[CONTRIBUTION FROM THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

Studies in the Chlorophyll Series. XIV. Potentiometric Titration in Acetic Acid Solution of the Basic Groups in Chlorophyll Derivatives[1]

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The nucleus of the chlorophyll molecule and of each of its derivatives contains four pyrrole or modified pyrrole rings. An important problem in connection with the fine structure of the nucleus is the determination of the relative basicities of the four pyrrole nitrogen atoms, which added together determine the basic character of the whole molecule. Willstätter[2] made use of variation in basicity in the chlorophyll series in his method of acid fractionation,[2] which has made possible the separation of chlorophyll derivatives in solution. Willstätter also obtained qualitative evidence of the greater basicity of two of the nitrogen atoms by the isolation of dihydrochlorides.

The first quantitative evidence on this problem has been obtained by conducting a series of potentiometric titrations in glacial acetic acid using a chloranil electrode and perchloric acid as titrating agent, according to the procedure developed in this Laboratory.[3] Formulating the reaction between the chlorophyll base (B) and the acid proton (H^+) as follows

$$B + H^+ \rightleftharpoons BH^+$$

the $pK'^{(HAc)}$ can be derived as usual

$$pK'^{(HAc)} = pH^{(HAc)} + \log (BH^+)/(B)$$

In the paper by Conant and Chow there is a discussion of $(pH)^{HAc}$ in terms of Hammett and Deyrup's acidity function. However, in the present paper the old use of the $(pH)^{HAc}$ scale is continued, and is given by the equation

$$(pH)^{HAc} = \frac{0.566 - E}{0.0591} \text{ at } 25°$$

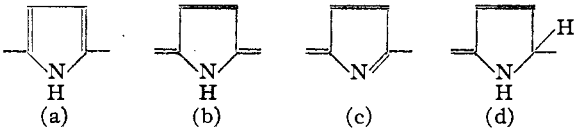
where E is the potential of the cell

Pt	$C_6Cl_4O_2$ (satd.)	Bridge	KCl	Calomel electrode aqueous (satd.)
	$C_6Cl_4(OH)_2$ (satd.)	LiCl (satd.)	(satd.)	
	HX	gelatin	in	
	in HAc	in HAc	H_2O	

The ionic strength of the solution was kept constant at $\mu = 0.2$ by adding the required amounts of the neutral salt trimethylammonium perchlorate.

Table I lists a series of compounds and the corresponding $pK'^{(HAc)}$ values obtained from the titration data. Figures 1 and 2 show titration curves for most of the compounds reported. The pK_1', pK_2' and pK_3' values correspond to titration mid-points for each group and are usually read directly from the titration curve at added aliquots of 0.5, 1.5 and 2.5 moles of titrating agent. For porphyrins, however, where there is only one sharp inflection at the mid-point of the curve, it is assumed that the pK_1' and pK_2' values are equal and an average value is reported for each.

Section (a) of the table shows the results for a few simple nitrogen compounds used for comparison with the chlorophyll derivatives. These include positively and negatively substituted pyrroles, a dipyrrylmethane, two N-methylmethanes and methylethylmaleic imide. The pK' values for all are about −2.3 and are the same within the limits of the experimental error (±0.3 pK' unit), and indicate that the pyrrole group is a very weak base. Regarding pyrrole as a substituted ammonia, the two α,β-unsaturated linkages seem to be the controlling factors in weakening the original strong basicity of the nitrogen atom. The titration curves of the methanes show one definitely very weakly basic group at $pK' = -2.0$ to -2.5. A second group is not definitely indicated but this is not surprising since the method is not sensitive in this very weakly basic region. It is possible also that this insensitivity may blot out substituent group effects which are really present. The method is entirely satisfactory, however, for the differentiation between the relatively strongly and very weakly basic groups in any one compound, and is most accurate in the region around a zero pK' value.



I

All of the chlorophyll derivatives examined contain at least one, and possibly two, of these

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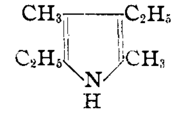
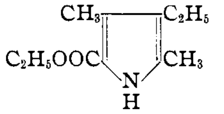
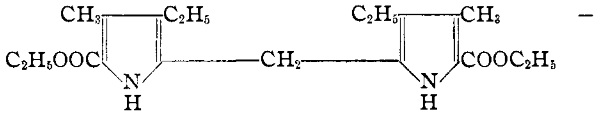
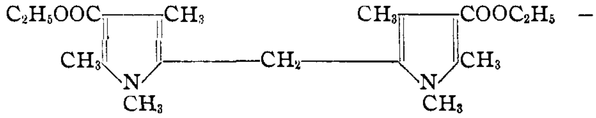
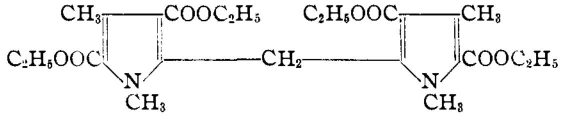
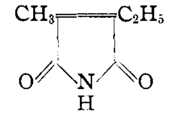
[1] This work was completed in July, 1933, but the publication has been delayed by unavoidable circumstances.

[2] Willstätter and Meig, *Ann.*, **350**, 1 (1906).

[3] Conant and Werner, THIS JOURNAL, **52**, 4449 (1930); Conant and Chow, *ibid.*, **55**, 3745 (1933).

Table I

Part (a)

Substances	$(pK_3')^{\text{HAc}}$
	−2.2
	−2.3
	−2.2
	−2.1
	−2.6
	−2.4

Part (b)

Substances	$(pK_1')^{\text{HAc}}$	$(pK_2')^{\text{HAc}}$	$(pK_3')^{\text{HAc}}$
Isorhodoporphyrin dimethyl ester	+1.8	+1.8	−1.9
Phylloporphyrin monomethyl ester	+2.3	+2.3	−2.0
Pyrroporphyrin monomethyl ester	+2.4	+2.4	−2.0
Mesoporphyrin	+2.4	+2.4	−2.0
Aetioporphyrin I	+2.6	+2.6	−1.7

The N–CH_3 methanes were prepared by Dr. A. Corwin and Dr. M. Quattlebaum.

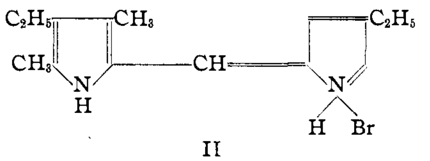
Part (c)

Methylphaeophorbide *a* from phaeophytin	+1.9	−1.4	−2.3
Methylphaeophorbide *a* from methyl chlorophyllides	+1.9	−1.4	−2.3
Dehydromethylphaeophorbide *a*	+2.2	−1.4	−2.4
Chlorin *k*	+2.3	−0.9	−2.0
Chlorin *g* monomethyl ester	+2.0	−0.9	−2.0
Chlorin *f* dimethyl ester	+2.2	−0.7	−2.0
Chlorin *a* trimethyl ester	+2.3	−0.2	−2.2
Pyrochlorin *e*	+2.2	−0.1	−2.0
Phaeopurpurin 7 dimethyl ester	+2.3	0.0	−2.3
Chlorin *e*	+1.9	+0.3	−2.2
Chlorin *e* trimethyl ester	+1.9	+0.4	−2.0

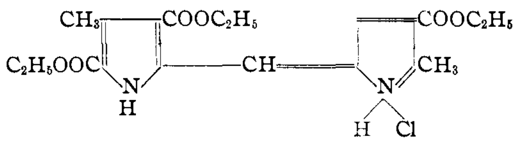
Part (d)

Methylphaeophorbide *b*	+0.3	−1.7	−2.3
Rhodin *k* dimethyl ester	+1.2	−0.8	−2.3
Rhodin *l* dimethyl ester	+1.2	−1.4	−2.1
Rhodin *g*	+1.9	+0.0	−2.0
Phaeopurpurin 18 monomethyl ester	+0.5	−1.8	−2.2
Rhodoporphyrin-γ-carboxylic anhydride	+0.3	+0.3	−2.2

very weakly basic groups to which we attribute the pyrrole or isopyrrole (maleic imide) structure (Formula I, (a) or (b)). They all have a pK_3' value of −1.9 to −2.4. With very few exceptions, all of these compounds also contain one rather strongly basic group with a pK_1' value of +1.8 to +2.3. The qualitative results obtained on titration of the two methenes, II and III



II



III

indicated the presence in these compounds of the same relatively strongly basic group with estimated pK' values of +2.2 and +2.0. The titration was carried out with one mole of sodium acetate to free the base, then with one mole of perchloric acid. Further addition of perchloric acid rapidly developed a black color in the solution due to oxidation, and further titration was impossible. This evidence indicates that this relatively strongly basic group in the methenes and in the chlorophyll derivatives may be an oxidized pyrrole or pyrrolenine ring, I(c), which has one α,β-unsaturated linkage and a tertiary nitrogen atom. An analogy for this is pyridine which is also a relatively strong base; its $pK'^{(\text{HAc})} = +2.93$,[4] the lower limit of basic strength in the glacial acetic acid system.

It must be remembered that in glacial acetic acid as a solvent all bases whose pK in water is greater than 4 or 5 are crowded together at the bottom of the scale at a $pK'^{(\text{HAc})}$ value of about +3. The method is satisfactory for measuring the basic strength of such compounds as urea which have a pK in water of about 0, and those which are so weak that they cannot be measured in water ($pK'^{(\text{HAc})} = -1$ to -2, *e. g.*, acetamide and acetanilide). The method becomes unsatisfactory again at $pK'^{(\text{HAc})}$ values much beyond this point.

(4) N. F. Hall, This Journal, **52**, 5124 (1930).

The porphyrins (Table I, Section (b)) stand out as a group from all other chlorophyll derivatives in that they contain *two* of these relatively strongly basic groups, as is shown by the sharp inflection in the titration curves at two equivalents of perchloric acid. The average pK' value is $+2.5$, which is practically the bottom of the scale, that is, they are strong bases with respect to the glacial acetic acid system.

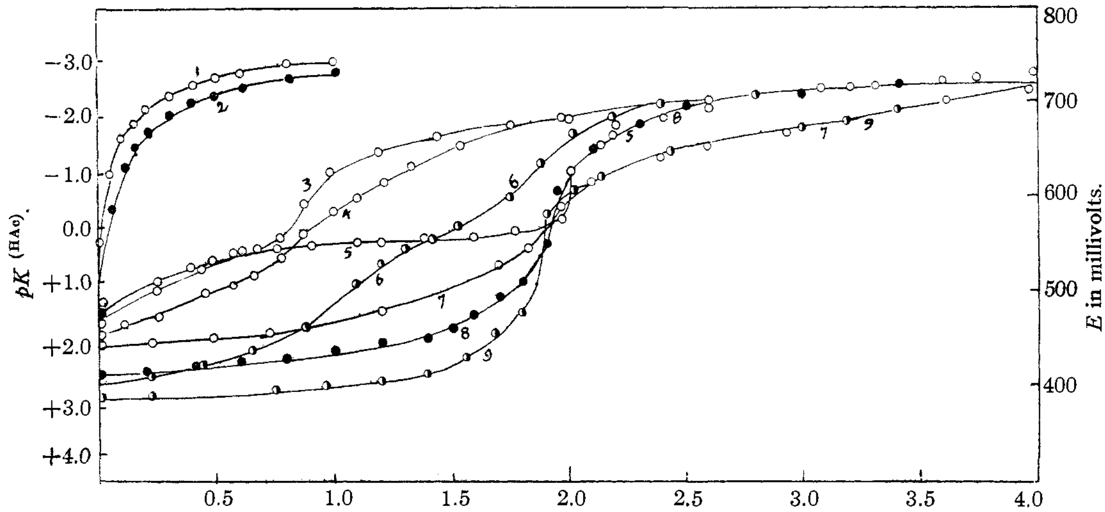


Fig. 1.—Abscissas are moles of perchloric acid added per mole of base: 1, ○ methyl ethyl maleic imide; 2, ● 2,4-dimethyl-3-ethyl-5-carbethoxypyrrole; 3, ○ phaeopurpurin *18* monomethyl ester; 4, ○ rhodin *l* dimethyl ester; 5, ○ rhodoporphryin-γ-carboxylic anhydride; 6, ◑ phaeopurpurin *7* dimethyl ester; 7, ○ isorhodoporphyrin dimethyl ester; 8, ● phylloporphyrin monomethyl ester; 9, ◑ aetioporphyrin I.

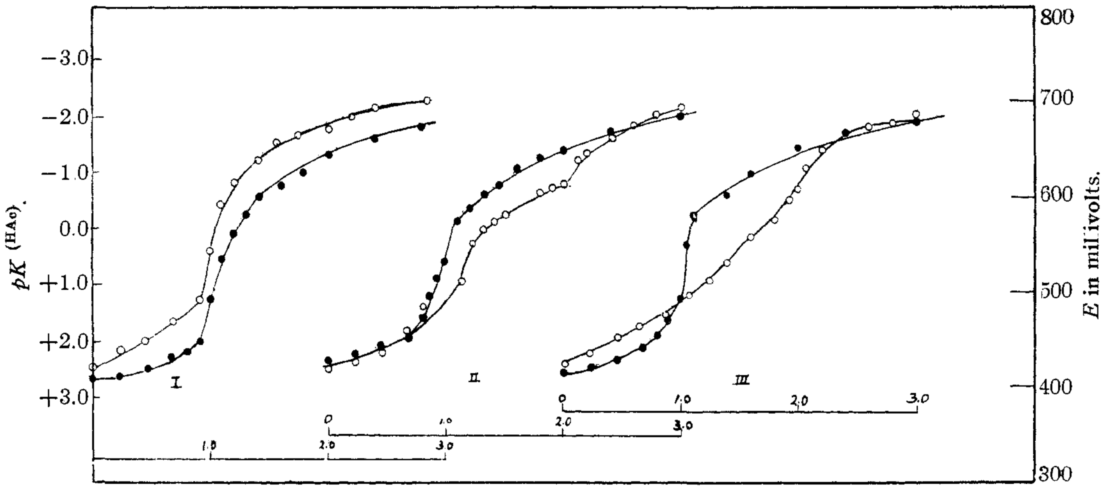


Fig. 2.—Abscissas represent moles of perchloric acid added per mole of base: I, ● chlorin *f* dimethyl ester; I, ○ methyl phaeophorbide *a* from methyl chlorophyllide; II, ● chlorin *g* monomethyl ester; II, ○ pyrochlorin *e*; III, ● chlorin *k*; III, ○ chlorin *e* triester.

The chlorins (Table I, Section (c)) differentiate themselves markedly from the porphyrins in that they have only *one* relatively strongly basic group ($pK' = +2.1$) 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 pK' of this group varies widely about a zero value ($pK_2' = -1.4$ to $+0.4$). Since this is the most accurate region of the $pK'^{(\mathrm{HAc})}$ scale, one may attribute these variations to substituent group effects. This is about the $pK'^{(\mathrm{HAc})}$ value of such substances as urea and acetoxime.

Our previous formula for chlorin *f*[5] (IV) is not consistent with these new facts since it contains two very weakly basic pyrrole, and two relatively

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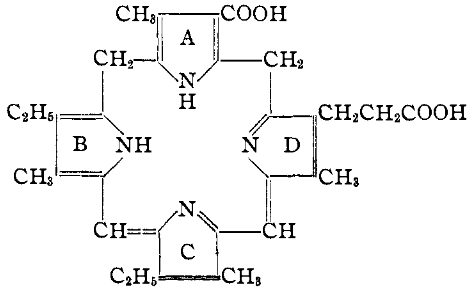
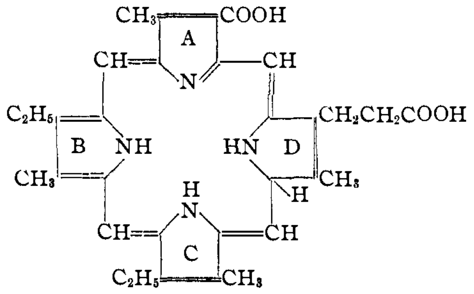
(5) Paper V, THIS JOURNAL, **53**, 2382 (1931).

strongly basic pyrrolenine, nuclei, such as the titration results show to be present in the porphyrins. We should now write for chlorin *f* and the other chlorins perhaps an isomeric form of this which contains one hydropyrrole nucleus (Formula I(d)) in place of one of the pyrrolenine rings but which is still an unconjugated dihydroporphyrin structure (V).



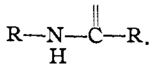
IV

Chlorin *f*



V

Chlorin *f* (revised)

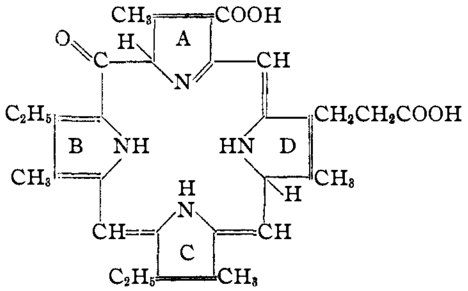
In this formula A is a pyrrolenine group of relatively strong basicity in the acetic acid system, whose $pK'^{(\mathrm{HAc})}$ is about +2.5. B is a pyrrole ring of very weak basicity even in acetic acid and has a $pK'^{(\mathrm{HAc})}$ of approximately −2.0. C is an iso-pyrrole or maleic imide type of ring which like B is also very weakly basic. D is a hydropyrrole grouping obtained by the addition of two hydrogen atoms to the carbon–nitrogen double bond of a pyrrolenine group. The structure of D might be considered comparable in basic properties to the amides which also contain the grouping  $R{-}\underset{H}{N}{-}\overset{\|}{C}{-}R$. As a matter of fact, the observed $pK'^{(\mathrm{HAc})}$ values for amides of the monobasic acids are roughly comparable to those found for this group in the chlorins. Such a formula will explain all the other facts, including the breaking of the conjugation of the porphyrin ring, as well as our other dihydro formula. It also supplies another hydrogen atom attached to nitrogen, which is necessary if the lactam formula for chlorophyll is to be used. Although in view of Fischer's and Stoll's preparation of oximes this formula now appears improbable.

The intermediate basic group in the chlorins varies in strength from a $pK'^{(\mathrm{HAc})}$ value of +0.4 to −0.9 (with an experimental error of ±0.2). It would not be profitable to attempt to correlate these variations with structure. When we pass to the true chlorophyll *a* compounds, the phaeophorbides, one relatively strongly basic and one very weakly basic group are present as in the chlorins. The intermediate group however is less basic than in the chlorins; 0.5 of a unit less than in chlorin *k* and 1.7 units less than in chlorin *e*. Whether this difference is significant of a radically different structure is difficult to say.

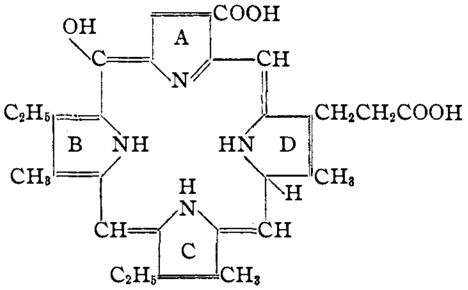
In Table I(d) are summarized the results for a few compounds of the *b* series and the peculiar substances, phaeopurpurin 18 and rhodoporphyrin-γ-carboxylic anhydride. The effect of anhydride formation is surprising. It decreases the basicity of the relatively strongly basic group by 1.5 to 2 units; in the case of the porphyrin *two* such groups are involved in the shift, in the case of the phaeopurpurins only one, using the chlorins or phaeopurpurin 7 as the reference substances. The intermediate group in phaeopurpurin 18 is also depressed by the same amount, going from about +0.0 in the chlorins, to −1.8 in phaeopurpurin 18. These marked changes in the character of two basic groups in each of these compounds make it seem certain that some rearrangement of the nuclear structure has taken place in addition to anhydride formation. Other facts pointing in this direction are the change of color and absorption spectrum.

It may be noted at this point that $pK'^{(\mathrm{HAc})}$ values are really accurate measures of basic strength while the acid numbers are not. The inconsistencies of the latter method are due to the relative solubility of two given compounds in ether, and of their hydrochlorides in the aqueous layer. For example, as might be expected, chlorin *e* and its triester show identical pK' values and are therefore of equal basic strength, although their acid numbers are five units apart. The porphyrins as a class have very similar basicities as judged by pK' values, yet their acid numbers vary from about +0.5 for phylloporphyrin to 7 for isorhodoporphyrin. The effect of the phytyl group on the acid number of phaeophytin is large as compared with methyl or free phaeophorbide because it makes the compounds more soluble in ether.

In the *b* series, rhodin *l* is the simplest compound and corresponds to chlorin *f* in the *a* series. A comparison of the $pK'^{(\mathrm{HAc})}$ values shows that two basic groups are here definitely weaker than in the *a* series ($pK_2'^{(\mathrm{HAc})} = 1.4$ as compared with -0.7). On the other hand rhodin *g* is very similar to chlorin *e*. Methyl phaeophorbide *b* is markedly different from the *a* compound in the relatively strongly basic group, which is depressed in basicity about 1.5 units. It appears that the extra oxygen atom in the *b* series has had an effect on the basicity of all the compounds. This would be unlikely if it were in the side chain of the propionic acid group as postulated by Fischer.



VI



VII

The new formula suggested above for chlorin *f* (V) would not allow of the substitution of a ketonic oxygen atom on a bridge carbon atom as we originally postulated. But this objection is easily taken care of by the above formula (VI) for the *b* series (rhodin *l*), in which a tautomeric form would be an hydroxy compound (VII) with the hydroxyl group on the bridge carbon atom.

Experimental

Potentiometric Titrations.—The acetic acid was purified by redistilling the commercial glacial acetic acid (m. p. 16.3°) through a 1.5-meter column filled with glass beads. The pure acid melted at 16.5°. The titration apparatus has been described by Conant and Werner. A typical experiment was as follows: 63 mg. of the trimethyl ester of chlorin *e* (10^{-4} mole) and 400 mg. of anhydrous trimethylammonium perchlorate (dried over phosphorus pentoxide) were dissolved in 10 cc. of glacial acetic acid (m. p. 16.5°) in the titration cell. An excess of the chloranil mixture was added just before the titration, so as to minimize oxidation. Three new platinum electrodes were used for every titration and they agreed within three millivolts. The potentials reached a constant value shortly after each addition of the small increments of the perchloric acid solution (0.20 *M*). The whole titration was accomplished in about half an hour.

After the titration, the total reaction mixture was poured into ether and the ethereal solution was washed with distilled water and ammonia to neutralize the acetic acid. The ether solution was then shaken gently with 0.05 *N* potassium hydroxide to test for alkali soluble material but none was ever observed. Fractionation showed the material to be homogeneous and the spectrum was unchanged. Thus under the experimental conditions no appreciable alteration of the chlorophyll compounds took place. For example, no chlorin *e* or isorhodoporphyrin was detected from the titration mixtures of methyl phaeophorbide *a* or from chlorin *f*, respectively.

Summary

1. Potentiometric titrations of the basic groups in certain pyrrole and chlorophyll derivatives have been carried out with perchloric acid in glacial acetic acid solution.

2. The titration curves of all the chlorophyll derivatives show three definite basic groups with corresponding $pK'^{(\mathrm{HAc})}$ values. The nature of these curves is markedly and characteristically different for classes of chlorophyll compounds, *i. e.*, porphyrins, chlorins and rhodins.

3. An attempt has been made to correlate these data with the nature of the pyrrole or modified pyrrole nuclei making up the nuclear structure of the various types of chlorophyll derivatives.

CAMBRIDGE, MASS.
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