due to the closer packing of the phthalocyanine molecules in the β-form, leading to stronger intermolecular interaction than in the α-form. As a result of the intensification of these interactions, conversion of the α-modification into the β-modification leads to the appearance, in the spectrum of the specimen, of new absorption bands, forbidden for the α-modification. The frequency of the stretching vibrations of the NH groups in the metal-free phthalocyanine decreases in the $\alpha \rightarrow \beta$ conversion, again indicating an increase in the intensity of the intermolecular interactions, in which the imino-groups evidently take part[20]. It is suggested that in the Cu-phthalocyanine crystal (β-modification) the pigment molecule is arranged in such a way that the copper atom interacts with two peripheral nitrogen atoms of neighbouring molecules situated on either side of the plane of the given molecule[74].

Solid chlorophyll, chlorophyllide, and bacteriochlorophyll may exist in either the amorphous or the crystalline state. The spectroscopic characteristics of these states differ appreciably. Thus the red absorption band for chlorophyll *a* lies at 670–675 mμm in the spectrum of the amorphous film and at 692 mμm and 740 mμm in the spectra of the two different crystalline modifications of chlorophyll *a*[75]. Marked differences between amorphous and crystalline specimens of chlorophyll *a* are observed in the infrared absorption spectra[23,24,31]. Unfortunately, the fact that no reliable interpretation has been given for these spectra means that as yet they tell us nothing of the nature of the interaction between the chlorophyll molecules in the solid state.

Association of chlorophyll molecules is observed not only in the solid state but also in solutions. Direct measurements of the molecular weights show that chlorophyll *a* in pure non-polar solvents ($CCl_4$, benzene, and chloroform) exists in the form of dimers, whereas chlorophyll *b* in benzene forms trimers. In polar solvents, or in non-polar solvents in the presence of small quantities of polar compounds, breakdown of the association complexes takes place and the pigments are present chiefly as monomers. Pheophytin does not form association complexes in either polar or non-polar solvents[26,27]. Association of the pigments leads to changes in their infrared absorption spectra. In particular, there is a decrease in the intensity of the 1700 $cm^{-1}$ band corresponding to the vibrations of the C=O keto-group in position 9 in the cyclopentanone ring, and a new band appears at 1665 $cm^{-1}$§. This band has been interpreted in different ways. We can apparently reject the interpretation which relates this band to the vibrations of enolised chlorophyll molecules[23-25,30,31], since the proportion of such molecules is small under ordinary conditions (see section II). According to another viewpoint[26-28], the 1665 $cm^{-1}$ band corresponds to the vibrations of the keto-group of the cyclopentanone ring, taking part in the formation of chlorophyll dimers according to a mechanism in which the keto-group is attached by a coordinate bond to the central Mg atom of a neighbouring pigment molecule:

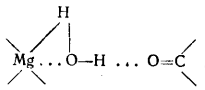



(XVIII)

Vacuum drying of layers of amorphous chlorophyll, however, leads to a decrease in the intensity of the band at 1665 $cm^{-1}$.[65,66,76] Moreover, the intensity of this band in the spectrum of crystalline chlorophyll (in this case the band is displaced to 1650 $cm^{-1}$) decreases when the specimen is vacuum-treated and increases again when the specimen is brought into contact with water vapour[31]. These experimental facts are difficult to explain on the basis of the theory that chlorophyll is associated according to mechanism (XVIII). Since the increase in the intensity of the 1665 $cm^{-1}$ band is always accompanied by a decrease in the intensity of the 1700 $cm^{-1}$ band, the suggestion that the 1665 $cm^{-1}$ band is due to keto-groups

§ Katz et al.[26] give for the position of this band in the spectrum the figure 1650 $cm^{-1}$.

perturbed as a result of intermolecular interactions is convincing. The structure of the chlorophyll association complexes in which this perturbation of the keto-groups takes place is not yet clear, however. In some cases a water molecule is apparently one of the components in the association complexes formed by the chlorophyll molecules. It may be suggested that in crystalline chlorophyll a water molecule attached to the central Mg atom of one of the pigment molecules interacts simultaneously, by means of its partially charged (positively) H atom (see section III, *2*), with the keto-group of a neighbouring pigment molecule according to the mechanism:




(XIX)

In the absence of combined water, association of chlorophyll possibly also takes place according to mechanism (XVIII). The absorption bands of keto-groups associated according to mechanisms (XVIII) and (XIX) have different positions in the spectrum: 1665 $cm^{-1}$ and 1650 $cm^{-1}$ respectively (when crystalline chlorophyll is vacuum-dried, the decrease in the intensity of the 1650 $cm^{-1}$ band is accompanied[31] by its displacement to 1665 $cm^{-1}$).

The important part played by water molecules in the association of chlorophyll is confirmed by the fact that crystallisation of the pigment takes place only in the presence of water[1]. The ability to form association complexes with the participation of water molecules is characteristic not only of Mg-derivatives of porphin, but also of certain other M-derivatives of this class of compound. It is suggested, for example, that similar association complexes are formed in the presence of moisture in an alcoholic solution of $Fe^{III}$-pheophorbide[77].

In the presence of polar molecules in chlorophyll solution, the 1665 $cm^{-1}$ absorption band disappears completely and the intensity of the 1700 $cm^{-1}$ band increases. This is attributed to the fact that the polar molecules form coordinate bonds with the Mg atom and lead to disaggregation of the chlorophyll molecules[26]. In addition to changes in the infrared spectrum, the disaggregation of the chlorophyll in solution also leads to significant changes in the electronic spectra: there is an increase in the intensity of the luminescence, which is generally characteristic only of monomeric molecules, and an increase in the intensity of the bands in the absorption spectrum[78,79]. Similar phenomena are observed not only for chlorophyll, but also for a number of other M-derivatives of the porphin series: Mg-phthalocyanine[79], bacterioviridin[80], Ca-, Mg-, and Zn-derivatives of mesoporphyrin, and Zn- and Ca-pheophytins[81].

It was previously mentioned that pheophytin does not associate in either polar or non-polar solvents. Association of the molecules of the pigment can be brought about, however, by adding to its solution a liquid in which the pigment is insoluble. In this way it has been possible to carry out spectroscopic studies of the association of pheophytin, and also chlorophyll, chlorophyllide, and Mg-phthalocyanine in polar solvents[11,82,83]. Gradual increase in the water content of the solution leads to the formation of first dimers and then polymeric aggregates. The red absorption band at 665 mμm for pheophytin in alcoholic solution is displaced to 695 mμm when dimers are formed[82]. The formation of chlorophyll dimers under these conditions leads to a displacement of the red band by only 2 mμm (from 666 mμm to 668 mμm); the formation of chlorophyllide dimers[82] leads to a displacement of 22 mμm (from 630 mμm to 652 mμm). It is suggested[11,82] that, when pheophytin dimerises, the planes of its molecules come into contact. A result of this interaction is the marked bathochromic shift of the pheophytin absorption band on dimerisation. The association of chlorophyll takes place with the direct participation of water molecules, which, by forming coordinate bonds with the Mg atom, do not allow the pigment molecules to approach one another to distances sufficiently short for $\pi$-electron interaction. The absence of the phytyl radical from the chlorophyllide molecule makes this interaction possible, despite the presence of combined water.

As expected, increase in temperature leads to disaggregation of the pigments in solutions[82]. Conversely, decrease in the temperature of the solutions to -100° and below leads to dimerisation of chlorophyll molecules even in such polar solvents as alcohol[84,85]. The degree of aggregation of the molecules of tetrapyrrole pigments in solutions also depends on the concentration. This was shown for the trimethyl ester of coproporphyrin by NMR spectroscopy[86].

The type of interaction between molecules of the porphin type in their associates are not limited to the cases examined above. It is evident that in view of the large number of and variety of substituents in the porphin derivatives, they may form association complexes of different structures, depending on the conditions and the properties of the substituents[11].

Thus the problem of the structure of the molecular compounds formed by pigments of the porphin type, and the problem of their role in biochemical processes, are far from being solved. Further spectroscopic studies, including NMR and ESR studies, are necessary. The value of spectroscopic studies will be greatly increased if they are accompanied by kinetic measurements and by chemical analysis of the pigments and the products of their interaction with the environment.

## IV. HYDROGEN EXCHANGE IN COMPOUNDS OF THE PORPHIN SERIES

Is it possible for the chlorophyll molecule to exchange its hydrogen atoms with the molecules of the medium, in particular with $H_2O$ molecules? It first became possible to answer this question, which is of fundamental importance for an understanding of the chemical properties and sensitising action of chlorophyll in photosynthesis, when tracer methods were developed. In the molecule of chlorophyll *a* (formula X, *a*) all the hydrogen atoms are joined to carbon atoms, which, unlike O, N, and S atoms, do not carry a free pair of electrons in a neutral molecule. In this case hydrogen exchange with water requires a high activation energy. The latter may be decreased in the presence of a catalyst or when the molecule contains electronegative substituents which increase the lability of the hydrogen atoms[87,88] in adjoining CH bonds.

The results of the first work on isotopic exchange between chlorophyll and $D_2O$ or $T_2O$ did not lead to an unambiguous conclusion regarding the possibility of hydrogen exchange between chlorophyll and water[89-95]. Even when isotope exchange was detected, it was not clear whether this involved the H atoms belonging to chlorophyll itself or the H atoms of the water molecules firmly bound to the pigment[94]. The possibility of H → D isotope

exchange between chlorophyll or pheophytin molecules and $D_2O$ molecules was established finally and unambiguously when the products of the interaction of the pigments with $D_2O$ were analysed by infrared spectroscopy[21,43], instead of by measurement of the radioactivity or by the mass-spectrometric method previously employed[89-92]. When films of chlorophyll or pheophytin were brought into contact with $D_2O$ vapour, their absorption spectra showed changes which could be attributed only to H → D exchange involving the hydrogen atoms belonging to the pigment molecules themselves. The nature of the spectroscopic changes taking place during exchange established that the location of the exchanging atoms is the same for the chlorophyll and pheophytin molecules. As expected, pheophytin shows deuterium exchange with the hydrogen atoms of the imino-groups[21]¶ in addition to exchange involving the labile atoms, which are also present in chlorophyll. It was found, moreover, that the nature of the spectroscopic changes in the H → D isotope exchange and hence the location of the exchanging atoms in the pigment molecules are the same for exchange[43] involving $CH_3OD$, $D_2O$, or $CH_3COOD$.

Unfortunately, difficulties in the interpretation of the infrared absorption spectra make it impossible to reach unambiguous conclusions regarding the location of the exchanging atoms in the molecules of chlorophyll and its analogues. The use of NMR spectroscopy has led to considerable progress towards a solution of this problem. Woodward and Škarić[17,96,97] found by NMR spectroscopy that chlorins, unlike porphyrins, are highly sensitive to electrophilic attack in the γ- and δ-positions, that is at the methine bridges adjoining the hydrogenated pyrrole ring of the pigment molecule. This property is revealed, in particular, by the fact that in the presence of $CH_3COOD$ chlorins readily undergo deuterium exchange involving the H atoms in the γ- and δ-positions. Such exchange does not take place in porphyrin molecules. This phenomenon is evidently due to increased electron density in the γ- and δ-positions in the chlorin molecules. Theoretical calculations of the electronic structure in fact show that all the methine bridges of porphin have the same electron density deficit. In the chlorin molecule this deficit is increased in the α- and β-positions but an excess of electron density appears in the γ- and δ-positions[98].

When powerful reagents are used ($CF_3COOD$, $D_2SO_4$), isotope exchange in the methine bridges becomes possible even for porphyrin molecules[99], apparently as a result of redistribution of the electron densities in the pigment molecules in the presence of the strong acids.

Woodward's work naturally led to experiments on isotope exchange in chlorophyll and its analogues using NMR spectroscopy. These experiments not only gave independent proof of the possibility of hydrogen exchange in the chlorophyll molecule but also made it possible to solve the problem of the location of the exchanging H atoms. It was shown[32,33,100-102] that, as a result of exchange with deuteriated alcohols, the peaks in the NMR spectra of chlorophyll, pheophytin, and other analogues of chlorophyll, corresponding to the protons in the δ-position and in position 10, disappear, that is, it is in these positions that H → D isotope exchange takes place. The rates of the isotope exchange are different for positions δ and 10. The rate of exchange is also influenced to a marked extent by the presence of the central magnesium atom in the structure of the molecule[33,101]; this is evidently due to a difference in the densities of the electron clouds close to the exchanging H atoms in the molecules of the metal-free and metal-containing pigments.

According to NMR spectroscopy, in the presence of deuteriated alcohols, the H atoms of all three unsubstituted methine bridges in molecules of bacteriochlorophyll (XI) undergo exchange with deuterium[101]. In the presence of deuteriated trifluoroacetic acid, the H atoms of the three methine bridges adjoining the hydrogenated rings in the molecules of octaethylbacteriochlorin with the *cis*-arrangement of the hydrogenated pyrrole rings undergo exchange with deuterium. The rate of exchange of the H atom adjoining two hydrogenated rings is greater than the rate of exchange of the H atoms which adjoin only one hydrogenated pyrrole ring[99].

The rate of isotope exchange in the molecules of chlorophyll and its analogues depends to a marked extent on the acid or basic nature of the medium in which the exchange takes place[21,43,101,102]. More detailed studies of the dependence of the kinetics of exchange on the properties of the pigment and the medium will reveal the mechanism of the isotope exchange, which is not yet clear.

Experiments not directly related to the problems under consideration have been carried out on the biosynthesis of chlorophyll in media containing tracer atoms. It has been possible to prepare completely deuteriated chlorophyll from seaweed grown in almost pure $D_2O$ (99.8%). The absorption spectra of this chlorophyll were recorded in the visible and infrared ranges[103]. Unfortunately, these spectra revealed nothing new for the interpretation of the infrared absorption bands of the pigment. The chlorophyll in chlorella grown in the presence of $T_2O$ shows radioactivity which does not decrease when the chlorophyll is illuminated or treated with alcohols or alkali. The radioactivity decreases, however, when the pigment is oxidised with silver using Fischer's method, in which it is assumed that the hydrogenated pyrrole ring of the chlorophyll molecule is oxidised[104]. If this is the case, it may be concluded that the hydrogenated chlorophyll ring is unable to undergo hydrogen exchange with alcohol, alkali, or water.

## V. HYDROGENATION AND DEHYDROGENATION OF THE PYRROLE RINGS IN COMPOUNDS OF THE PORPHIN SERIES

Despite the fact that chlorophyll has recently been synthesised[105-107], the route by which it is formed in the green leaf is not yet clear. It has been proved, for example, that, when etiolated leaves containing protochlorophyll are illuminated, the protochlorophyll is converted almost quantitatively into chlorophyll[1]. Attempts to reproduce this reaction under artificial conditions[108,109], and attempts to carry out the hydrogenation of chlorophyll to bacteriochlorophyll, however, have not yet been successful. In order to investigate the possibility and mechanism of hydrogenation of the pyrrole rings in the molecules of

¶ Isotope exchange in the imino-groups of molecules of the porphin series has been observed repeatedly in studies of the infrared spectra of these compounds[19,21,41]. Since, however, the ability to undergo rapid hydrogen exchange in N–H bonds is a general property of NH-containing molecules, and since there is no reason to distinguish in this connection the tetrapyrrole pigments from other NH-containing compounds, exchange in the imino-groups is not examined here.

chlorophyll and its analogues, it is natural to begin the study with the simplest compound of the porphin series.

1. Dark hydrogenation. The hydrogenation of some porphyrins has been carried out in alcoholic solutions in the presence of sodium [15,16,110-117], in a pyridine + + acetic acid mixture in the presence of zinc [117], in ether or dioxan in the presence of Raney nickel [15], and in pyridine containing hydrazine [118]. Theoretical calculations [119], chemical analysis, and comparison of the spectra of the porphyrin hydrogenation products with the spectra of compounds of known structure have established that these products are the corresponding chlorins, bacteriochlorins, and hexahydro- and octahydro-porphyrins. The reaction of chlorophyll with hydrazine in pyridine solution takes place in two stages. Spectroscopic data indicate that the cyclopentanone ring of the pigment molecule is broken in the first stage and that hydrogenation of the molecule takes place in the slower second stage, leading to the formation of the analogue of bacteriochlorophyll with neighbouring hydrogenated pyrrole rings and a closed cyclopentanone ring [118].

An important feature is that the hydrogenation of the tetrapyrrole pigments was carried out in solutions containing dissolved oxygen. The hydrogenation of tetraphenylporphin, Zn-tetraphenylporphin, and chlorophyll by hydrazine takes place readily in air but not in degassed solutions [118]. The rate of hydrogenation depends on the temperature of the solution and the structure of the pigment molecule. In particular, the reaction rates are different for the metal-free and metal-containing molecules and in the case of the latter they also depend on the nature of the central metal atom [115,117].

2. Photochemical hydrogenation. As in the case of dark hydrogenation, the photochemical hydrogenation of porphyrins in the presence of reducing agents leads to the formation of the corresponding chlorins, bacteriochlorins, and more extensively hydrogenated derivatives [120-123]. The difference lies in the mechanism of the reaction, which takes place readily in degassed solutions under illumination but which in the dark takes place only in the presence of dissolved oxygen (as shown by experiments with tetraphenylporphin, Zn-tetraphenylporphin, and chlorophyll [118]). It may be assumed that the dark hydrogenation is possible only in the presence of molecular complexes formed by the pigment or reducing agent with oxygen, whose formation decreases the activation energy for the introduction of additional H atoms into the pigment molecule. The photochemical hydrogenation evidently involves an optically excited state of the molecule [120], in which the molecule becomes active in the absence of oxygen.

It has already been pointed out that in experiments *in vitro* it has not yet been possible to carry out the hydrogenation of the pyrrole rings of protochlorophyll and chlorophyll without changing the structure of the remainder of the molecule. The assumed formation of the analogue of bacteriochlorophyll with the *cis*-arrangement of the hydrogenated pyrrole rings by the action of hydrazine on chlorophyll [118] is accompanied by irreversible rupture of the cyclopentanone ring of the pigment molecule. It was recently found that, when a concentrated degassed alcoholic solution of chlorophyll containing phenylhydrazine is illuminated, the main absorption bands of the pigment are displaced towards shorter wavelengths [124]; this is analogous to the displacement observed on going from chlorin to bacteriochlorin with the *cis*-arrangement of the hydrogenated pyrrole rings [15,119]. The spectrum of the original chlorophyll is regenerated when the solution is brought into contact with air [124]. It may be assumed that this reaction leads to the formation of the *cis*-isomer of bacteriochlorophyll with a closed cyclopentanone ring, which is insufficiently stable and is oxidised to the original chlorophyll on contact with atmospheric oxygen.

If the properties of the medium are close to those characteristic of green plants, it is possible to select conditions under which protochlorophyll undergoes photochemical reduction to chlorophyll [109]. It is believed that appropriate selection of the medium will make it possible to carry out the further hydrogenation of chlorophyll to bacteriochlorophyll with the *trans*-arrangement of the hydrogenated pyrrole rings, which is characteristic of this pigment. This hydrogenation apparently takes place when the water-soluble "667 protein chlorophyll" is illuminated, leading to a decrease in the intensity of the absorption bands of the original pigment and the production of new bands [125] at 362, 567, and 743 mμm, closely resembling the bands due to natural bacteriochlorophyll.

As in the case of dark hydrogenation, the ability to undergo photochemical hydrogenation depends to a marked extent on the structure of the pigment molecule. For example, illumination of Zn-tetraphenylporphin in the presence of reducing agents leads to its ready conversion into dihydro- and tetrahydro-derivatives [118,120], whereas photochemical hydrogenation of metal-free tetraphenylporphin and tetraphenylchlorin does not take place under the same conditions [34,35].

3. Dehydrogenation of chlorins and bacteriochlorins. The hydrogenation of the pyrrole rings of tetrapyrrole pigments is reversible: the action of oxidising agents under suitable conditions may lead to the conversion of bacteriochlorins into chlorins and of chlorins into porphins. This change can be carried out in the dark or photochemically. For example, unsubstituted chlorin is dehydrogenated to porphin when its solutions are heated in the presence of various quinones [126]. Metal derivatives of chlorin and bacteriochlorin are dehydrogenated much more rapidly than the metal-free pigments [16,126]. Dehydrogenation of bacteriochlorins takes place more readily than the dehydrogenation of chlorins. For example, the *cis*-isomer of octaethylbacteriochlorin is converted immediately into octaethylchlorin by treatment with mineral acids [16], and tetraphenylbacteriochlorin is oxidised to tetraphenylchlorin even by passage through a chromatographic column of talc.[15]

The photochemical dehydrogenation of chlorins by quinones apparently takes place when the pigment molecules are in an optically excited triplet state [119,121,127-129].

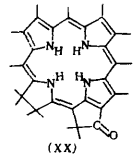
## VI. REVERSIBLE PHOTOCHEMICAL REDUCTION OF COMPOUNDS OF THE PORPHIN SERIES

From the time of Krasnovskii's discovery (1948) of the reversible photochemical reduction of chlorophyll [8,130], this reaction has been extensively studied. In particular, it has been established that this reaction is characteristic not only of chlorophyll, but also of many other compounds of the porphin series, and that the final result of the photoreduction is the attachment to the pigment molecule of two or more H atoms which can be readily removed in the presence of an oxidising agent, or even without oxidising

agents, with the regeneration of the original pigment molecule. Up to the present, however, no agreement has been reached regarding the molecular structure of the products of the photoreduction of chlorophyll and other tetrapyrrole pigments. Without going into questions of the mechanism of photoreduction (the formation of primary and secondary photoreduced forms, the influence of the properties of the medium, the part played by complex formation in photoreduction, the influence of the structure, particularly of the central metal atom, on the ability of the pigment molecule to undergo photoreduction, and other topics), we shall concentrate attention in this section on the molecular structure of the photoreduced forms of compounds of the porphin series.

For the red photoreduced form of chlorophyll, Rackow and König proposed a structure in which, as a result of the photoreaction, one H atom is attached to one of the central N atoms and the second is attached to the cyclopentanone ring of the pigment molecule so that the C=O keto--group of the cyclopentanone ring is converted into a C–O–H carbinol group [131]. Serious objections to this structure can be put forward, however. Firstly, the ability to undergo photoreduction is shown not only by chlorophyll but also by a whole series of other tetrapyrrole pigments which do not contain a cyclopentanone ring. Secondly, the infrared absorption band corresponding to the stretching vibrations of the C=O group of the cyclopentanone ring in pheophytin does not disappear from the spectrum after photoreduction of the pigment [132], as would have been expected if the keto-group had been converted into a carbinol group.

Sidorov and Terenin [132] were able to isolate the photoreduced form of pheophytin from solution and to measure its infrared absorption spectrum. On the basis of the spectroscopic data obtained, they proposed the following structure (XX) for the photoreduced form of pheophytin:

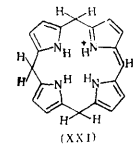


(XX)

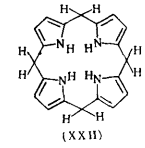
Objections to this structure were also put foward, since it was shown that in the photoreduction of mesoporphyrin and tetraphenylporphin the degree of luminescence polarisation of the pigment increases, and this usually corresponds to a decrease in the symmetry of the structure of the luminescent molecules [133,134]. The molecule with structure (XX), however, has a higher symmetry than that of the original pheophytin molecule. Infrared spectroscopy cannot yet give us sufficient information to resolve unambiguously the problem of the structure of the photoreduced porphins. There is also insufficient foundation, however, for the objections to structure (XX) on the basis of polarisation measurements. The molecules of the reduced pigments show an increased tendency to form complexes with the molecules of the medium, leading to appreciable perturbation of the electronic structures of the interacting molecules [122]. These perturbations can undoubtedly lead to breakdown of the symmetry of the molecules and hence to an increase in the degree of luminescence polarisation. From this follows that a high degree of luminescence polarisation, indicating low symmetry of the electronic structures, does not provide sufficient grounds for excluding the possibility of high symmetry for the atomic configurations of the molecules of the photoreduced forms.

On the basis of recent data, it may be suggested that on photoreduction different compounds of the porphin series give products with different molecular structures or, in other words, that the location of the H atoms attached as a result of the photochemical reaction is different for different tetrapyrrole pigment molecules [35,123,135]. The following main classes of porphin molecule can be distinguished, depending on the structure of the photoreduced forms: porphyrins, chlorins, metal derivatives of the porphin series, and phthalocyanines. The number of these classes apparently does not limit the number of possible structures for the photoreduced forms.

Most workers at present ascribe to the molecules of the photoreduced forms of porphyrins a structure of the phlorin type (XIII) [34,97,123,136,137]. The photoreduction of porphyrins often does not stop at the attachment of only two H atoms but proceeds further, leading to the formation of tetrahydro- and hexahydro-porphyrins. According to Mauzerall [136], the latter have the structures of porphomethene (XXI) and porphyrinogen (XXII) respectively:



(XXI)



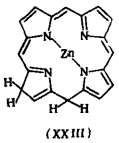
(XXII)

In a study of the properties of various compounds of the porphin series, Gurinovich concluded that the introduction of substituents in the pyrrole rings (positions 1–8) in porphin makes the photoreduction of the pigments more difficult. Tetrabenzoporphin and phthalocyanine do not undergo photoreduction under conditions in which other porphin derivatives are fairly readily reduced [133,138]. This fact, the polarisation studies discussed above [133,134], and certain general chemical considerations led to the suggestion that in the photoreduction the H atoms are attached not to methine bridges, where substitution does not reduce the reactivity of the pigment with respect to photoreduction, but to the pyrrole rings. In the process the two H atoms are attached not to a single pyrrole ring, as in the case of the hydrogenation of porphin to chlorin, but to two rings, with a corresponding redistribution of the double bonds in the molecule [133,134,138,139]. Without denying that hydrogenation of the pyrrole rings is possible in principle in the photoreduction of compounds of the porphin series, one must point out some unsatisfactory features of the arguments put forward in support of this photoreduction mechanism. It is natural that pigments with different structures should have different reactivities with respect to photoreduction under identical conditions. The fact that tetrabenzoporphin and phthalocyanine do not undergo photoreduction under the usual conditions for this reaction does not mean that these pigments cannot be reduced under any conditions. For example, Mg-phthalooyanine gives a photoreduced form under certain conditions [8]. It is then difficult to imagine that the reduction takes place at the pyrrole rings, which in the phthalocyanines are fused with benzene rings.

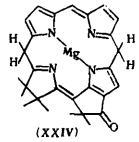
The infrared absorption spectra indicate that the structures of the photoreduced forms of tetraphenylporphin and tetraphenylchlorin differ significantly [34,35]. The photoreduction of tetraphenylporphin leads to the appearance of

an absorption band in its spectrum at about 2600 $cm^{-1}$, which can be assigned to the stretching vibrations of the newly-formed C–H bonds. This absorption band is not observed in the photoreduction of tetraphenylchlorin. The infrared spectrum of the photoreduced form of tetraphenylchlorin resembles in many respects the spectrum of its hydrochloride. This leads to the idea that, like the protons in the formation of the hydrochloride, in the photoreduction of the tetraphenylchlorin molecule the H atoms are attached to its two central N atoms. In other words, the molecule of the photoreduced form of tetraphenychlorin has a structure analogous to that proposed for the photoreduced form of pheophytin (XX). The most probable structure of the photoreduced form of tetraphenylporphin is the phlorin structure. Whether this difference is general for all porphyrins and chlorins is an open question. It is believed that differences should exist in the structures of the photoreduced forms of metal-free pigments and their complexes with metals.

Seely and Talmadge [123] consider it improbable that in the photoreduction of M-derivatives of the porphin series the H atom is attached to a central nitrogen atom joined to the M atom. For the photoreduced form of Zn-porphin, they proposed the structure (XXIII), assuming this to be one of the intermediate forms in the conversion of Zn-porphin into Zn-chlorin. There are as yet no direct experimental facts confirming or refuting structure (XXIII) proposed for the photoreduced form of Zn-porphin. It may, however, be held that this form is not necessarily an intermediate in the conversion to Zn-chlorin. This is indicated by the absence of an intermediate photoreduced form in the photohydrogenation of Zn-tetraphenylporphin to Zn-tetraphenylchlorin in the presence of benzoin or hydrogen sulphide [120–122], although it is known that under illumination in the presence of hydrazine Zn-tetraphenylporphin gives a photoreduced form [122] similar to the photoreduced form of Zn-porphin. It must apparently be assumed that the photohydrogenation of porphins to chlorins may take place without the formation of an intermediate reduced form similar to the photoreduced form of Zn-porphin and Zn-tetraphenylporphin. The photoreduced forms of M-derivatives of porphin, which show an increased reactivity, may undergo various changes depending on the conditions. Chlorins are only one of the most stable final products of these changes.




(XXIII)




(XXIV)

After comparing the spectra of the photoreduced form of ethyl chlorophyllide *a* with the spectra of biliverdin and mesobiliviolin, Seely and Folkmanis [135] concluded that the molecule of photoreduced ethylchlorophyllide does not contain a ring of conjugated bonds covering more than two pyrrole rings. On this basis the photoreduced form of ethyl chlorophyllide was assigned the structure (XXIV) with two hydrogenated methine bridges in the $\beta$- and $\delta$-positions. According to the suggestion of Seely and Folkmanis, the photoreduced form of pheophorbide (metal-free chlorophyllide) has two spectroscopically different tautomeric structures: the phlorin structure and a structure similar to structure (XXIV).

To summarise this section, it may be concluded that there are as yet insufficient experimental data for a final solution of the problem of the structure of the photoreduced forms and that all proposed molecular structure schemes are to some extent hypothetical. Further progress in the determination of these structures requires the continuation of systematic spectroscopic studies of the photoreduction of compounds of the porphin series, accompanied by kinetic measurements and chemical analyses.


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