STUDIES ABOUT CHLOROPHYLL. I.
THE PHYSICO-CHEMICAL BEHAVIOUR OF CHLOROPHYLL.

T. Vrbaški

Chlorophyll is in the living plant photoactive to a high degree, but is at the same time extremely fast to light. In assimilating the carbon dioxide the pigment absorbs the light energy necessary for conducting a complicated chemical process, in transfering it to the reacting system (CO_2,H_2O) without undergoing a permanent chemical transformation. The oxygen itself which is set free by the assimilation, probably in a very reactive state, causes no oxidation of the chlorophyll in the leaf. Chlorophyll, however, behaves in a different manner in vitro after being extracted from green plants by means of organic solvents. Such extracts in acetone, methyl alcohol, ethyl alcohol etc. contain chlorophyll besides other pigments depending on the kind of the effected extraction. It is either linked with phytol or dissolved as methyl or ethyl chlorophyllide respectively, but always in a molecular solution. If this solution is irradiated by visible light in the presence of oxygen or air, a rapid chemical transformation of chlorophyll takes place and the solution is bleached[1]).

The impressing fastness to light of the chlorophyll in vivo as contrasted to its ability of photooxidation in vitro is explained by R. Willstätter and A. Stoll[2]) with the assumption that the chlorophyll in the leaf is bound with protein and that this compound, destroyed by the extraction of the pigment, prevents its photooxidative transformation. In fact W. N. Lubimenko[3]) as A. Stoll and E. Wiedemann[4]) succeded in isolating from the green leaves the light fast chloroplastine, which may be considered as a complex compound of chlorophyll with the proteins. Aqueous suspensions of chloroplastine are practically entirely fast to light. But they cannot, according to the last experiments of the authors mentioned above, perform an assimilation of carbon dioxide in vitro. In a certain sense these investigations about chlorophyll deepened

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[1]) R. Willstätter and A. Stoll, Assimilationsbuch, p. 415. H. Gaffron, Ber., **60**, 755, 2229 (1927); K. Weber, Ber., **69**, 1026 (1936).

[2]) A summary of investigations about chlorophyll has been given by A. Stoll and E. Wiedemann in L. Zechmeisters Fortschritte d. Chemie org. Naturstoffe, Springer Verlag Wien 1938, vol. I. p. 231.

[3]) W. N. Lubimenko, Comp. rend., **173**, 365 (1921).

[4]) A. Stoll, Naturwiss., **24**, 53 (1936); A. Stoll and E. Wiedemann, La Chimica e l'Industria, **16**, 356 (1938); Compare to Je. A. Boitschenko, Chem. Zentr., **1943**, II, 815

our knowledge about the existing relations between the pigments of the leaf and the blood after the chemical constitution of the respective pigments has been determined. Just as the haemiglobine represents a complex protein compound of haemine with a colloidal character, so the chloroplastine is likewise a colloidal protein compound of the chlorophyll. On the other hand some authors succeeded in uniting haemines and haemes with organic nitrogen bases and similar compounds into loose complexes or molecular compounds.[5]) Parahaematines and haemochromogenes obtained in this way may be considered as a kind of artificial haemiglobines or haemoglobines respectively. Especially in regard to their catalytic activities there are some analogies between the natural and artificial molecular compounds of haemines.

Considering all these facts it may be supposed that chlorophyll will also yield with organic nitrogen bases or with similar substances molecular compounds which like the chloroplastine itself should be fast to light. Their photochemical autoxidation might proceed considerably slower even in solutions, than it would be the case with the free dyestuff.

Such molecular compounds of chlorophyll, analogous to the parahaematines and haemochromogenes, may be considered as »artificial chloroplastines«. The fastness to light of the chlorophyll in the presence of nitrogen compounds, which may form such chloroplastines, can be considered as a physico-chemical proof of their existence.

In order to prove this idea experimentally photochemical bleaching experiments were carried out with chlorophyll solutions in the presence or absence of different organic and inorganic nitrogen compounds. Having achieved in several instances a far reaching fastness of the chlorophyll to light, I investigated in detail the stabilization of chlorophyll in the presence of nitrogen compounds against the thermo-catalytic oxidation with H_2O_2.

The existence of the above mentioned molecular compounds was finally proved by means of other physico-chemical investigations. These experiments and their results will be briefly discussed here.

EXPERIMENTAL.

The chlorophyll solution was prepared from the dry meal of nettle bush's leaves *(Urtica dioica)*, obtained according to

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[5]) W. Langenbeck, Ber., **65**, 845 (1932;) W. Langenbeck, R. Hutschenreuter and W. Rotting, Ber., **65**, 1750 (1932).

Willstätter and Stoll[6]) by drying fresh leaves at 40°C and subsequent grinding. The chlorophyll solutions were obtained with methyl alcohol using the so called »rapid extraction« according to Willstätter and Stoll[6]) which avoids to a certain degree an alcoholysis of the chlorophyll and the subsequent splitting off the phytol. Since the meal of the leaves of the nettle plant contains only a small quantity of chlorophyllase — a phytol splitting ferment — a splitting of chlorophyll could hardy take place during the short time of extraction. The solutions used in the experiments contained therefore the dyestuff chiefly as a phytol ester. For several experiments the crystallized chlorophyll prepared according to Willstätter and Stoll, a product of the factory Sandoz-Basel, was used, which represented apparently a pure methyl chlorophyllide. The most important part of the investigations, the quantitative photochemical bleaching experiments, were achieved by measuring photoelectrically the reacting velocity. As the light source served a Nitra-Osram projection lamp (500 W), the light of which was concentrated on a reaction vessel (a cuvette with planparallel glass sides 10×10×1 cm) by means of a round retort filled with water. Behind the reaction vessel two optical glass filters (RG_4 and BG_{10} by Schott & Gen.) and a selenium photoelement were placed. The photocurrent was registered by a mirror galvanometer, (multiflexgalvanometer by B. Lange, Berlin, sensibility $2{,}6 \times 10^{-9}$ amp. per scale part).

By this experimental arrangement the spectrally undecomposed light of the light source acted on the solution of chlorophyll during the bleaching process. During the measurement only a narrow spectral region between 650 and 750 mμ approximately was used, corresponding to the absorption region of chlorophyll in the red part of the visible spectrum. The galvanometric deflexions were therefore proportional to the actual transparency of the solution in the spectral region mentioned above. They permitted a simple calculation of the absorption and the concentration of the chlorophyll respectively or the change of their quantities during the reaction time. The apparatus was standardized with solutions of pure crystallized chlorophyll of known concentration. The temperature in all experiments was kept at 25±0.2°C. The oxygen had a free access to the reacting solution which was stirred from time to time. The intensity of the light's source remained unchanged in all the experiments. Besides the photochemical bleaching, bleaching was also effected by means of H_2O_2.

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[6]) R. Willstätter and A. Stoll, Untersuchungen über Chlorophyll, Springer Verlag Berlin 1913, p. 112 ff.

Simple qualitative experiments showed that the heterocyclic nitrogen compounds as well as the nitrogen bases have no influence on the photochemical bleaching of the chlorophyll. On the other hand the same compounds are able to create haemochromogenes or parahaematines respectively. Some of these compounds increase the bleaching to a slight degree, while others affect the reaction very little or are entirely without effect.

Nitro- and nitrosocompounds, on the contrary, are able to inhibit to a high degree the photochemical bleaching of the chlorophyll. Used in appropriate concentrations they may stabilize entirely the chlorophyll solution to light. o-Nitrobenzaldehyde, o-nitrophenol, sodium nitrite, nitrosomethylcarbamide, m-nitrochlorbenzene, m-nitrobenzaldehyde, m-dinitrobenzene, α-nitronaphtalene, trinitronaphtalene, p-nitrophenol, p-nitrotoluene, nitrobenzene, p-nitrosodimethylaniline, nitrosobenzene and N-nitroso-p-nitrosophenylpiperazine behave in the same way. The compounds are given in order of their increasing efficiency. The next group which also inhibits the bleaching is the group of mono- and polyphenols and their ethers especially phenol, resorcinol, hydroquinonedimethylether, pyrogallol, hydroquinone, pyrocatechol and polyhydroquinone (also given in the order of their increasing efficiency). The compounds known in chemical kinetics as inhibitors or antioxygenes[7]) do not stabilize the chlorophyll solutions perfectly, even when used in the highest concentrations.

At last it should be pointed out that the so called acceptors of the bleaching reaction stabilize also more or less the chlorophyll solutions. Acceptors are compounds which in small concentrations favour the bleaching, but used in greater concentrations inhibit it. Such acceptors are: thiosinamine, diethylthiosinamine, diphenylthiocarbamide and diphenylsulfone[8]).

Of the quantitative measurements of the velocity of bleaching, the experiments made with the addition of nitro- and nitrosocompounds will be discussed first, because these compounds may cause a perfect stabilization of chlorophyll to light. The figure 1 represents some photochemical velocity curves, obtained in the presence of nitrosobenzene. The velocity of the bleaching without the addition of nitrosobenzene is shown by curve 0, while with increasing concentrations of nitrosobenzene (curve 1—5) the velocity is much reduced. A nitrosobenzene concentration of 5.10^{-3} mol/l stops entirely the photooxidation

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[7]) K. Weber, Inhibitorenwirkungen, Stuttgart 1938.

[8]) H. Gaffron, Ber., **60**, 755, 2229 (1927); Biochem. Z., **264**, 251 (1933); K. Weber, Ber., **69**, 1029 (1936).

and stabilizes the chlorophyll solution. From such velocity curves, which were also determined experimentally for alien compounds, the relative elementary velocity (v) of bleaching for different concentrations of added compounds can be calculated easily. This may be determined by drawing the tangent through

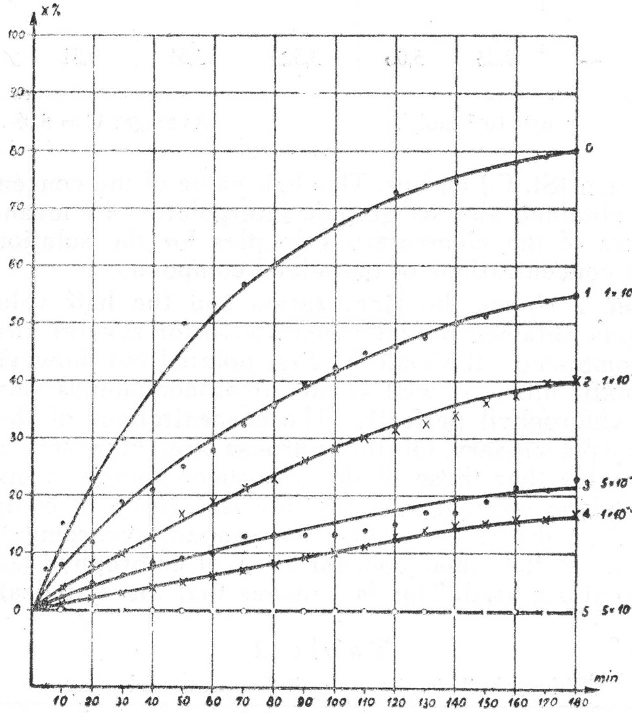


Figure 1

the zero point of each velocity curve and estimating the cutting point of this tangent with a certain value of the abscissa (after a reacting time of 20 minutes). Elementary velocities obtained in this way decrease with the increasing concentration of the added compound (table 1 for p-nitrosodimethylaniline) and attain at a certain concentration a half value of the elementary bleaching velocity of the chlorophyll solution free from the added compound. The half value concentration in mol/l (H. V. C.) represents a relative measure for the efficiency of stabilizators and characterizes them especially in the case when, in consequence of the small solubility of the added compound, it is not possible to determine experimentally the concentration at which total

Table 1.

Chlorophyll + p-nitrosodimethylaniline

c mol/l	—	$1 . 10^{-5}$	$5 . 10^{-5}$	$1 . 10^{-4}$	$5 . 10^{-4}$	$1 . 10^{-3}$	$5 . 10^{-3}$
v	23	21	13	7	5	2	0
$C . 10^5$	—	9,23	5,06	3,52	13,31	9,31	—

H. V. C. = $6{,}0 . 10^{-5}$ mol/l. Average: C = $8{,}08 . 10^{-5}$

stabilization (St. C.) ensues. This half value of the concentration may be obtained also by graphic interpolation by means of all the values of the elementary velocities for the solutions with different concentrations of the added compound.

Table 2 shows the stabilization and the half value concentrations obtained as described above for several nitro- and nitrosocompounds. It should be first pointed out, however, that the aromatic nitro- as well as the nitrosocompounds may stabilize the chlorophyll perfectly. The concentrations of the nitrocompounds necessary for this purpose are, however, considerably greater that those of the nitrosocompounds. Considering the half value concentrations of the last group of compounds, it is evident that these values correspond, according to their magnitude, to the molar concentration of chlorophyll ($2{,}5 . 10^{-5}$) in the solutions used. This fact means that the stabilization re-

Table 2

Chlorophyll + nitro- or nitrosocompounds resp.

Added compound	Concentration of stabilization St. C. mol/l	Half value of concentration H. V. C. mol/l	C
nitrobenzene	2,0	0,090	0,780
p-nitrotoluene	—	0,013	$3{,}21 . 10^{-2}$
α-nitronaphtalene	—	0,018	$1{,}86 . 10^{-2}$
nitrosobenzene	$5 . 10^{-3}$	$2 . 10^{-5}$	$9{,}02 . 10^{-5}$
p-nitrosodimethylaniline	$5 . 10^{-3}$	$6 . 10^{-5}$	$8{,}08 . 10^{-5}$
N-nitroso-p-nitroso-phenylpiperazine	$3{,}4 . 10^{-3}$	$0{,}85 . 10^{-5}$	$5{,}68 . 10^{-5}$
nitrosomethylcarbamide	—	0.165	—

presents a process by which one molecule of chlorophyll reacts alternately with one molecule of the nitrogen compound. Assuming as the main process of stabilization the formation of a molecular compound (artificial chloroplastine) in the form of a chemical equilibrium, this may be formulated as follows:

$$\underset{\text{chlorophyll}}{[\mathrm{Ch}]} \cdot \underset{\text{stabilizator}}{[\mathrm{No}]} \rightleftarrows \underset{\text{molecular compound}}{\mathrm{ChNo}} \qquad (1)$$

According to the law of mass action the following expression results for the constant of the chemical equilibrium:

$$\frac{[\mathrm{Ch}] \cdot [\mathrm{No}]}{[\mathrm{ChNo}]} = C \qquad (2)$$

If c_0 is the starting concentration of chlorophyll and n_0 the concentration of the stabilizator, the following expressions results for the concentrations in the equilibrium:

$$[\mathrm{Ch}] = c_0 - [\mathrm{ChNo}] \qquad (3)$$

$$[\mathrm{No}] = n_0 - [\mathrm{ChNo}] \qquad (4)$$

The concentration of the molecular compound in equilibrium may be calculated by means of the measured starting velocities of bleaching v. Between these velocities and the concentration of the uncombined chlorophyll (c_0-[ChNo]) there is obviously a proportionality, and the following expression can be obtained

$$[\mathrm{ChNo}] = c_0 - \frac{c_0 v}{v_0} \qquad (5)$$

in which v_0 is the starting velocity of the photochemical autoxidation in the presence of the stabilizator, and v the same velocity in the presence of the corresponding concentration of the stabilizator n_0.

If now the equilibrium concentrations are computed for different starting concentrations of the stabilizators according to the equations (3), (4) and (5), the constant of the equilibrium C may be easily evaluated from the equation (2). The obtained constant values for the different concentrations of the stabilizator indicate the existence of molecular compounds according to the relation (1).

The calculation of the equilibrium constants as described yields for the employed nitro- and nitrosocompounds mean values. They are also given in the table 2. Smaller values of the constants correspond, according to the relation (2), to a greater concentration of the molecular compound and also to a better stabilizing effect. Of all the examined compounds N-nitroso-p-nitrosophenylpiperazine gives the best stabilization and very easily forms molecular compounds. Moreover it is remarkable that the half value concentration of this nitrosocompound is smaller than the half value of the used chlorophyll concentration. This fact indicates that the nitrosocompound, at least partially, may also combine with two molecules of chlorophyll for one molecule of the stabilizator. This statement will be clearer, if one takes into consideration that the molecule of phenylpiperazine has two nitrosogroups. The *C* values for p-nitrosodimethylaniline, given in the table 1, show with what an exactness the equilibrium constant may be calculated from the reaction velocities. There are high oscillations of single values round the average value. This is not surprising if one takes into account that the exactness of the velocity measurements, necessary for calculating the values of constants, cannot be very great. But the exactness of the measurements and the calculation of the equilibrium constant is sufficient to confirm, at least qualitatively, that the equilibriums of the described manner will indeed occur. Their position in the presence of aromatic nitrosocompounds, seems to be strongly shifted to the side of the molecular compound. This fact has the same meaning as the statement that in the examined solutions there are really present artificial chloroplastines, which represent molecular compounds of chlorophyll with aromatic nitrosocompounds. The aromatic nitrocompounds seem to be able to form molecular compounds also with chlorophyll. The corresponding equilibriums are only strongly shifted to the side of the dissociation of the chloroplastines, which is evident from the much smaller effect of stabilization. Eventually the half value concentration of the used aliphatic nitrosocompound is so great, and its stabilizing effect so small that in this case there can be no formation of a molecular compound. The quantitative measurement of the inhibiting effect of mono- and polyphenols on the photochemical bleaching of chlorophyll yields much higher half value concentrations (table 3) than in the case of aromatic nitrosocompounds.

Under such experiment conditions which yield half of the reacting velocity of the solutions without any addition, nearly 200 hydroquinone molecules or 50.000 phenol molecules come for each molecule of chlorophyll. Moreover the chlorophyll may not be totaly stabilized even when using the strongest concentrations of phenol. Finally the law of mass action (equation 2) is not satisfied as the constant *C*, computed according to this law, increases with the inhibitor concentration nearly in the same measure as the concentration itself. This feature is evi-

Table 3

Chlorophyll + phenol

Inhibitor	H .V. C. mol/l
phenol	1,66
hydroquinone	$5 . 10^{-3}$
pyrocatechol	$7,5 . 10^{-3}$
resorcinol	$2,8 . 10^{-2}$
toluhydroquinone	$8,5 . 10^{-2}$
pyrogallol	$5,0 . 10^{-2}$

dent from the values for the inhibitor hydroquinone given in table 4.

Table 4

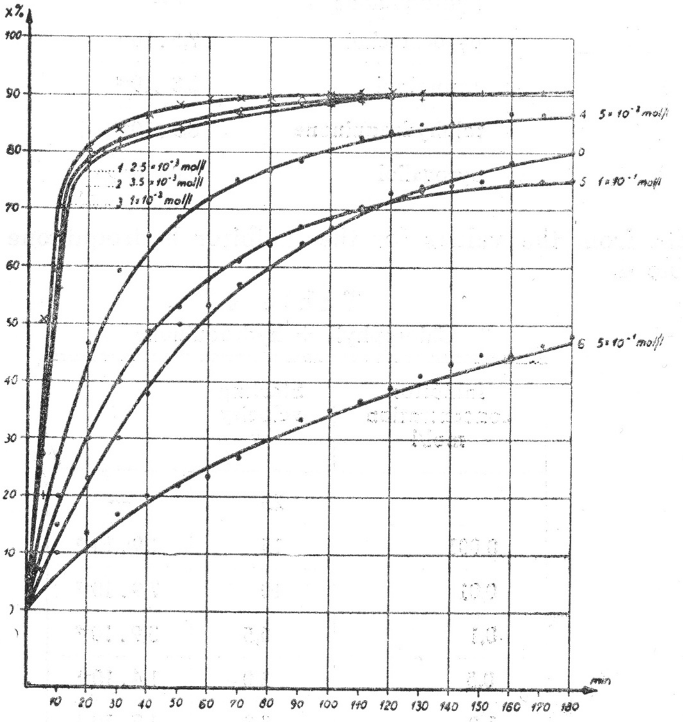
Chlorophyll + hydroquinone

Inhibitor concentration mol/l	Starting velocity v	C
—	23	—
0,001	15	$1,9 . 10^{-3}$
0,01	10	$7,9 . 10^{-3}$
0,1	6,5	$3,9 . 10^{-2}$
0,5	5,0	$1,4 . 10^{-1}$
1,0	3,3	$1,7 . 10^{-1}$

All these statements mean that the phenols cannot form any molecular compounds with the chlorophyll, they react rather like true inhibitors (negative catalysts). They only reduce the reaction velocity, perhaps by mutual effect on the stimulated chlorophyll molecule by irradiation of light during the time of exposure.

The oxidated forms of the polyphenols too, the quinones (benzoquinone, toluquinone, thymoquinone) can inhibit in small concentrations the autoxidation of chlorophyll. In greater concentrations they promote the autoxidation, this being in connection with their oxidative effect.

Different velocity curves for bleaching in presence of thiosinamine are pointed out in figure 2 as an example of a characteristic acceptor effect. Smaller concentrations of this compound ($2{,}5 \cdot 10^{-3} - 1 \cdot 10^{-2}$ mol/l) increase considerably the bleaching velocity, especially in the first reaction phases. With further increase of the concentration of thiosinamine ($5.10^{-2} - 1.10^{-1}$ mol/l) the promoting effect first decreases, but with still greater concentrations of the acceptor (5.10^{-1} mol/l) a partial stabilization of the chlorophyll results. All these concentrations, especially the concentration of the stabilization, are much greater than the corresponding concentration of the chlorophyll. There can surely be no question of a molecular compound between the chlorophyll and the acceptor, but the case presents a special kinetic influence of the acceptor concentration. These special kinetic influences cause the chlorophyll to become a photochemical sensibilizator for the autoxidation of thiosinamine at greater concentrations. The available oxygen will in this case be trans-



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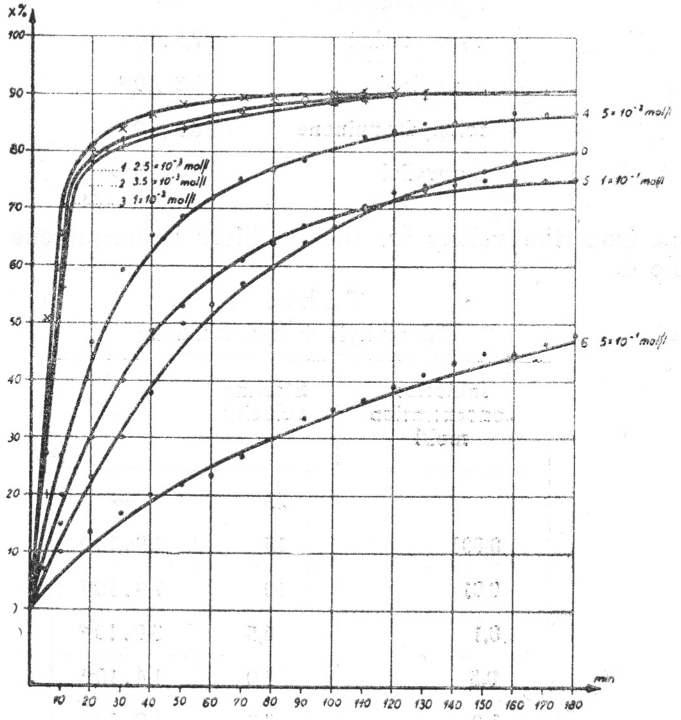

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Simultaneously with the investigations of photochemical bleaching measurements of light absorption of the irradiated,

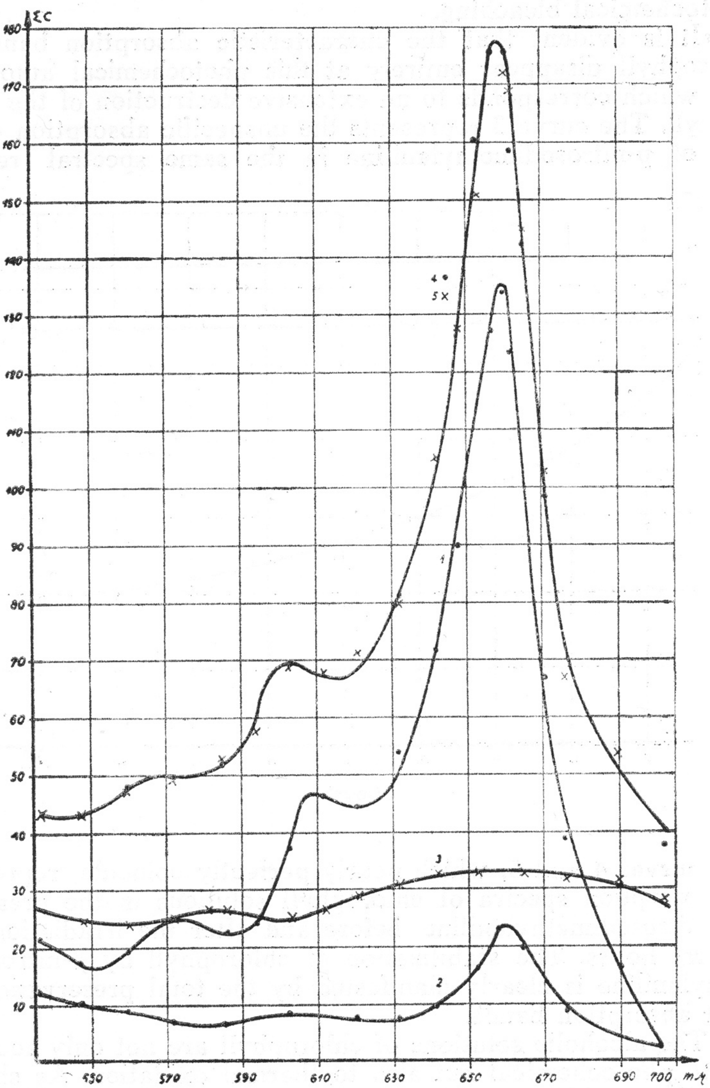

Figure 3

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[9]) Compare besides the quoted investigations also K. Weber, Atti. X. Congresso intern. chim. V. 215, 1938.

not irradiated, stabilized and unstabilized solutions of chlorophyll were achieved. The figure 3 represents absorption spectra obtained by means of the König-Martens spectral photometer. The curve 1 represents a spectrum of a not irradiated chlorophyll solution. The curve 2 a spectrum of the same solution after photochemical bleaching.

It is evident that the characteristic absorption bands of chlorophyll disappear entirely at this photochemical autoxidation which corresponds to an extensive destruction of the chlorophyll. The curve 3 represents the unspecific absorption capacity of p-nitrosodimethylaniline in the same spectral region.

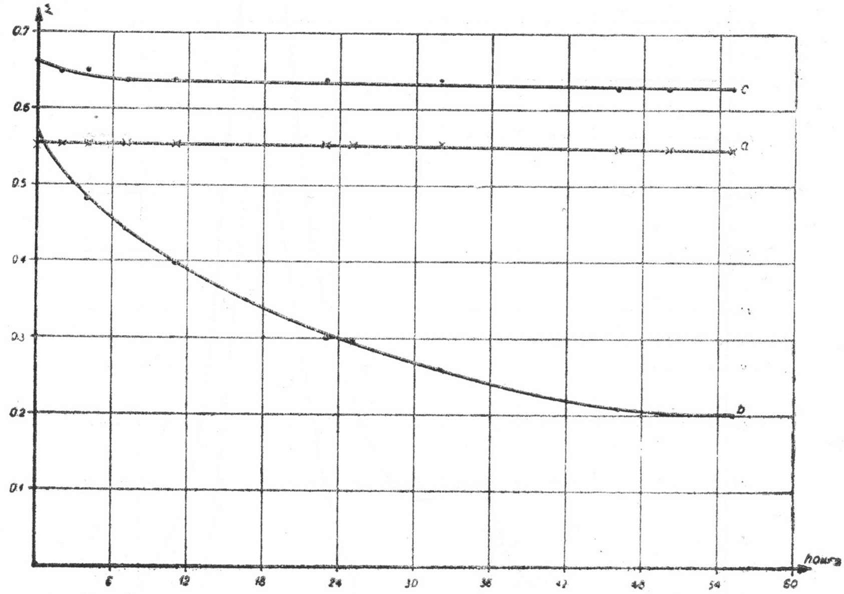

Figure 4

The curves 4 and 5, which nearly perfectly coincide, represent the absorption spectra of chlorophyll solutions in the presence of p-nitrosodimethylaniline before and after the irradiation for several hours. The stabilization of chlorophyll by p-nitrosodimethylaniline is clearly manifested by the total preservation of their absorption bands.

The alcoholic solutions of chlorophyll are not only accessible to photochemical but also to thermal oxidation. As shown by K. Noack[10]) especially H_2O_2 reacts in this sense in the presence of ferric hydroxide as a catalyst. Colloidal aqueous chlo-

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[10]) K. Noack, Biochem. Z., 316, 166, (1943).

rophyll solutions remain unchanged, however, by this oxidative effect. In the solutions the pigment is again combined with a protein molecule. It should be interesting therefore to test the behaviour of the »artificial chloroplastines« towards this thermal oxidation. The figure 4 gives the results of several measurements. They clearly show that the aromatic nitrosocompounds can stabilize the chlorophyll perfectly against the thermal oxidation with H_2O_2 in presence of ferric hydroxide. The abscissae of this diagram represent reacting times, the ordinates the extinction coefficients of the solutions of chlorophyll in the red spectral region[11]. The values of the curve a) correspond to a chlorophyll solution free from any oxidative substance and from any stabilizator, which remained unchanged during 60 hours at the experimental temperature of $30^0 \pm 0{,}2^0$C. The curve b) represents the values of the extinction coefficients which refer to the same chlorophyll solution in the presence of H_2O_2 and of the catalyst. A pronounced oxidative effect is evident. The curve c) corresponds finaly to a chlorophyll solution when an oxidative compound, catalyst and stabilizator, p-nitrosodimethylaniline have been added. A hardly noticeable oxidation proves clearly the perfect stabilizing effect of nitrosocompound. Other investigations also clearly allow the assumption of the existence of an artificial chloroplastine.

It may be pointed out at last that other investigations also show the existence of molecular compounds between chlorophyll and nitrosocompounds as for example, the investigation of light absorption of chlorophyll solutions in the absence and the presence of stabilizator in the ultraviolet and the infrared spectral region, the investigation of fluorescence of chlorophyll as well as the chromatographic investigations. The results of these investigations are, however, not so convincing as the kinetic investigation of the photochemical or thermal oxidation of chlorophyll. The results of these investigations will be discussed in a subsequent study.

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IZVOD

Studija o klorofilu, I.
Fizikalno-kemijska svojstva klorofila

T. Vrbaški

Dokazano je fizikalno kemijskim metodama rada, da je klorofil u stanju stvarati sa stanovitim aromatskim dušikovim spojevima kompleksne

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[11] These extinction coefficients were measured by means of the photoelectric colorimeter by B. Lange.

ili molekularne spojeve, koji su stalni prema djelovanju svijetla, a nazvani su »umjetni kloroplastini«. Ovi umjetni kloroplastini mogu se smatrati analognim spojevima parahematinima i hemokromogenima, koji pretstavljaju labav kompleksni ili molekularni spoj hemina ili hema sa organskim bazama i sličnim spojevima.

Primijenjene metode rada jesu fotokemijsko i termičko katalitičko izbljeđivanje klorofila, optička mjerenja spektralnih apsorpcija u raznim spektralnim područjima, mjerenja fluorescencije kao i kromatografska ispitivanja.

Zanimljivi rezultati postignuti su na području kemijske kinetike t. j. na području fotokemijske oksidacije i sprečavanje fotooksidacije alkoholne otopine klorofila. U pogledu djelovanja upotrebljenih stranih tvari na sam tok fotooksidacije klorofila mogu se iste svrstati u slijedeće skupine:

1. Tvari koje potpuno ili skoro potpuno sprečavaju fotokemijsku oksidaciju klorofila. To su t. zv. stabilizatori, koji djeluju na taj način da se kemijski vezuju na nenadraženu molekulu klorofila i stvaraju fotostabilne kompleksne ili molekularne spojeve, koji pokazuju veliku otpornost prema djelovanju svijetla. To su aromatski nitrozo- i nitrospojevi. Otopina klorofila stabilizira se potpuno već kod vrlo male koncentracije nitrozospoja. Tako koncentracija stabilizacije za p-nitrozodimetilanilin iznosi $5 . 10^{-3}$ mol/l. Polovična koncentracija P. K., t. j. koncentracija stabilizatora kod koje brzina fotokemijskog izbljeđivanja klorofila padne na polovicu, također je vrlo mala za nitrozospojeve, te iznosi za nitrozobenzol $2{,}0 . 10^{-5}$ mol/l. Koncentracija klorofila u metilalkoholnoj otopini iznosi kod toga samo $2{,}5 . 10^{-5}$ mol/l. Ova činjenica upućuje na pretpostavku, da kod stabilizacije otopine klorofila otpada na jednu molekulu klorofila samo jedna molekula stabilizatora, dočim kod najjačeg inhibitorskog djelovanja, gdje nikada ne dolazi do potpune stabilizacije, otpada na jednu molekulu klorofila 200 do 50.000 molekula inhibitora. Izračunate vrijednosti za konstantu ravnoteže K kod raznih koncentracija stabilizatora ostaju približno konstantne, te se ta okolnost može uzeti kao dovoljan dokaz da se ravnoteža stvarno uspostavlja i da je njezin položaj u prisutnosti aromatskih nitrozospojeva jako pomaknut na stranu molekulskog spoja između klorofila i stabilizatora.

U prisutnosti nitrospojeva izgleda da također dolazi do stvaranja sličnih molekulskih spojeva samo ovoj ravnoteži pripada neki srednji položaj, što također proizlazi iz znatno manjeg stabilizirajućeg djelovanja, mnogo veće koncentracije stabilizacije i polovične koncentracije, koje iznose za nitrobenzol 2,0 mol/l i 0,090 mol/l.

Što se tiče mjesta kao i kemijske skupine, na koju se ti nitrozospojevi vezuju na molekuli klorofila, ne može se iz dobivenih rezultata ništa pobliže zaključiti.

2. Pravi inhibitori, koji smanjuju brzinu fotokemijske oksidacije klorofila, ali ga pri tome ne stabiliziraju potpuno. To su prije svega hidrokinon, toluhidrokinon, pirokatehin i pirogalol kao i neki drugi polioksibenzoli.

Zakonitosti inhibitorske pojave uglavnome su ispunjene, iako opća inhibitorska jednadžba Baur Ouellet-a nije uvijek zadovoljena. O mehanizmu ovog djelovanja može se samo to reći, da se po svoj prilici radi o kinetičkoj, a ne statičkoj pojavi t. j. da se svijetlom aktivirane molekule za vrijeme podražaja dezaktiviraju djelovanjem inhibitora, a nikako se ne stvara između dotičnog inhibitora i klorofila neki donekle stabilan spoj.

3. Akceptori, koji u manjim koncentracijama pospješuju fotokemijsku autooksidaciju klorofila, a u većim djeluju stabilizirajuće. To su

poznati spojevi kao na pr. tiozinamin, dietiltiozinamin, feniltiozinamin, dialiltiosemikarbazid i mnogi drugi. Sprečavanje fotooksidacije klorofila u prisutnosti većih koncentracija akceptora ne može se smatrati pravim inhibitorskim djelovanjem i ako čisto formalno i u ovom slučaju nastupa smanjivanje brzine fotokemijske reakcije.

Konačno mogu se smatrati četvrtom skupinom takove tvari, koje same naginju fotooksidativnoj pretvorbi djelovanjem svijetla u prisutnosti klorofila. U ovu skupinu spadaju aromatski amini i diamini: anilin, o-, m- i p-fenilendiamin, kao i neki kinoni: p-benzokinon, tolukinon i timokinon. Ovdje se očito radi o senzibiliziranoj fotooksidaciji kod koje klorofil ima ulogu senzibilizatora.

Spektralno fotometrijska ispitivanja osvijetljene i stabilizirane otopine klorofila pokazala su, da se osvjetljavanjem karakteristična vrpca apsorpcije klorofila niti najmanje ne mijenja, te ostaje u cijelosti uščuvana i nakon višesatnog osvjetljavanja.

Kinetički pokusi oksidacije klorofila u otopini metilnog alkohola pomoću vodikovog peroksida u prisutnosti željeznog (III) hidroksida izrazito potvrđuju osnovnu zamisao. Ovim pokusima je na vrlo jednostavan način dokazana analogija fizikalno kemijskog odnosa prirodnog bjelančevinastog klorofila kloroplasta i umjetnog kloroplastina prema termičko oksidativnom sistemu vodikovog peroksida u prisutnosti katalizatora željeznog (III) hidroksida.

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