

## Chlorophyll

THE green colouring matter of plants is a wax-like material of complex chemical structure to which the name chlorophyll was given by Pelletier and Caventou in 1817. It is insoluble in water but soluble in alcohol, ether and other organic solvents. Early investigations of chlorophyll which are important are those of Brewster and Stokes, on the absorption spectrum and fluorescence, and of Edward Schunck on the chemical side. Schunck studied particularly the action of acids on chlorophyll and found that important changes in its physical and chemical properties resulted. The first really fundamental investigations on the chemical structure of chlorophyll were those of Willstätter and his collaborators<sup>1</sup> which showed that there are two green pigments present in leaf-green, namely, chlorophyll-*a*, with the formula  $C_{55}H_{72}O_5N_4Mg$ , a bluish-black solid giving greenish-blue solutions; and chlorophyll-*b*, a greenish-black solid giving pure green solutions.

On hydrolysis, chlorophyll yields an alcohol, phytol,  $C_{26}H_{46}O$ , which has been synthesised, and a complex containing four pyrrole nuclei, the composition of which has been determined by examining the decomposition products phytychlorin-*e* from chlorophyll-*a* and phytyrhodin-*g* from chlorophyll-*b*, these two substances being usually called now chlorin-*e* and rhodin-*g*. By the action of acids on chlorophyll, a product free from magnesium, called phæophytin, is obtained, separable into two components, *a* and *b*. This in its turn, on treatment with hydrochloric acid, yields two phæophorbides, *a* and *b*. By the further degradation of chlorophyll and its derivatives many products are obtained, known as porphyrins.

Since Willstätter's pioneer work, the investigation of chlorophyll and its derivatives has been continued mainly by Hans Fischer<sup>2</sup>, who has carried out some important syntheses, and by J. B. Conant<sup>3</sup>, and their collaborators. The results obtained by these two groups of investigators sometimes differ in points of detail<sup>4</sup>. The present article has the object merely of recording some recent new work on the subject, the present position of which must be sought in the sources given in the references.

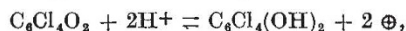
A method which has been used in attempts to determine the relations between different substances is hydrogenation. Dietz and Werner<sup>5</sup> now propose to discard this method as leading to very puzzling results difficult to reconcile with other reactions of the substances. Thus, two porphyrins which appear to be isomeric, rhodoporphyrin and isorhodoporphyrin, differ by 0.8–1.2 molecules of hydrogen absorbed, whilst they are interconvertible in 50 per cent sulphuric acid at room temperature. Transformation also occurs slowly in cold concentrated hydrochloric acid and more rapidly in glacial acetic acid with dry hydrogen bromide. If the difference in hydrogen absorption is regarded as indicating a difference in hydrogen content, the acid transformations must be reductions, which seems very unlikely.

The hydrogenation results show only a small difference between the chlorins and true porphyrins. If these results are accepted, chlorophyll and the chlorins are to be regarded as dihydroisoporphyrins in basic structure and also isomeric with true porphyrins, as Fischer postulates. The American workers, however, while continuing to assume that the fundamental nucleus of chlorophyll and the

chlorins is that of a dihydroisoporphyrin, postulate that it is also that of a dihydroporphyrin, these being of an equal state of hydrogenation.

A very interesting new method of attack has now been developed by Conant, Chow and Dietz<sup>6</sup>, namely the potentiometric titration in acetic acid solution of the basic groups in chlorophyll derivatives. The chlorophyll nucleus and those of its derivatives contain four pyrrole or modified pyrrole rings. An important problem in connexion with the fine structure of the nucleus is the determination of the relative basicities of the four pyrrole nitrogen atoms, which add together to determine the basic character of the whole molecule. Willstätter had utilised the variation in basicity in the chlorophyll series in the method of acid fractionation, which made possible the separation of chlorophyll derivatives in solution, and had also obtained qualitative evidence of the greater basicity of two of the nitrogen atoms by the isolation of dihydrochlorides.

Conant and his collaborators have now supplied quantitative evidence on this problem by a series of potentiometric titrations in glacial acetic acid using a chloranil electrode and perchloric acid as titrating agent, a method which had previously been studied<sup>7</sup>. This electrode makes use of an oxidation-reduction system similar to that in the quinhydrone electrode. Chloranil and its reduction product provide the system in equilibrium with hydron:



and a pH scale can thus be derived from it. When the solvent is glacial acetic acid, this pH scale will not, of course, be related directly to the standard hydron activity as defined for aqueous solutions, but it may be defined in terms of an acidity function by a method discussed by the authors. For comparative purposes this is a secondary consideration, and the values of pH in acetic acid may be calculated by a formula similar to that used for the quinhydrone electrode in aqueous solutions, a particular value being taken for the standard potential. If the reaction between chlorophyll base (*B*) and acid proton ( $H^+$ ) is formulated as  $B + H^+ \rightleftharpoons BH^+$ , the value of  $pK^1$ , the dissociation constant of the chlorophyll base, will then be given by:

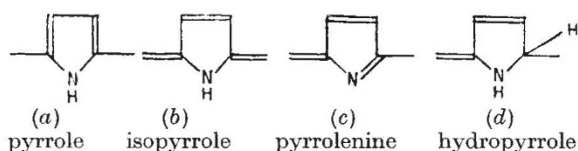
$$pK^1 = pH + \log (BH^+)/ (B)$$

in which all symbols refer to the acetic acid solutions. The ionic strength of the solution (which influences the activity coefficients of the solutes) was kept constant at  $\mu = 0.2$  by adding the required amounts of the neutral salt trimethylammonium perchlorate. The values of  $pK_1^1$ ,  $pK_2^1$  and  $pK_3^1$ , for the dissociation of the first, second and third basic groups, corresponding to titration mid-points for each range, were read directly from the titration curves at added aliquots of 0.5, 1.5 and 2.5 mols of titrating agent, in accordance with the usual *approximate* theory for calculating *pK* from the *pH* titration values.

In this way, the values of  $pK^1$  for a series of simple nitrogen compounds (positively and negatively substituted pyrroles, a dipyrromethane, two *N*-methyl methanes and a methylethylmaleic imide) were first determined. These are all about  $-2.3 \pm 0.3$ , indicating that the pyrrole group is a very weak base. If pyrrole is regarded as a substituted ammonia, the two  $\alpha, \beta$ -unsaturated linkages appear to control the



diminution in basic character of the ammonia nitrogen. All the chlorophyll derivatives examined contain at least one and possibly two of the very weakly basic groups (a) or (b):



and they gave  $pK_3^1$  values of  $-1.9$  to  $-2.4$ .

With very few exceptions, all these compounds also contain one rather strongly basic group with a  $pK_1^1$  value of  $+1.8$  to  $+2.3$ , which, as titrations of two methenes indicated, was probably the oxidised pyrrole or pyrrolenine ring (c), having one  $\alpha, \beta$ -unsaturated linkage and a tertiary nitrogen atom. Pyridine has also a  $pK^1$  value of  $+2.93$ , which is the lower limit of basic strength in the glacial acetic acid system. This system gives satisfactory results for basic strengths of compounds such as urea, which have  $pK$  in water of about zero, and compounds so weakly basic (for example, acetanilide and acetamide) that they cannot be measured in water.

The porphyrins stand out as a group from all other chlorophyll derivatives in containing two relatively strong basic groups, of average  $pK^1$  of  $+2.5$ . The chlorins are differentiated from the porphyrins in having only one relatively strongly basic group, with  $pK^1 = +2.1$ , and one group intermediate in basicity between the pyrrole and pyrrolenine groups, possibly an oxidation or reduction product of the latter in which the character of the nitrogen atom is changed. The basicity of this group is influenced by substituent groups and is comparable with the basicity of urea

and acetoxime. This result leads to a correction of a previous formula for chlorin *f*, which is now supposed to contain a hydroxypyrrole nucleus (d) in place of one of the two pyrrolenine rings previously assumed by Conant.

The true chlorophyll-*a* compounds, the phaeophorbides, are found to contain one relatively strongly basic and one very weakly basic group, as in the chlorins. The intermediate group, however, is less basic than in the chlorins; but whether this difference is significant of a radically different structure is difficult to say. In the *b* series, rhodin-*l* is the simplest compound and corresponds with chlorin-*f* in the *a* series. The two basic groups in rhodin-*l* are weaker than in the *a* series, but rhodin-*g* is very similar to chlorin-*e*. Methyl phaeophorbide-*b* is markedly different from the *a* compound in the relatively strongly basic group. It appears that the extra oxygen atom in the *b* series affects the basicity of all the compounds, which would be unlikely if it were in the side chain of the propionic acid group, as postulated by Fischer.

The interpretation of the results of the new potentiometric titration experiments is still incomplete and rather tentative; but it is clear that the method promises to throw light on the structure of compounds containing basic groups, and its extension from the chlorophylls and porphyrins into other fields is obvious.

<sup>1</sup> Summarised in R. Willstätter and A. Stoll, "Untersuchungen über Chlorophyll", Berlin, 1913.

<sup>2</sup> Pedler Lecture, *J. Chem. Soc.*, 245; 1934.

<sup>3</sup> Many papers in *J. Amer. Chem. Soc.*, 1929 to date.

<sup>4</sup> Critical summary of the literature by K. F. Armstrong, *Chemistry and Industry*, 809; 1933.

<sup>5</sup> E. M. Dietz and T. H. Werner, *J. Amer. Chem. Soc.*, 56, 2180; 1934.

<sup>6</sup> J. B. Conant, B. F. Chow and E. M. Dietz, *J. Amer. Chem. Soc.*, 56, 2185; 1934.

<sup>7</sup> J. B. Conant and T. H. Werner, *J. Amer. Chem. Soc.*, 52, 4436; 1930; J. B. Conant and B. F. Chow, *ibid.*, 55, 3745; 1933.

## Fuel Research in Great Britain\*

THE work of the Fuel Research Board touches many aspects of the technology of fuel, and the annual report for the year ending March 1934 therefore provides a useful review of the problems before the coal and other fuel industries.

In the first place, the report emphasises the steady accumulation of information by the survey of the coal-fields of Great Britain, the value of which becomes increasingly evident as the demands on the properties of fuel become more exacting.

Reference is made to the fall in demand for large lump coal in recent years. Formerly, collieries made great efforts to avoid breaking coal because the consumer was prepared to pay, for size, a premium which was disproportionate to the calorific value of the coal itself. Actually the consumer paid his premium for a fuel the cleanness of which was visible to the eye. Now more than 77 million tons of coal is washed and its quality can be guaranteed, irrespective of size. Industrial fuel is nearly always wanted in small pieces, especially when firing is automatic. The modern house has little room for storage, and the householder wishes to avoid the trouble and dirt of breaking coal. One can foresee a time when the large lumps will become unsaleable,

and already some collieries are seeking the best and most efficient manner of breaking down lump coal without the undue formation of dust. Many difficult problems arise when coals are broken, such as the best treatment of wash water containing dust, and the staff of the Fuel Research Board is engaged on their examination.

A section of the report deals with the liquid fuels from coal. A small fraction of the needs of Great Britain is covered by the by-products of coal carbonisation, that is, benzole and coal tar oils. Most of the liquid fuel is imported from distant parts where Nature has provided a bounteous though, from our point of view, ill-placed supply of oil. The geographical distribution of petroleum provides food for speculation as to what the distribution of man might have been had he known of the existence of the oil earlier, or had understood how to obtain, control and distribute the natural gas which accompanies oil in such abundance. Industries might have been very differently situated, and it is improbable that men would have toiled against the hazards of coal-getting if such an ideal fuel as methane were available without effort. Even as it is, Governments all over the world are exerting themselves to turn solid into liquid fuels by processes which are technically speaking heroic but, judged by ordinary standards, uneconomic. The studies of the Fuel

\* Department of Scientific and Industrial Research. Report of the Fuel Research Board for the Year ended 31st March 1934, with Report of the Director of Fuel Research. Pp. vii+178. (London: H.M. Stationery Office, 1934.) 3s. net.