The large order of nearly 1600 gauzes was quickly subscribed and no more now remains than sufficient to supply this laboratory; but if a few laboratories care to club together, the same or better terms could doubtless be secured from the weavers, who have kindly assisted us with advice and coöperation in the production of this new article. When one considers the possibility of damage by students' use, the subjection of the red-hot gauze to accidental flooding with concentrated acids or alkalies, the record of not a single damaged gauze in three years is quite remarkable. Satisfaction with this innovation increases with the lapse of time.

AMHERST COLLEGE.
Dec. 7, 1914.

ARTHUR JOHN HOPKINS.

CHLOROPHYLL.

BY RICHARD WILLSTÄTTER.

Received December 23, 1914.

The Method of Investigation.[1]

Some years ago, chlorophyll was unknown as a substance in a chemical respect. It was doubtful whether there was one chlorophyll or several pigments related to each other, or a great number of green leaf dyestuffs. The first questions of the analysis were unsolved; it was yet undecided which elements belonged to the chlorophyll molecule. Through the investigations of F. Hoppe-Seyler,[2] E. Schunck and L. Marchlewski,[3] and M. Nencki,[4] the chief fact found was that the decomposition of chlorophyll leads to pyrrol derivatives which are related to the derivatives from blood pigment.

After some investigations of Hoppe-Seyler[5] and A. Gautier[6] (1879) chemists no longer attempted to isolate chlorophyll, for this appeared impossible principally on account of the changeability, the chemical indifference and the easy solubility of the dyestuff diluted, as it was, with so many colorless and yellow accompanying substances. But it was possible, without investigating chlorophyll itself, to deduce the peculiarities of its constitution from the consideration of the derivatives which are obtained by the reactions with acid and alkali.

---

[1] A more detailed report is given by R. Willstätter and A. Stoll, "Untersuchungen über Chlorophyll," from the Kaiser Wilhelm-Institut für Chemie, Berlin, 1913 (J. Springer).

[2] *Z. physiol. Chem.*, **3**, 339 (1897); **4**, 193 (1880); **5**, 75 (1881).

[3] Complete abstracts of the numerous researches performed by E. Schunck and L. Marchlewski are published in three monographs of L. Marchlewski, "Die Chemie des Chlorophylls," Hamburg, 1895; chapter "Chlorophylle," in Roscoe-Schorlemmer-Brühl, Vol. VIII, 848 (1901); "Die Chemie der Chlorophylle," Braunschweig, 1909.

[4] *Ber.*, **29**, 2877 (1896); M. Nencki and J. Zaleski, *Ibid.*, **34**, 997 (1901); M. Mencki and L. Marchlewski, *Ibid.*, **34**, 1687 (1901).

[5] *Z. physiol. Chem.*, **3**, 339 (1879).

[6] *Compt. rend.*, **89**, 861 (1879).

If alkali hydroxide is allowed to act on chlorophyll, it is changed into salts of chlorophyll, green color. From the neutral chlorophyll, an acid is obtained which forms water-soluble salts. Therefore, without a considerable optical change by the reaction with alkalies, a component which was bound with an acid group is hydrolytically split off. This reaction is the saponification of an ester.

There is another part of the molecule against which the gentle action of acid is directed. The chlorophyll color changes thereby to an olive, at the same time the fluorescence is weakened, a salt-forming group is not formed, showing that saponification is avoided. Therefore, in the splitting with acid, it is possible to preserve and to find in the products of hydrolysis that component which is separated by alkalies, and conversely, the alkali derivatives of the dyestuff must likewise exhibit a characteristic atomic group, which is destroyed with extraordinary ease by acid. According to this guiding idea,[1] it was possible, before chlorophyll itself was known, to determine its characteristics from the analysis of the decomposition products formed by acid and alkali, and indeed so completely, that the analysis at the end gave nothing new when we succeeded in preparing the natural dyestuff pure.

The chlorophyll-green acids, the chlorophyllins, which are formed by alkaline hydrolysis in an alcoholic extract of the leaves, are decomposed very easily, but they can be separated successfully in a fairly pure condition from a mixture with other products of the saponification. They were taken up from their ethereal solution by disodium phosphate and liberated again from it by monosodium phosphate. By analysis, they have been shown to be magnesium compounds.[2] In them the metal is bound to the nitrogen, the linkage being of a complex nature. The magnesium-containing group is very sensitive towards acids, but it is uncommonly stable against alkalies. It remains intact consequently even in the more far-reaching transformations of the molecule in which the carboxyl groups are split off, one after the other. The presence of magnesium has been proven and the assumed manner of its binding has found confirmation in the results of the continued action of alkalies,[3] particularly by the decomposition of the chlorophyllins on heating with concentrated alcoholic alkali to 240°. This yields a series of well-crystallizing, beautifully colored, intensely fluorescent decomposition products, the so-called phyllins with three, two and finally one carboxyl group. All these compounds, of which a number have received names such as glauko- and rhodophyllin, from

---

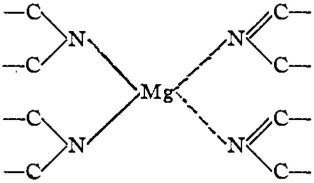
[1] R. Willstätter and F. Hocheder, *Ann.*. **354**, 205 (1907).

[2] R. Willstätter, *Ibid.*, **350**, 48 (1906).

[3] R. Willstätter and A. Pfannenstiel, *Ibid.*, **358**, 205 (1907); R. Willstätter and H. Fritzsche, *Ibid.*, **371**, 33 (1909); R. Willstätter and M. Utzinger, *Ibid.*, **382**, 129 (1911); R. Willstätter, M. Fischer and L. Forsén, *Ibid.*, 400, 147 (1913).

their beautiful blue and red color, and of which we have prepared a dozen as homogenous crystals, contain magnesium and at the same time are free acids.

The assumption that a complex is formed by magnesium with the nitrogen atoms has been doubted in the past years, but recently its correctness was proven beyond doubt by the decomposition of the phyllins to the carboxyl-free fundamental substance, the aetiophyllin,[1] the composition of which is expressed by the formula $C_{31}H_{34}N_4Mg$. In consequence of the diminution in the size of the molecule, the ash content has risen to 8% of magnesium oxide. There are only nitrogen-containing groups available, which bind the magnesium with two valencies and fasten it in a complex with secondary valencies. The constitution of the remaining phyllins is analogous, and their oxygen atoms have no part in the binding of the metal complex:



The magnesium content of chlorophyll is constant.[2] And this has been found, without exception, in land and water plants of the most different classes. For a long time it has been known that magnesia is indispensable as a mineral constituent of plants, but now, for the first time, the definite function of the metal has been established. Inasmuch as it is not a case of catalytic influence, such as is ascribed to iron for chlorophyll formation, but a stoichiometric participation of the magnesium in the building up of chlorophyll, the attention of the agricultural chemists should be directed to the question whether magnesia is provided by nature in sufficiency for the useful plants. The effect of an addition of magnesium salt on the chlorophyll formation and growth with different kinds of cultures was investigated a short time ago by E. Mameli[3] in the Botanical Institute at Pavia and a favorable influence of added magnesium was found.

All the phyllins lose magnesium by the action of mineral acids, as well as by acetic acid; the carboxylic acids of the phyllin series pass, thereby, into polybasic or monobasic amino acids which display characteristic basic properties besides the acid ones. They form a natural group with the phylloporphyrin discovered by F. Hoppe-Seyler,[4] later investigated,

---

[1] R. Willstätter and M. Fischer, *Ann.*, 400, 182 (1913).

[2] R. Willstätter and A. Pfannenstiel, *Ibid.*, 358, 205 (1907); R. Willstätter and H. J. Page. *Ibid.*, 404, 237 (1914).

[3] *Atti della Soc. Ital. per il Progr. d. Scienze*, 5, 793 (1911); *Atti dell' Ist. Bot. dell' Università di Pavia*, [2] 15, 1 (1912).

[4] *Z. physiol. Chem.*, 4, 193 (1880).

but not obtained in a pure condition, by E. Schunck.[1] Therefore, they are named porphyrins and that prefix is added which belongs to the corresponding phyllin. From the oxygen-free aetiophyllin, the simplest porphyrin, aetioporphyrin, of the formula $C_{31}H_{36}N_4$ is formed.

Analogous to the behavior of the phyllins towards acid is the action of acids on the solutions of chlorophyll itself. For the investigation of this reaction, the observation was fundamental that, by gentle decomposition with oxalic acid, a chlorophyll derivative, the so-called phaeophytin which is not readily soluble in alcohol and free from colorless and yellow accompanying substances, is precipitated almost quantitatively from an alcoholic extract of the leaves. It no longer contains magnesium, and the splitting off of the metal is the only change which has occurred, if the preparation of the plant material, the extraction, and the treatment with acid is made with all the precautions which are suggested by the experience of this work.

The phaeophytin recalls to mind the substances which F. Hoppe-Seyler[2] in 1879 and at the same time A. Gautier[3] obtained as products of the unintentional decomposition of chlorophyll by the plant acids, contained in the extracts. The old preparations,[4] however, were not free from accompanying substances and not intact in the easily changeable atomic groups of the dyestuff molecule. Hoppe-Seyler found in such a substance, the chlorophyllan, a phosphorus content of 1.4% and was thereby led to the supposition that chlorophyll might belong to the lecithins. The hypothesis, cautiously expressed by Hoppe-Seyler, is ardently upheld by J. Stoklasa[5] up to the present day, who states to have found phosphorus and potassium in chlorophyll, and indeed more phosphorus than in lecithin. This experience cannot be corroborated.[6] The long and uncertain way by which chlorophyllan used to be obtained is now replaced by a method which permits us to separate the chlorophyll of any origin as a pure derivative. It can now be obtained on a kilogram scale by the ordinary means of the laboratory; the meal of dried common nettle leaves is a suitable starting material (yield 4 to 6 g. phaeophytin from 1 kg.).

The phaeophytin is a wax-like substance without acid properties and of a weak basic nature. As regards the color of its solutions, it is quite different from chlorophyll, but the similarity of this decomposition product to chlorophyll appears as soon as a metal is introduced into the molecule,

---

[1] *Proc. Roy. Soc.*, 50, 302 (1891); E. Schunck and L. Marchlewski, *Ann.*, 284, 81 (1894); *Proc. Roy. Soc.*, 57, 314 (1895).

[2] *Z. physiol. Chem.*, 3, 339 (1897).

[3] *Compt. rend.*, 89, 861 (1879).

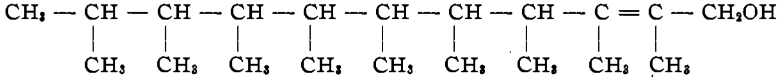
[4] See R. Willstätter and M. Isler, *Ann.*, 390, 287 and 337 (1912).

[5] *Ber. Deutsch. bot. Ges.*, 26, 69 (1907) and 27, 10 (1909): J. Stoklasa, J. Sebor and E. Senft, *Beihefte z. Bot. Zentralbl.*, 30, Erste Abt., Heft 2, 167 (1913).

[6] R. Willstätter and E. Hug, *Ann.*, 380, 177, 209 (1911).

in which it becomes bound as a complex.[1] Many metals enter very easily, for example, copper and zinc by the action of their acetate salts on the alcoholic solution of phaeophytin. The complex formation is a phenomenon so evident that the smallest traces of certain metals can be shown by the aid of chlorophyll derivatives. It was much more difficult to reintroduce the magnesium on account of the sensitiveness of the magnesium complex to acids. This has been attained[2] finally by the action of the Grignard solutions, for example, of magnesium methyl iodide on phaeophytin or on porphyrins. Another method, namely, the action of magnesium oxide and alkali in the heat, is not applicable for the reformation of chlorophyll on account of its instability towards alkali, but is very suitable for the formation of the phyllins from the porphyrins. In the same manner, iron can be introduced into the porphyrins by heating with methyl alcoholic potassium hydroxide and iron oxide.

Phaeophytin[3] behaves like a wax upon saponification with alkalies and produces, besides high molecular nitrogen-containing acids with 34 atoms of carbon, a nitrogen-free alcohol with twenty carbon atoms. It has been isolated and called phytol. It has the formula $C_{20}H_{39}OH$ and is an unsaturated primary alcohol with an open chain of carbon atoms. Investigations[4] concerning its decomposition have made it probable that its carbon skeleton has many side-chains and that it contains many methyl groups. For its structure, the following constitutional formula, which is hypothetical in its details, may serve provisionally:



$$CH_3-\underset{\substack{|\\ CH_3}}{CH}-\underset{\substack{|\\ CH_3}}{CH}-\underset{\substack{|\\ CH_3}}{CH}-\underset{\substack{|\\ CH_3}}{CH}-\underset{\substack{|\\ CH_3}}{CH}-\underset{\substack{|\\ CH_3}}{CH}-\underset{\substack{|\\ CH_3}}{CH}-\underset{\substack{|\\ CH_3}}{C}=\underset{\substack{|\\ CH_3}}{C}-CH_2OH$$

It is probable that relations exist between isoprene, the well-known building stone of the terpenes and caoutchouc, and this alcoholic component of chlorophyll.

Consequently the chlorophyll derivative, formed by acid, has yielded the information which was sought concerning the chief transformation which the alkalies produce upon the molecule of chlorophyll; they saponify the phytolester group. The action, however, is not limited to this group; for the phaeophytin, and as a result the chlorophyll, contains further a $COOCH_3$ group which also is decomposed by the hydrolysis.

Phytol is of general occurrence[5] in nature, amounting to one-third of

---

[1] R. Willstätter and F. Hocheder, *Ann.*, **354**, 208 (1907); R. Willstätter, A. Stoll and M. Utzinger, *Ibid.*, **385**, 180 (1911).

[2] R. Willstätter and L. Forsén. *Ibid.*, **396**, 180 (1913).

[3] R. Willstätter and F. Hocheder, *Ibid.*, **354**, 205 (1907).

[4] R. Willstätter, E. W. Mayer and E. Hüni, *Ibid.*, **378**, 73 (1910); O. Schuppli, Dissertation, Zürich, 1912.

[5] R. Willstätter, F. Hocheder and E. Hug. *Ann.*, **371**, 1 (1909); R. Willstätter and A. Oppé, *Ibid.*, **378**, 1 (1910); R. Willstätter and H. J. Page, *Ibid.*, **404**, 237 (1914).

the weight of chlorophyll, and the phytol occurs constantly as a component of chlorophyll. This knowledge was obtained in an indirect manner by working up different plants. In the beginning, the phaeophytin preparations of different origin showed considerable fluctuations. The phytol content never exceeded 33% but not infrequently remained below that figure and in many cases sank to zero. Now it has been found that just those plants containing a chlorophyll poor in phytol proved to be an excellent material for the isolation of chlorophyll in a peculiar form of beautiful crystals.[1] These crystals were the same which the Russian botanist J. Borodin[2] discovered under the microscope in 1881. Borodin observed the formation of crystals under certain conditions, namely, when drying alcohol-moistened sections of leaves. He left the question open, however, whether these crystals originated from chlorophyll or from transformed chlorophyll. N. A. Monteverde[3] continued the investigation and isolated such crystals in a small quantity in order to characterize them spectroscopically. But the substance had not been analyzed and chemically investigated. In the monographs concerning chlorophyll, Borodin's research was not mentioned. The discovery, therefore, remained useless until it was made a second time in the chemical laboratory. In 1907, I obtained, together with Benz, the so-called crystallized chlorophyll on a large scale, but at a time when the analysis of chlorophyll had been already completed by indirect methods. The production of crystallized chlorophyll on a preparative scale was of special value for investigations, until it became possible to separate the natural dyestuff itself in a pure condition.

The formation of the crystallized compound and the corresponding deficiency of phytol could be explained only by the observation[4] that by rapid extraction of many leaves the phytol content is normal. But by slow extraction, when the extract remains in contact with the meal of dried leaves for some time, it is too low. According to Willstätter and Stoll,[5] the chlorophyll in the green plant parts is accompanied by an enzyme, the chlorophyllase, belonging to the esterases. This is not inactive in alcoholic media, as is generally assumed for enzymes, but it causes the displacement of the phytol by the alcohol applied as a solvent and thereby effects alcoholysis of the chlorophyll. The enzyme occurs pretty generally; for example, it is contained in large quantity in *Galeopsis* and *Heracleum*, but its quantity appears to fluctuate within wide limits. After the dynamics of this enzyme reaction had been sufficiently studied,

---

[1] R. Willstätter and M. Benz. *Ann.*, 358, 267 (1900).

[2] *Botan. Z.*, 40, 608 (1882).

[3] *Aceta Horti Petropolitani*, 13, Nr. 9, 123 (1893).

[4] R. Willstätter and A. Oppé. *Ann.*, 378, 1 (1910).

[5] *Ann. Chem.*, 378, 18 (1910).

extensive application of the action of chlorophyllase was made for preparative purposes. There is no longer anything accidental about the preparation of crystallized chlorophyll. From fresh, as well as from dried leaves, almost the total chlorophyll can be separated in the form of the ethyl or methyl compound (ethyl or methyl chlorophyllide) or through hydrolysis in the form of the corresponding free carboxylic acid, the chlorophyllide.[1] The reversal of the hydrolysis, the partial synthesis[2] of chlorophyll from the two components, has also been carried out, namely, from chlorophyllide with the alcohol phytol by esterification under the catalytic influence of chlorophyllase. The usual methods of ester formation could not be applied in this case on account of the sensitiveness of chlorophyll.

Inasmuch as the phaeophytin is the most suitable form of chlorophyll for investigation as well as for the comparison of the leaf green from different plants, the characterization of chlorophyll, apart from the determination of magnesium and phytol, requires above all the knowledge of the nitrogen-containing acids which result, besides the phytol, upon saponification of phaeophytin. Although this is a pure chlorophyll substance, it is not a homogeneous compound; its acid component contains substances which differ in basic properties and color.[3] In the beginning, the investigation led to a great number of such decomposition products which form two groups; the one, phytochlorins, olive-green in indifferent solution, the other, phytorhodins, beautifully red. On account of their great number, the single compounds are designated with group names and letters added to them.

We would have scarcely succeeded in throwing light on the mixture of complicated composition which is met by the first transformations of chlorophyll if the basic nature of the decomposition products, in their unprecedented differentiation, had not placed in our hands a never-failing method[4] for the determination and separation of chlorophyll derivatives. This method (Willstätter and Mieg) depends on the different distribution of these dyestuffs between ether and hydrochloric acid. The proportion in which these bases are distributed between ether and dilute acids varies in a most unusual manner with the acid concentration. The concentration of the acid, which under ordinary conditions extracts a large quantity of the substance from ether, is so important for preparative purposes that particular weight must be attached to it. We call "the

---

[1] R. Willstätter and A. Stoll, *Ann.*, **387**, 317 (1912).

[2] R. Willstätter and A. Stoll. *Ibid.*. 380, 148 (1911).

[3] R. Willstätter and F. Hocheder. *Ibid.*, **354**, 212 and 242 (1907); see M. Tswett, *Biochem. Z.*, **5**, 6 (1907); **6**, 373 (1907); 10, 404 (1908); *Ber.*, 41, 1352 (1908).

[4] R. Willstätter and W. Mieg, *Ann.*, 350, 1 (1906); R. Willstätter, M. Fischer and L. Forsén, *Ibid.*, 400, 151, 178 (1913): R. Willstätter and M. Fischer, *Z. physiol. Chem.*, **87**, 439 (1913); R. Willstätter and A. Stoll, "Untersuchungen über Chlorophyll," Chap. XIV.

hydrochloric acid number" the percentage content of that acid which by shaking removes approximately two-thirds of the dissolved substance from an equal volume of an ethereal solution.

HYDROCHLORIC ACID NUMBERS.

	Traces are extracted by acid of per cent.	Almost entirely extracted by acid of per cent.	Hydrochloric number.
Phaeophytin *a*	25	32	29
Phaeophytin *b*	30	..	35
Methylphaeophorbid *a*	13	18	16
Methylphaeophorbid *b*	17	23	21
Phaeophorbid *a*	12	17	15
Phaeophorbid *b*	16	22	$19\frac{1}{2}$
Phytochlorin *e*	$\frac{1}{2}$	4–5	3
Phytochlorin *f*	7	12	10
Phytochlorin *g*	8	12–13	10–11
Phytorhodin *g*	6	11	9
Phytorhodin *i*	11	20	15–16
Phytorhodin *k*	9	18	$14–14\frac{1}{2}$
Glaukoporphyrin	2	6	4–5
Cyanoporphyrin	1	5	4
Rhodoporphyrin	2	4	3
Rubiporphyrin	$2\frac{1}{2}$	$6\frac{1}{2}$	$4\frac{1}{2}$
Pyrroporphyrin	$\frac{1}{2}$	3	$1\frac{1}{2}$
Phylloporphyrin	$\frac{1}{16}$	$1\frac{1}{2}$	$\frac{3}{4}$
Aetioporphyrin	1	4	3

The decomposition products of phaeophytin, occurring in mixtures, were therefore separated by fractionation of their ethereal solution with hydrochloric acid of varying concentration. But the appearance of complicated mixtures was only a result of certain transformations, which the chlorophyll, being very changeable in alcoholic solution, underwent under the experimental conditions, as, for example, too slow extraction or too slow precipitation with acid. The preliminary treatment of the plant material and particularly its extraction and the treatment of the extract with acid had to be improved and to be made more uniform. By looking for the causes of the changes appearing in the solutions and by learning to avoid them, the differences between our preparations have become less frequent and more insignificant and we have finally succeeded in obtaining two, and only two, well-crystallizing and characteristic decomposition products from phaeophytin:[1] Phytochlorin *e* of the composition $C_{34}H_{34}O_5N_4$ and phytorhodin *g* of the composition $C_{34}H_{34}O_7N_4$.

Phytochlorin *e* is a tricarboxylic acid with two free carboxyl groups and one bound as a lactam. Phytorhodin *g* is a tetracarboxylic acid; of its carboxyl groups only two or three are found in a free condition.

The common appearance of a green and red decomposition product

---

[1] R. Willstätter and M. Isler, *Ann.*, 380, 154 (1911); R. Willstätter and M. Utzinger, *Ibid.*, 382, 129 (1911); R. Willstätter and M. Isler, *Ibid.*, 390, 269 (1912).

by the subsequent hydrolysis of chlorophyll with acid and alkali opened up an important question.[1] Is it caused by the decomposition of a large molecule into two fragments? As an argument against this we find the molecular weight of phaeophytin to be of similar magnitude to that of phytochlorin and phytorhodin. Then again, it might be conceivable that one of the decomposition products represents an earlier, the other a subsequent step of decomposition. But phytochlorin and phytorhodin cannot be converted into each other, and they are formed in quite definite weight proportions. It followed, therefore, from the formation of phytochlorin *e* and phytorhodin *g* that phaeophytin, and consequently chlorophyll, is a mixture of two components from which the one furnishes phytochlorin *e* by decomposition, the other phytorhodin *g*. On this basis, we have succeeded in obtaining the separation of the components from the mixture by physical and chemical means.

One method[2] which can be applied with chlorophyll solutions, with crystallized chlorophyll and with phaeophytin, consists in the rearrangement of the given proportion by unequal distribution of the dyestuff between several solvents, immiscible with one another; for example, water containing methyl alcohol with petroleum ether or, in the case of the difficultly soluble phytol-free compounds, methyl alcohol with ether-petroleum ether. This rearrangement of the relation of the components can be repeated and so utilized by numerous repetitions of the operations that, finally, the two components of the magnesium-bearing free dyestuffs are in pure condition.

The other method,[3] which of course is only applicable with magnesium-free compounds, consists in the fractionating with hydrochloric acid, according to the method of Willstätter and Mieg. The phaeophytin is so weak a base and so sensitive in its phytolester group toward hydrolytic agents, that this separation was not obvious and offered particular obstacles. It served its purpose, nevertheless, and confirmed the results of the first method of separation.

These results have corroborated a view which G. G. Stokes[4] expressed as early as 1864, unfortunately in only a few short words. Stokes recognized chlorophyll spectroscopically as a mixture and he endeavored to separate it by distribution between alcohol and carbon disulfide, a method subsequently rediscovered and developed by H. C. Sorby[5] and G. Kraus,[6]

---

[1] R. Willstätter and M. Isler, *Ann.*, 380, 154 (1911); 390, 269 (1912).

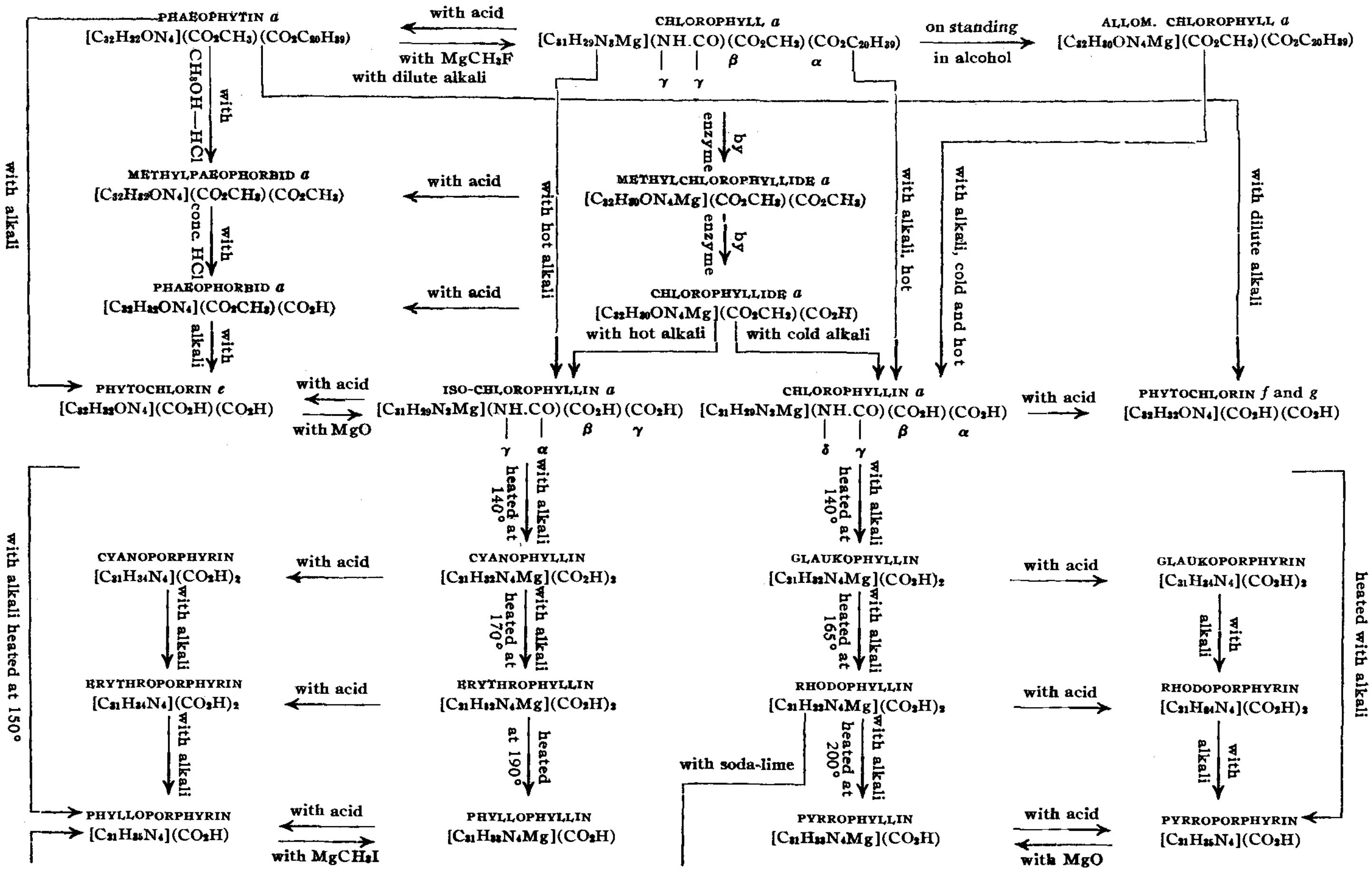
[2] R. Willstätter and M. Isler, *Ibid.*, 390, 269 (1912); R. Willstätter and A. Stoll, *Ibid.*, 387, 317 (1911) and "Untersuchungen über Chlorophyll," Chap. VI.

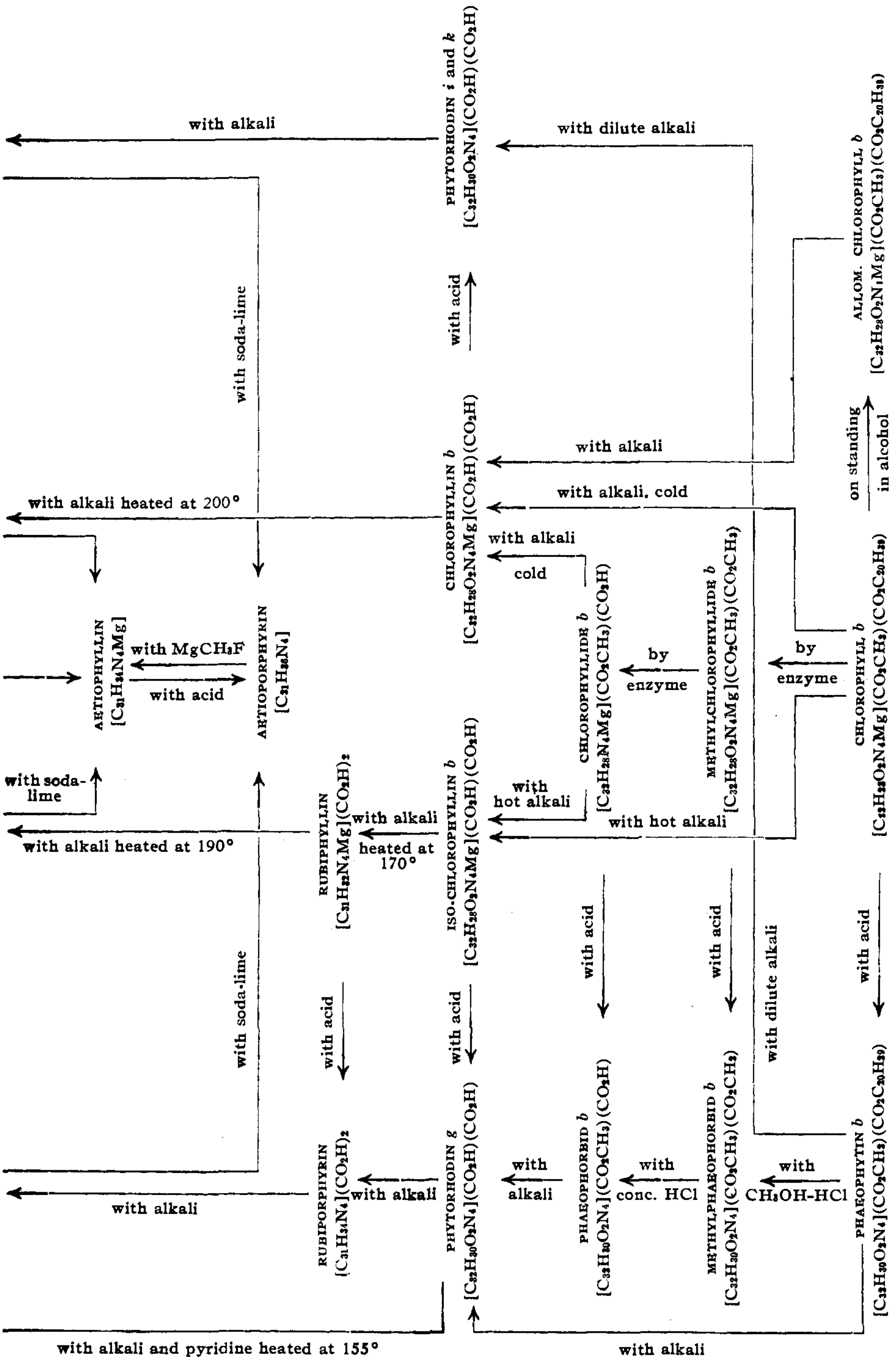
[3] R. Willstätter and A. Stoll, "Untersuchungen über Chlorophyll," Chap. XV.

[4] G. G. Stokes, *Proc. Roy. Soc.*, 13, 144 (1864).

[5] H. C. Sorby, *Ibid.*, 15, 433 (1867); 21, 442 (1873); *Quarterly Journ. of Microscopical Science*, 11, 215 (1871); *Ibid.*, 8, 64 (1871).

[6] G. Kraus, *Zur Kenntnis der Chlorophyllfarbstoffe und ihrer Verwandten*, Stuttgart, 1872.


PHAEOPHYTIN *a*
$[C_{32}H_{32}ON_4](CO_2CH_3)(CO_2C_{20}H_{39})$
with acid
with MgCH$_3$F
with dilute alkali
CHLOROPHYLL *a*
$[C_{31}H_{29}N_3Mg](NH.CO)(CO_2CH_3)(CO_2C_{20}H_{39})$
γ γ β α
on standing
in alcohol
ALLOM. CHLOROPHYLL *a*
$[C_{32}H_{30}ON_4Mg](CO_2CH_3)(CO_2C_{20}H_{39})$
with CH$_3$OH—HCl
with alkali
METHYLPAEOPHORBID *a*
$[C_{32}H_{32}ON_4](CO_2CH_3)(CO_2CH_3)$
with acid
by enzyme
METHYLCHLOROPHYLLIDE *a*
$[C_{32}H_{30}ON_4Mg](CO_2CH_3)(CO_2CH_3)$
with hot alkali
with alkali, hot
with alkali, cold and hot
with dilute alkali
with conc. HCl
by enzyme
PHAEOPHORBID *a*
$[C_{32}H_{32}ON_4](CO_2CH_3)(CO_2H)$
with acid
CHLOROPHYLLIDE *a*
$[C_{32}H_{30}ON_4Mg](CO_2CH_3)(CO_2H)$
with hot alkali
with cold alkali
with alkali
PHYTOCHLORIN *e*
$[C_{32}H_{32}ON_4](CO_2H)(CO_2H)$
with acid
with MgO
ISO-CHLOROPHYLLIN *a*
$[C_{31}H_{29}N_3Mg](NH.CO)(CO_2H)(CO_2H)$
γ α β γ
CHLOROPHYLLIN *a*
$[C_{31}H_{29}N_3Mg](NH.CO)(CO_2H)(CO_2H)$
δ γ β α
with acid
PHYTOCHLORIN *f* and *g*
$[C_{32}H_{32}ON_4](CO_2H)(CO_2H)$
with alkali heated at 140°
with alkali heated at 140°
with alkali heated at 150°
CYANOPORPHYRIN
$[C_{31}H_{34}N_4](CO_2H)_2$
with acid
CYANOPHYLLIN
$[C_{31}H_{32}N_4Mg](CO_2H)_2$
GLAUKOPHYLLIN
$[C_{31}H_{32}N_4Mg](CO_2H)_2$
with acid
GLAUKOPORPHYRIN
$[C_{31}H_{34}N_4](CO_2H)_2$
heated with alkali
with alkali
with alkali heated at 170°
with alkali heated at 165°
with alkali
ERYTHROPORPHYRIN
$[C_{31}H_{34}N_4](CO_2H)_2$
with acid
ERYTHROPHYLLIN
$[C_{31}H_{32}N_4Mg](CO_2H)_2$
RHODOPHYLLIN
$[C_{31}H_{32}N_4Mg](CO_2H)_2$
with acid
RHODOPORPHYRIN
$[C_{31}H_{34}N_4](CO_2H)_2$
with alkali
heated at 190°
with soda-lime
with alkali heated at 200°
with alkali
PHYLLOPORPHYRIN
$[C_{31}H_{35}N_4](CO_2H)$
with acid
with MgCH$_3$I
PHYLLOPHYLLIN
$[C_{31}H_{33}N_4Mg](CO_2H)$
PYRROPHYLLIN
$[C_{31}H_{33}N_4Mg](CO_2H)$
with acid
with MgO
PYRROPORPHYRIN
$[C_{31}H_{35}N_4](CO_2H)$


PHYTORHODIN *i* and *k* [C₃₂H₃₀O₂N₄](CO₂H)(CO₂H)
with alkali
with dilute alkali
ALLOM. CHLOROPHYLL *b* [C₃₂H₂₈O₂N₄Mg](CO₂CH₃)(CO₂C₂₀H₃₉)
with soda-lime
with acid
on standing in alcohol
with alkali
CHLOROPHYLLIN *b* [C₃₂H₂₈O₂N₄Mg](CO₂H)(CO₂H)
with alkali heated at 200°
with alkali. cold
with alkali cold
METHYLCHLOROPHYLLIDE *b* [C₃₂H₂₈O₂N₄Mg](CO₂CH₃)(CO₂CH₃)
CHLOROPHYLLIDE *b* [C₃₂H₂₈N₄Mg](CO₂CH₃)(CO₂H)
by enzyme
by enzyme
CHLOROPHYLL *b* [C₃₂H₂₈O₂N₄Mg](CO₂CH₃)(CO₂C₂₀H₃₉)
AETIOPHYLLIN [C₃₁H₃₄N₄Mg]
with MgCH₃F
with acid
AETIOPORPHYRIN [C₃₁H₃₆N₄]
with soda-lime
RUBIPHYLLIN [C₃₁H₃₂N₄Mg](CO₂H)₂
with alkali heated at 170°
ISO-CHLOROPHYLLIN *b* [C₃₂H₂₈O₂N₄Mg](CO₂H)(CO₂H)
with hot alkali
with hot alkali
with alkali heated at 190°
with soda-lime
with acid
with acid
with acid
with acid
with dilute alkali
with acid
RUBIPORPHYRIN [C₃₁H₃₄N₄](CO₂H)₂
with alkali
PHYTORHODIN *g* [C₃₂H₃₀O₂N₄](CO₂H)(CO₂H)
with alkali
with alkali
PHAEOPHORBID *b* [C₃₂H₃₀O₂N₄](CO₂CH₃)(CO₂H)
with conc. HCl
METHYLPHAEOPHORBID *b* [C₃₂H₃₀O₂N₄](CO₂CH₃)(CO₂CH₃)
with CH₃OH-HCl
PHAEOPHYTIN *b* [C₃₂H₃₀O₂N₄](CO₂CH₃)(CO₂C₂₀H₃₉)
with alkali and pyridine heated at 155°
with alkali

and much applied since then, particularly to show that yellow pigments accompany the green dyestuff. Recently, the botanist M. Tswett[1] in Warsaw has confirmed in a new, original way the view of Stokes, namely, by a separation of the natural pigment on an analytical scale by means of fractional adsorption from its solutions. It was impossible up to the present time, however, to find whether the dyestuff had changed or not by the extraction and during the following manipulations. Such changes occur easily and explain considerable errors which can be found in the work of Stokes, Sorby and Tswett. According to Stokes,[2] Sorby[3] and Tswett,[4] a third chlorophyll component occurs as a pigment of *Phaeophyceae*. An investigation[5] of the pigments of seaweeds, carried our recently, has led to the result that the chlorophyll of this algae is identical with that of land plants, with the exception that component *b* is reduced in quantity. The supposed third chlorophyll dyestuff was in fact a decomposition product of chlorophyll.

Whether the chlorophyll molecule remains intact in its details must be tested by decomposition to the typical splitting products (phytochlorin *e* and phytorhodin *g*). This characterization of the constituents of the leaf-green was the condition for the final isolation of the chlorophyll in an unchanged and pure state in form of a mixture or as individual components.

Isolation of Chlorophyll and Separation into Its Components.

The isolation of chlorophyll (Willstätter and Hug, 1911) was controlled by the colorimetric determination of the degree of purity of its solutions and depended on systematic increase of the purity in the course of a "un-mixing process," in which the distribution of the substances, contained in the extracts, between several solvents is applied in a particular manner, in order to separate the yellow and, still more, the colorless substances accompanying the chlorophyll. Solutions of about 70% chlorophyll result by the un-mixing method from the extracts which, in consequence of the great quantity of accompanying colorless substances, contain only 8–16% chlorophyll. Then, finally, an unexpected observation aided in the solution of the problem. When the chlorophyll has reached a certain degree of purity, it is still easily soluble in alcohol containing petroleum ether but, surprisingly, no longer soluble in pure petroleum ether. If the ethyl or methyl alcohol is removed by washing, the chlorophyll is precipitated and it can be purified by further precipitations from ether by petroleum

---

[1] *Ber. Deutsch. bot. Ges.*, **44**, 1125 (1911); "Die Chromophylle in der Pflanzen- und Tierwelt," Warsaw, 1910 (Russian).

[2] *Proc. Roy. Soc.*, **13**, 144 (1864).

[3] *Ibid.*, **21**, 442 (1873).

[4] *Ber. Deutsch. bot. Ges.*, **24**, 235 (1906).

[5] R. Willstätter and H. J. Page, *Ann.*, **404**, 237 (1914).

ether. In the beginning, this procedure[1] was laborious and the yield was small. In newer researches with A. Stoll,[2] I have perfected the procedure by variations in the methods of extraction and separation.

The materials for our work on a large scale are mostly dried and pulverized leaves. It has been shown that a considerable water content of the solvent essentially facilitates and accelerates the extraction of the total leaf dyestuff. The pigments in the chloroplasts, even after drying, are found in a colloidal condition and are difficultly soluble. They are coagulated by a solvent which dissolves salts out of the material and are

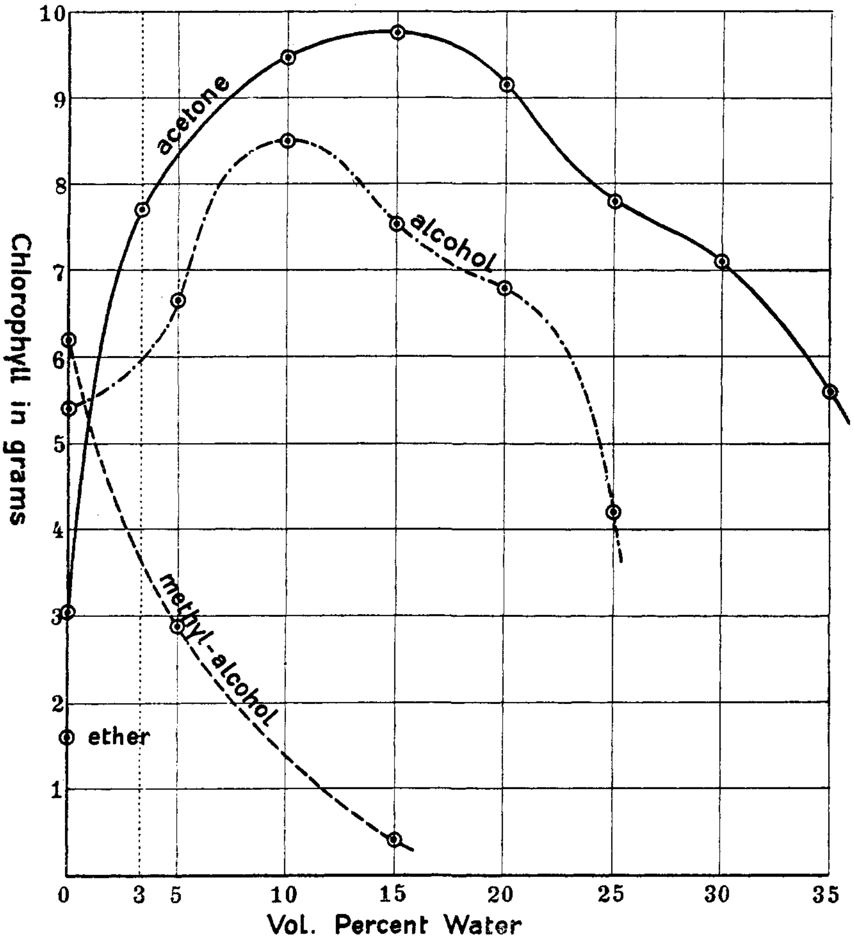


Fig. 1.

---

[1] R. Willstätter and E. Hug, *Ann.*, 380, 177 (1911).

[2] R. Willstätter and A. Stoll, "Untersuchungen über Chlorophyll," Chaps. III and VI.

therefore made more easily soluble. Besides, the quantity of the accompanying substances going into solution is increased and it is no longer the solvent itself but a mixture with the accompanying substances which becomes the particular means of extraction for the green of the leaf and, indeed, one so excellent, that the dyestuffs are quickly, easily and almost quantitatively extracted by it. It appears as if actually the whole chloroplast substance was carried off by the solvent with suitable water content. The best solvents are 85 to 90% alcohol and especially 80 to 85% acetone; the following figures show the influence of the water percentage on the chlorophyll content of the extract.

At the present time we can isolate pure chlorophyll in a few hours without much trouble from a few kilograms of dried leaves with a yield of about 80% of the quantity, that is, about 6.5 g. from a kg. of dried leaves. The procedure can also be carried out with fresh leaves, and likewise, as in the whole course of the work, no difference between the freshly plucked and the dried leaves resulted in the production of the chlorophyll preparations and in their properties. In a lecture hour, a quarter of a gram of pure chlorophyll can be isolated from a quarter of a kilogram of fresh common nettles. The leaf dyestuff can be isolated, at present, as easily as any kind of a plant constituent, as an alkaloid or a sugar.

With our methods of separation the two components are divided in an unequal manner between methyl alcohol and petroleum ether. By systematic fractionating, the rearrangement of the proportion of the components to each other is continued so far that finally the two components[1] result quite homogeneous from the mixture. The one component, chlorophyll *a*, is bluish green, the second, chlorophyll *b*, yellowish green. Their composition, in spite of their optical difference,[2] is very similar. The distinction is due to a different degree of oxidation. With the chlorophyll itself, we find confirmed what the formulae of phytochlorin *e* and phytorhodin *g* predicted, namely, that the compounds of Series *b* are derived from the compounds *a*, and probably in such a manner that two atoms of hydrogen are replaced by an atom of oxygen. This substitution, corresponding to the formulas: chlorophyll *a* $C_{55}H_{72}O_5N_4Mg$, *i. e.*, $[C_{32}H_{30}ON_4Mg](CO_2CH_3)(CO_2C_{20}H_{39})$ and chlorophyll *b* $C_{55}H_{70}O_6N_4Mg$, *i. e.*, $[C_{32}H_{28}O_2N_4Mg](CO_2CH_3)(CO_2C_{20}H_{39})$ means a difference of a molecule of oxygen.

Such a result does not appear certain, considering the size of the chlorophyll molecule. The difference, as far as the analyses show, could be CO_2

---

[1] R. Willstätter and M. Isler, *Ann.*, 390, 269, 327 (1912); R. Willstätter and A. Stoll, "Untersuchungen über Chlorophyll," Chap. VI.

[2] "Ueber die Absorptionsspektra der Komponenten und ersten Derivate des Chlorophylls," R. Willstätter, A. Stoll and M. Utzinger, *Ann.*, 385, 156 (1911); R. Willstätter and A. Stoll, "Untersuchungen über Chlorophyll," Chap. XXV.

instead of O_2 and that would be important for the explanation of the chemical function of chlorophyll. The assumed relation between the two components, however, is made more likely by the behavior of the derivatives of chlorophyll *b* toward the Grignard reagent,[1] by which they are converted into components of Series *a*, and further through the course of the decomposition which, in the *b* series, leads to the same aetiophyllin and aetioporphyrin.[2]

The Carotinoids.

In the preparation of the leaf-green, the separation from the yellow accompanying substances, which are extraordinarily widely spread in the plants and are associated with chlorophyll in the chloroplasts, was an important problem. The common occurrence of the yellow dyestuffs with the green, points to a significant physiological role of these carotinoids. In every green leaf, there occur two well-crystallizing nitrogen-free pigments of many common properties, but differing in their behavior towards solvents. One of these, as A. Arnaud[3] has made probable, is identical with the carotin of carots which has been known a long time. The analyses of Willstätter and Mieg[4] have shown that it is an unsaturated hydrocarbon of the formula $C_{40}H_{56}$. Its companion, xanthophyll,[5] was yet unknown in substance, in spite of the fact that it predominates in regard to quantity in the leaves. According to its composition, $C_{40}H_{56}O_2$, and its properties, it is to be considered as an oxide of carotin. The hydrocarbon is considerably soluble in petroleum ether, the oxygen compound, on the other hand, only in alcohol. A great affinity for oxygen, which they greedily absorb, particularly in their solutions, is peculiar to both the yellow pigments.

A third carotinoid, the fucoxanthin, is found in the olive-colored seaweeds and it has been recently isolated in pure crystals;[6] its composition is $C_{40}H_{54}O_6$. It is similar to carotin and xanthophyll in a chemical respect, but distinguished from them by the considerable basic property of its oxygen atoms, bound in the manner of an ether. It forms a characteristic blue hydrochloride which is to be considered as an oxonium salt. The constitution of these yellow pigments has not been explained hitherto, as their decomposition produced only amorphous substances.

Comparative Investigation of the Leaf Pigments.

The description and analysis of chlorophyll and its yellow companions furnish the conditions for the determination of the quantitative relation

---

[1] R. Willstätter and A. Stoll, "Untersuchungen über Chlorophyll," p. 331.

[2] R. Willstätter and M. Fischer, *Ann.*, 400, 182 (1913).

[3] *Compt. rend.*, 100, 751 (1885); 102, 1119 and 1319 (1886); 104, 1293 (1887); 109, 911 (1889); *Bull. soc. chim.*, 48, 64 (1887).

[4] *Ann.*, 355, 1 (1907).

[5] R. Willstätter and W. Mieg, *Ibid.*, 355, 1 (1907).

[6] R. Willstätter and H. J. Page, *Ibid.*, 404, 237, 253 (1914).

between all the components of the leaf dyestuff and for the comparison of the leaf-green in the most different kinds of plants. The material for the comparison, in respect to the chemical characteristics of chlorophyll, was furnished by over 200 plants from numerous classes of cryptogamia and phanerogamia. Our method consisted in the examination of phaeophytin, which was separated by quick extraction of the leaves and quick precipitation with acid, by its phytol content as well as by its basic decomposition products, phytochlorin *e* and phytorhodin *g*, and for completion the decomposition of the chlorophyll alkali salts to the crystallizing rhodophyllin, in which the ash amounts to 7% of magnesium oxide. The result[1] is the identity of chlorophyll in all plants investigated. We find only one single leaf-green consisting of the two components, *a* and *b*, of chlorophyll. We also observe a great regularity in the quantitative relation between these; chlorophyll *a* predominates, at the rate of almost three molecules to one molecule of chlorophyll *b*. Only the seaweeds (*Phaeophyceae*) form an exception[2] to this quantitative relation, containing besides the chlorophyll *a*, only a diminishingly small quantity of component *b*.

The molecular proportion of the green to the yellow pigments is also approximately constant, that is 3 : 1, and the relation of carotin to xanthophyll, with insignificant fluctuations in light leaves, stands 0.6 : 1.

An example might illustrate the results: In 1 kg. of dried elder leaves (corresponding to 4 kg. of fresh leaves) are contained: 8.48 g. chlorophyll, that is, 6.22 g. chlorophyll *a;* 2.26 g. chlorophyll *b;* 1.48 g. carotinoids, that is, 0.55 g. carotin; 0.93 g. xanthophyll. These quantities correspond to the following molecular proportions: For 1 molecule chlorophyll $(a + b)$ there occurs 0.35 molecule of carotinoids. For 1 molecule chlorophyll *a* there occurs 0.36 molecule of chlorophyll *b*.

Questions of Constitution.

If we consider the decomposition of the two chlorophyll components to aetiophyllin and aetioporphyrin, we see many intermediate products on the way to the mother substance, namely, the magnesium containing acids, the green, blue and red phyllins which are formed by the saponification of chlorophyll and by further heating with alkali. From them are derived the magnesium-free compounds, among which, the phaeophytins, phytochlorin *e* and phytorhodin *g*, and the porphyrins are important.

Phaeophytin is formed from chlorophyll *a* by the loss of magnesium and yields phytochlorin *e* on treatment with alkali. The latter is also formed when alkali and then acid acts on chlorophyll.

---

[1] R. Willstätter and M. Isler, *Ann.*, 380, 154 (1911); 390, 269 (1912); R. Willstätter and A. Stoll, "Untersuchungen über Chlorophyll," Chaps. IV and V.

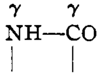
[2] R. Willstätter and H. J. Page, *Ann.*, 404, 237 (1914).

$$\begin{tikzcd}
\begin{matrix}\text{Chlorophyll } a \\ {[\mathrm{MgN_4C_{32}H_{30}O}]\mathrm{CO_2CH_3.CO_2C_{20}H_{39}}}\end{matrix} \arrow[r, "\text{by acid}"] \arrow[d, "\text{by alkali}"'] & \begin{matrix}\text{Phaeophytin } a \\ {[\mathrm{N_4C_{32}H_{32}O}]\mathrm{CO_2CH_3.CO_2C_{20}H_{39}}}\end{matrix} \arrow[d, "\text{by alkali}"'] \\
\begin{matrix}\text{Iso-chlorophyllin} \\ {[\mathrm{MgN_4C_{32}H_{30}O}]\mathrm{CO_2H.CO_2H}}\end{matrix} \arrow[r, "\text{by acid}"] & \begin{matrix}\text{Phytochlorin } e \\ {[\mathrm{N_4C_{32}H_{32}O}]\mathrm{CO_2H.CO_2H}}\end{matrix}
\end{tikzcd}$$

The relation between chlorophyll and the chlorophyllin-salts is not a simple one. Two alkali salts, obtained in a homogeneous form, result from each chlorophyll component, according to the conditions of the hydrolysis. One fluoresces, like chlorophyll itself, while the other is lacking fluorescence (chlorophyllin and isochlorophyllin[1]). The reaction consists not only in the saponification of two ester groups but, first and foremost, in a specific transformation which is recognized by the appearance of the so-called brown phase. Only a correct explanation of this brown phase[2] can give the key for the theory of the first steps of decomposition and of the changes which chlorophyll so easily and spontaneously undergoes in its solutions. Let us picture in our minds that, by the action of alkalies on chlorophyll and chlorophyllids, the green color at first changes to an intense brown, to a yellowish brown with the component *a*, to a red with *b*, and then in some minutes, the original chlorophyll color returns in the alkaline medium. The reaction has the appearance of a complete decomposition and a new formation of chlorophyll. Of course, it must be understood that a group, essentially responsible for the chromophoric complex, is changed by hydrolysis and that a new similar one is formed in its place.

We attempt to explain this behavior of chlorophyll as a "re-lactamization," as the opening of an existing lactam ring and the closing of a new ring, similar, but nevertheless stable in alkali. The formation of a new lactam ring can result in several ways: the different series of chlorophyllins and the hardly avoidable occurrence of the weak basic phytochlorins and phytorhodins are explained by it.

The original group may be marked:



$$\overset{\gamma}{\mathrm{NH}}\text{—}\overset{\gamma}{\mathrm{CO}}$$

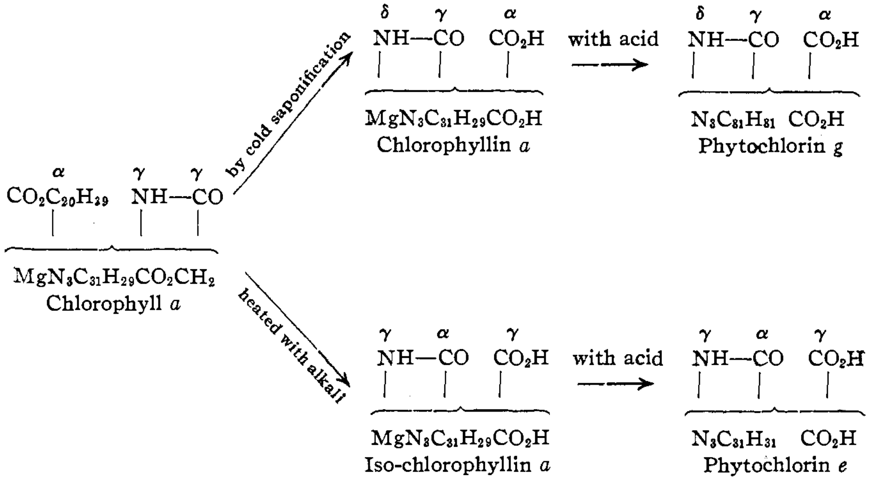
It can so relactamize that, for example, the carboxyl γ enters into combination with another nitrogen group, called δ, or the relactamization can re-

---

[1] R. Willstätter and M. Utzinger, *Ann.*, 382, 129, 157 (1911); R. Willstätter, M. Fischer and L. Forsén, *Ibid.*, 400, 147 (1913); R. Willstätter and A. Stoll, "Untersuchungen über Chlorophyll," Chap. XVIII.

[2] R. Willstätter and M. Utzinger, *Ann.*, 382, 128, 135, 139 (1911); R. Willstätter and A. Stoll, *Ibid.*, 387, 317, 326 (1911).

sult in such a manner that another carboxyl, namely, α, unites, for example, with the nitrogen γ. This transformation of the lactam groups takes place in a different manner if the hydrolysis is carried out in the cold or with very concentrated alkali at a higher temperature. Chlorophyll also, by standing in alcoholic solution, changes for the same reason. This change, which can be induced by traces of an acid, has been called allomerization.[1]



Since the ring-groups, newly formed by the first attack of the alkali on chlorophyll, are more stable than the original ones, the different course of the relactamization causes remaining differences in the products of further decomposition. Corresponding series of simpler composed phyllins and porphyrins therefore originate from the two fundamental derivatives of each chlorophyll component, the chlorophyllin and the isochlorophyllin. There are altogether four series of acids[2] produced by the alkaline decomposition which lead finally to a single end-product.

From isochlorophyllin *a* arise the dicarboxylic acid cyanophyllin, blue in solution; the dicarboxylic acid erythrophyllin, red in solution; the monocarboxylic acid phyllophyllin, blue, tinged red in solution.

From chlorophyllin *a* result the dicarboxylic acid glaukophyllin, blue in solution; the dicarboxylic acid rhodophyllin, bluish red in solution; the monocarboxylic acid pyrrophyllin, blue, tinged red in solution.

Willstätter and Fischer[3] have recently succeeded in splitting off the last carboxyl group from the phyllins and porphyrins by heating them in very small quantities with soda lime. As a result the same aetiophyllin ($C_{31}H_{34}N_4Mg$) and aetioporphyrin ($C_{31}H_{36}N_4$) have been obtained from

---

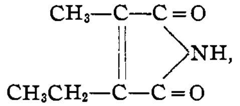
[1] R. Willstätter and A. Stoll, *Ann.*, 387, 357 (1911).

[2] R. Willstätter, M. Fischer and L. Forsén, *Ibid.*, 400, 147 (1913).

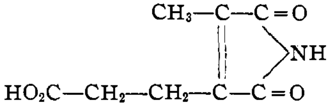
[3] *Ann.*, 400, 182 (1913).

the derivatives of chlorophyll *a* and *b*. These nuclei substances are well-crystallizing compounds, which fit into the group picture of the phyllins and the porphyrins previously investigated.

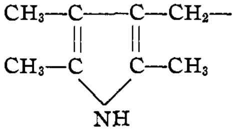
In order to simplify the consideration of the structure of chlorophyll, I would like to restrict myself to a discussion of the formula of aetioporphyrin. It must take into account the results of the oxidation and the reduction of chlorophyll derivatives. By oxidation,[1] phylloporphyrin furnishes compounds which are well known from the investigation of W. Küster[2] on the decomposition of Haemin. I refer to methyl-ethyl-maleic-imid,



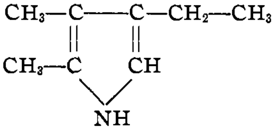
of which more than one molecule is obtained and further to haematic acid, which is produced in molecular quantity:



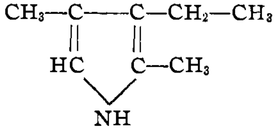
The reduction[3] of the porphyrins yields haemopyrrol, which was chiefly known as a reduction product of the blood dyestuff. M. Nencki and L. Marchlewski[4] have been the first to obtain it from a chlorophyll derivative. Together with Asahina, I have shown that it has a surprisingly complicated composition, being a mixture of three components, at least. We have isolated from it a tetra-substituted pyrrol, the phyllopyrrol. Hans Fischer and Bartholomaeus,[5] further O. Piloty[6] and L. Knorr,[7] have contributed to the explanation of different haemopyrrol components of which the following are now known exactly:



Phyllopyrrol



Iso-haemopyrrol



Kryptopyrrol

---

[1] R. Willstätter and Y. Asahina, *Ann.*, **373**, 227 (1910).

[2] *Z. physiol. Chem.*, **28**, 1 (1899); **29**, 185 (1900); **44**, 391 (1905); **54**, 501 (1908); **61**, 164 (1909); *Ann.*, **315**, 174 (1900).

[3] R. Willstätter and Y. Asahina, *Ann.*, **385**, 188 (1911).

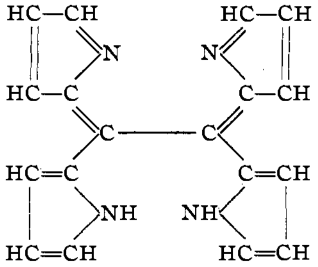
[4] *Ber.*, **34**, 1687 (1901).

[5] *Ibid.*, **44**, 3313 (1911); **45**, 1979 (1902); **45**, 466 (1912); *Z. physiol. Chem.*, **77**, 185 (1912); 80, 6 (1912).

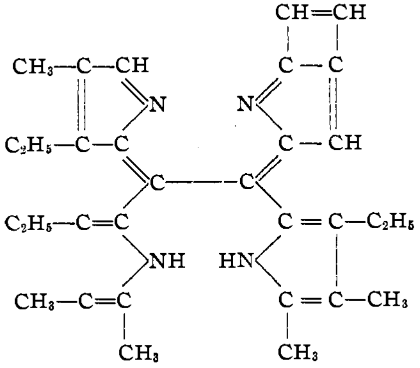
[6] *Ann.*, **366**, 237 (1909); **377**, 314 (1910); O. Piloty and E. Quitmann, *Ber.*, **42**, 4693 (1909); O. Piloty and J. Stock, *Ann.*, **392**, 215 (1912) and *Ber.*. **46**, 1008; O. Piloty and K. Wilke, *Ibid.*, **46**, 1597 (1913).

[7] L. Knorr and K. Hess, *Ibid.*, **44**, 2758 (1911); **45**, 2626 (1912).

The aetioporphyrin therefore is composed of four pyrrol nuclei. The number of its hydrogen atoms is strikingly low, which implies that the pyrrols must be so united and substituted by double bonds or further closing of rings that eight atoms of hydrogens less are required than if there were simple bonds. If the ethyl groups, necessary for the explanation of the oxidation products, are taken into consideration, it does not appear possible to formulate the aetioporphyrin without the assumption of a carbon ring. Several suggestions are met with in the literature on haemin by W. Küster[1] and O. Piloty[2] as to the combination of the pyrrol nuclei, but the question has not been solved by them. I consider the following formula probable to represent a simple union to a dyestuff of four pyrrol nuclei, of which two are salt-forming and two complex-forming:



If we substitute this parent substance, in respect of the oxidation products, with three methyl and three ethyl groups and, in respect of the reduction products, with at least another methyl group, there remains finally only so much hydrogen left for the last three carbon atoms that either two double bonds or two carbon rings or one of both must be assumed. If the assumption of a cyclopentane or hexane ring, corresponding to the course of the reduction, is avoided, we arrive, with some probability, at the following formulae for aetioporphyrin and aetiophyllin:

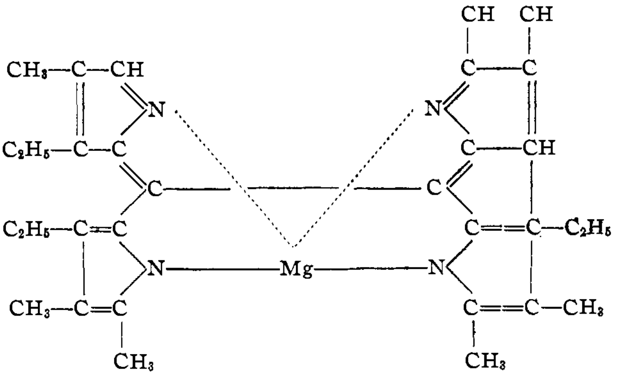


Aetioporphyrin, $C_{31}H_{36}N_4$

---

[1] *Z. physiol. Chem.*, 82, 463 (1912).

[2] *Ann.*, 388, 313 (1912); 392, 215 (1912).



Aetiophyllin, $C_{31}H_{34}N_4Mg$.

Several details of this formula, as the position of two methyl groups, are arbitrary. The cyclobutene ring could also be attached to the pyrrol in the β,β-position.

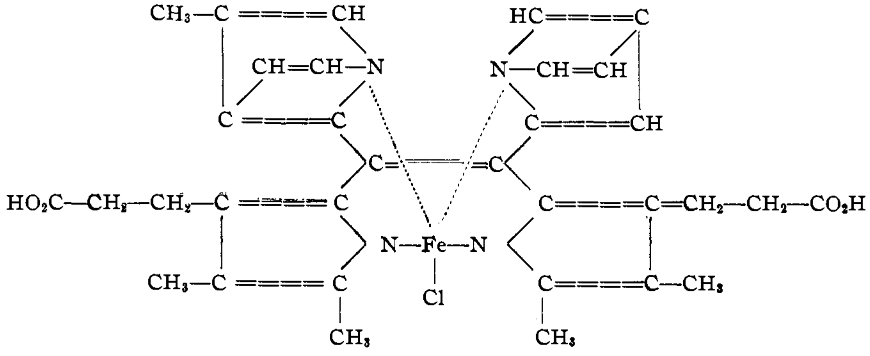
The question of the constitution seems to be the more important, as I have succeeded together with M. Fischer,[1] a short time ago, in breaking down the blood dyestuff to aetioporphyrin. There has been obtained, for the first time, a transformation product, from haemin and chlorophyll, whose molecule still stands in close relation to the dyestuff itself. Up to this time, only the decomposition products of haemin and chlorophyll, simple pyrrol derivatives, produced by oxidation and reduction, have been identical. The porphyrins from both pigments exhibited great similarity, which Hoppe-Seyler as well as Schunck and Marchlewski recognized, but nevertheless show considerable differences, for example, in their basic properties.

The porphyrins result from haemin through an entirely different reaction than from chlorophyll, and indeed not simply by the withdrawing of the metal. Haematoporphyrin, the product of the action of hydrobromic acid on haemin, is a dihydroxy acid. It has been possible to reduce haematoporphyrin to a new porphyrin, which I have called haemoporphyrin, by heating with methyl alcoholic potassium hydroxide and pyridine. This product differs in its composition from haematoporphyrin by minus two hydroxyl groups. Like the rhodoporphyrin, with which it is isomeric, it can be decarboxylated by heating with soda lime. From the composition $C_{31}H_{36}N_4$ of the aetioporphyrin so obtained, it follows that haemoporphyrin corresponds to the formula $C_{33}H_{36}O_4N_4$, and consequently haemin does not correspond to the generally adopted formula $C_{34}H_{32}O_4N_4$-FeCl but to the formula $C_{33}H_{32}O_4N_4FeCl$. While the aetioporphyrin of the formula $C_{31}H_{36}N_4$ already appears strikingly poor in hydrogen, haemin, $C_{33}H_{32}O_4N_4FeCl$, is derived from a parent substance, $C_{31}H_{34}N_4$, still poorer

---

[1] *Z. physiol. Chem.*, 87, 423 (1913).

by two hydrogen atoms, which renders it necessary to assume double bonds as well as peculiar carbon rings. Upon the basis of a new research[1] on the reactions leading from haemin to haematoporphyrin, I have tried to develop a constitutional formula of haemin explaining the behavior upon oxidation and reduction and also the porphyrin formation. In many details it has not been proved but, as I hope, it may serve to guide further investigation.



Haemin, $C_{33}H_{32}O_4N_4FeCl$.

Although chlorophyll and haemin have been traced back to the same aetioporphyrin, which can be called a parent substance, we do not draw the conclusion of a near constitutional relationship between chlorophyll and haemin from the investigations of the decomposition. In one, there is magnesium, in the other iron, in one ester formation with phytol, in the other a combination with globin. In addition to such differences, corresponding to the function of a different kind, there are further significant dissimilarities in the real nucleus of the dyestuff which have disappeared only by far-reaching decomposition. On the way from haemin to aetioporphyrin there are two transformations, which essentially change the construction of the molecule, namely, the transition from haemin to haematoporphyrin and from this to haemoporphyrin, which is isomeric with the porphyrins of chlorophyll. Between chlorophyll and aetiophyllin and even between chlorophyll and the first dibasic porphyrins, as cyanoporphyrin or erythroporphyrin, there are two steps, essentially transforming the molecule. They cannot be compared with the reactions of haemin.

Future investigations on the constitution of chlorophyll will still find important problems. The relations between the two chlorophyll components and the transformations which lead from chlorophyll to the chlorophyllins and from the chlorophyllins to the dibasic phyllins, are to be explained and the structure of aetioporphyrin is still to be investigated in important details. Of still greater importance are the questions which

---

[1] R. Willstätter and M. Fischer, *Z. physiol. Chem.*, 87, 423 (1913).

plant physiology asks and their solution will perhaps be aided by the isolation and the study of the components of the leaf dyestuff. These questions refer to the possibility of a chemical function of chlorophyll and to its nature.

BERLIN-DAHLEM, GERMANY.

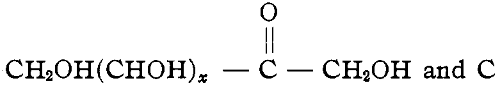
[CONTRIBUTION FROM THE KENT CHEMICAL LABORATORY OF THE UNIVERSITY OF CHICAGO.]

ON THE ESTERS, AS WELL AS THE MONOMOLECULAR β- AND γ-LACTONES, OF *d*-MANNONIC AND *d*-GLUCONIC ACIDS; ON ORTHO-BIS-*d*-GALACTONIC ACID, *d*-GALACTONIC γ-LACTONE AND ITS MONO-HYDRATE.

BY OSCAR F. HEDENBURG.

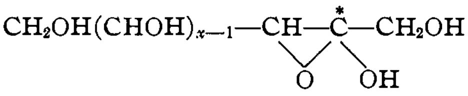
Received December 5, 1914.

A very condensed description of the properties and methods of preparation of the various compounds named in the above title has very recently been published by Nef,[1] who also discusses in detail the significance of the discovery of α-, β- and γ-lactones of the polyhydroxy acids, $C_nH_{2n}O_{n+1}$, in coming to a decision on the question of the constitution of the α- and β-modifications of aldose and ketose sugars, as well as of the α- and β-glucosides. He has reached the conclusion that the various crystalline forms of the simple sugars,[2] including also malt and milk sugars, are to be represented either by the free ketose or aldose structure,



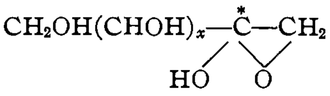
$$CH_2OH(CHOH)_x - \overset{\overset{O}{\|}}{C} - CH_2OH$$

and $CH_2OH(CHOH)_{x+1} - CHO$ (or at times by a corresponding ketose or aldose monohydrate structure, analogous to chloral hydrate, etc.), or, by a monomolecular α-lactone formula,



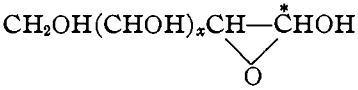
2,3-α-Ketose lactone.

, or



1,2-α-Ketose lactone.

and



α-Aldose lactone.

Generally only one of these two, or, in the case of the ketoses, three forms,

---

[1] *Ann.*, 403, 273-9, 306–331 (1914).

[2] In many cases the hydrazones, oximes, anilides and osazones of aldose and ketose sugars are known to show multarotation; this is unquestionably due to the existence of two or more analogous structural isomers, which have frequently been isolated, but have hitherto almost invariably been regarded as space isomers.

J. U. NEF.