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Photochemical Changes of Chlorophyll *a* as Reflected in Absorption Spectra

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The present work deals with an observation of changes in optical spectra of chlorophyll solutions induced by intense illumination. The dependence upon illumination intensity and spectral composition of light is examined for four organic solvents – methanol, acetone, benzene and cyclohexane. The first part of the work is a summary of up-to-date theoretical and experimental knowledge closely connected with absorption spectra of chlorophyll. To make the concluding discussion easier it has been found suitable to mention, in this part of the work, also the basic characteristics of chlorophyll *a* from the standpoint of chemical structure. In the second part experimental results are given, and the third part is devoted to discussion on the dependences discovered. A photochemical reaction of chlorophyll *a* induced by intense continuous illumination in the presence of air has been identified as photooxydation. The final products of illumination of chlorophyll *a* are probably protochlorophyll *a* and protopheophytin *a*.

I. Basic properties of chlorophyll *a* and its solutions in organic solvents

1. 1. CHEMICAL STRUCTURE OF CHLOROPHYLL *a*

Chlorophyll, due to its chemical structure, belongs to the ample group of porphyrines. The base of *a* chlorophyll molecule is a porphine skeleton (Fig. 1a)

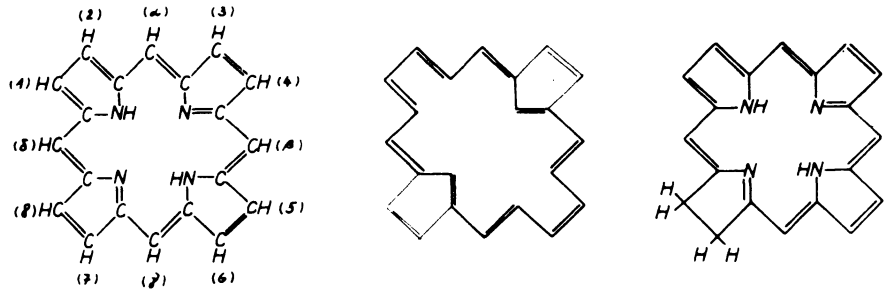

Fig. 1. Molecule of porphine (*a*) with conjugated system marked (*b*) and molecule of chlorine (*c*)

composed of four pyrrol nuclei connected by means of methine bridges to form a perfectly conjugated circular system. Atoms of hydrogen of porphine molecules in positions *1* — *8*, α — δ and one of the atoms in the centre of a molecule can be substituted with different organic radicals. Compounds obtained in this way are called porphyrines.

Porphyrines can be divided into proper porphyrines, dihydroporphyrines (chlorines) and tetra hydroporphyrines (bacteriochlorines). In the structural formula of porphyrine there are two double („half isolated") bonds (Fig. 1a, bonds 3—4 and 7—8), not included in the closed conjugated system. Proper porphyrines retain porphine skeleton and differ in lateral substituents. In molecules of dihydroporphyrines both "half-isolated" double bonds are replaced by simple bonds resulting from hydrogenation.

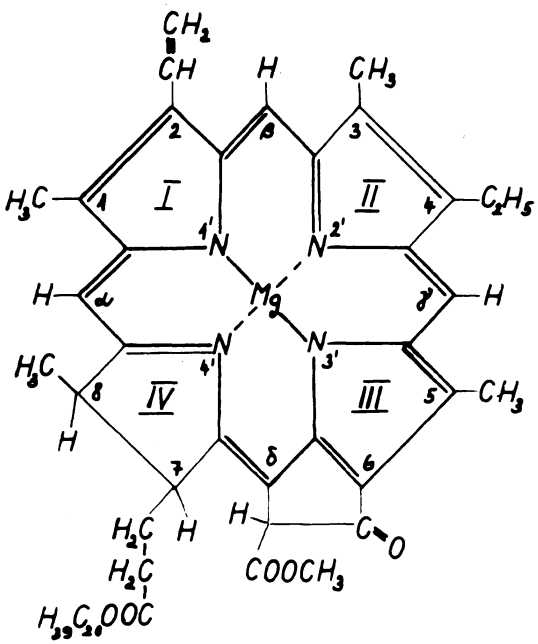

Fig. 2. Molecule chlorophyll *a*

Chlorophylls can be grouped with chlorines or metallochlorines, as two of central nitrogen atoms of pyrrols are bonded with magnesium. Besides the hydrogenated bond (Fig. 1a, bond 7—8), typical of chlorines, a chlorophyll molecule (Fig. 2) has other substantial features: a cyclopentanone ring linked with conjugated system and a long phytol chain. Two most important chlorophylls marked *a* and *b* differ in the substituent in position *3* (Fig. 2). Chlorophyll *a* has in this position a methyl and *a* chlorophyll *b* carbonyl groups.

The structure of chlorophyll molecule illustrated in Fig. 2 was verified by a number of experiments, the most important of which are infrared spectra and the chemical synthesis of molecule. A complete synthesis of chlorophyll molecule representing about thirty partial stages was made for the first time in 1960 by R. S. Woodward [1].

1. 2. ABSORPTION SPECTRA OF CHLOROPHYLL *a*

From a superficial comparison of absorption spectra of porphyrines as e. g. pheophytin, phtalocyanin, or chlorophyll, no similarity is clearly visible. A detailed

analysis of dependences between chemical structure and character of absorption spectra makes it possible to find the similarity and determine a number of empiric rules attributing certain changes in absorption spectra to certain chemical changes. As shown by Gurinovitch, Sevchenko and Solovyeff [1, 2] in a comprehensive work devoted to the spectroscopy of porphyrines, a number of chemists and physicists were keen on the problems as early as in the twenties of this century. Works by Conant and Kamerling, Hellström, Haurowitz are mentioned, and especially a complex study of porphyrines by A. Stern [3, 4, 5, 6]. Stern investigated a number of porphyrines and by following the dependences between the spectrum character and a system of lateral substituents of porphyrine nucleus, he formulated a number of empirical rules.

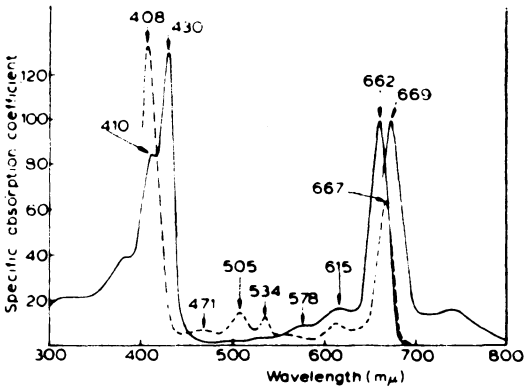

Fig. 3. Absorption and Fluorescence spectra of chlorophyll *a* (—) an pheophytin *a* (....) in ether (7)

Absorption spectra of porphyrines have four weak, relatively narrow, approximately equidistant bands in the visible region. On the boundary between ultraviolet and visible regions a very intense absorption band, called Soret band, appears in all porphyrines. Stern marked the four porphyrine absorption bands with Roman numerals I, II, III and IV, starting by the long wavelength region in the order given by the decreasing intensity.

The absorption spectrum of chlorophyll (Fig. 3) has a fairly intense long – wave band and a number of absorption bands, the intensity of which decreases towards the short wavelength. Soret band is wide, with a perceptible split.

The results of measurements of fluorescence and polarization spectra make it possible to interpret individual absorption bands. Fluorescence spectra are mirror symetric with respect to the absorption spectrum of an oscillator of the lowest energy, and consequently only bands coresponding to the lowest electronic transition can appear in the fluorescence spectrum. With respect to that, it can be said that the absorption bands I (662 nm) and II (615 nm) belong to the first electronic transition, the band II being vibration satellite of the band I. The fluorescence spectrum (Fig. 3) does not contain mirror symetric image of the band III (578 nm) which means that this band must be caused by another electronic transition.

A fairly more detailed information on the electronic substance of absorption bands can be gathered from polarization measurements. The dipole moments associated with the π – electronic transitions of large planar, conjugated molecules like porphin lie in the plane of the molecule, unlike dipole moments $n \rightarrow \pi^*$ transi-

tions which can be orientated perpendicularly to the plane of molecule. Whether an absorption band corresponds to a transition parallel or perpendicular to the plane of the molecule may be determined by measuring the dichroism of the pigment dissolved in an artificially oriented system, such as a "liquid crystal" or a stretched polyvinyl film. It was found for chlorophyll [8] that in the whole visible region absorption occurs, taking place in plane of molecule, and thus all these absorption bands are with great probability caused, to a considerable measure, by $\pi - \pi^*$ electronic transitions.

In polarization spectra a connection can be found between the absorbing and emitting oscillator by measuring the degree of fluorescence polarization as a function of the wavelength of the exciting light. The degree of polarization is different depending on excitation in various absorption bands due to the existence of a certain angle α between an absorbing and a corresponding oscillator. From the formula by Levshin – Perenov [9]

$$P = \frac{3\cos^2\alpha - 1}{\cos^2\alpha + 3}$$

a limit value $P = -0{,}33$ follows for mutually perpendicular oscillators and for mutually parallel oscillators a value $P = 0{,}5$. Within the absorption band system of a single transition (main band and vibrational levels), the polarization value should be constant.

The value of polarization, measured for chlorophyll in castor oil [9] is non-negative in nearly the whole visible region, and consequently the angle between an absorbing and emitting oscillator is small. That corresponds essentially to the results of measuring dichroism as to major participation of π —electrons in absorption of light by a chlorophyll molecule. The intense Soret bands located between 400 nm and 500 nm and the long wave bands in the red and near infrared region both result from $\pi - \pi^*$ transitions in the porphyrin ring in which the optical electrons move toward the periphery. The satellite bands of lower intensity represent transitions from vibrational substates. $\pi - \pi^*$ transitions in which electrons located on the carbon atoms of the pyrrole move towards the periphery of the porphyrin ring give rise to the Soret band, while $\pi - \pi^*$ transitions in which electrons flow from the pyrrole nitrogens to the peripheral carbon lead to the long wavelength transitions [10]. A number of authors suppose that in the $n - \pi^*$ transitions, which play a most important role in the fluorescence and phosphorescence of chlorophyll, n-electrons are located on the carbonyl group of cyclopentanone ring.

Thus it follows from polarization spectra and mirror symmetry of fluorescence that the absorption bands I and II arise from the first electronic transition. The connection of the second electronic transition with any absorption band is not, for the time being, quite unambiguous. A local minimum 630 nm can be observed in the polarization spectrum [7], becoming even more evident with decreasing tempe-

rature. In solvents forming solvates with chlorophyll molecule a band appears between the bands I and II in absorption spectrum in a position corresponding to the above mentioned minimum of polarization about $\lambda = 630$ nm, with disappearance of the characteristic "grove" of fading bands, including the maximum around 578 nm. It remains a question whether the solvation results only in the strengthening of a previously hidden transition ($\lambda = 630$ nm), or only in a shift of the maximum from the position 578 nm to 630 nm. The disappearance of "grove" during the solvation rather gives a shift of the band 578 nm, but the magnitude of the shift (52 nm) is too big to have resulted from a shift of electronic level during the solvation [9]. A more acceptable interpretation is evidently as follows: in the usual spectrum of chlorophyll (non-solvated) the second electronic 0 — 0 transition ($\lambda = 630$ nm) is suppressed, covered by the band I, and manifests itself in the spectrum only through its vibration satellite in the position $\lambda = 578$ nm (band III).

The most outstanding derivative of chlorophyll produced in an acid medium by replacing an atom of magnesium by two hydrogens is pheophytin. Chlorophyll and pheophytin spectra in ether (Fig. 3) differ substantially only in the blue – green region (450—550 nm) of visible spectrum. The long wave band (I) of pheophytin is shifted by about 5 nm toward the long wavelengths and has a lower intensity. The first vibration maximum (II) is shifted from 615 nm to 610 nm. The absorption band of chlorophyll 578 nm (III) which was aligned with the second electronic transition, is, in the case of pheophytin, evidently shifted down to 534 nm and is much more intense. In the position 505 nm a new band appears in pheophytin which, according to Goedheer [7], can be attributed to $n - \pi^*$ comprising n — electrons of pyrrole nitrogens. Soret's band is, in pheophytin, shifted somewhat to the blue and is less clearly divided into two components than is the Soret's band of chlorophyll.

An interesting information on absorption of light by chlorophyll can be gathered from the so-called quasi-linear spectra. They are based on Spolski phenomenon [1, 11], representing a quasi-linear split of molecular electronic spectra of organic molecules built into a crystal matrix, at very low temperatures. In 1969 Litvin, Personov and Korotayev succeeded in obtaining quasi-linear spectra of chlorophyll *a* by means of decreasing the temperature to 4°K.

The position, intensity and width of absorption bands of solutions of chlorophyll *a* depend on the physical parameters of solvents. With respect to the fact that the distribution of π — electrons of large planar molecules, e. g. chlorophyll, is suitable for the origin of aggregated forms [1, 16] a formation of dimers takes place e. g. in non-polar solvents at higher concentrations, due to the binding of the central magnesium atom of one molecule with the carbonyl group of the second molecule of chlorophyll. In pure polar solvents chlorophyll is solvated with two molecules of solvent, bound to the magnesium in the center of the porphyrin ring by the most nucleophilic atom of the solvent [12]. Under certain experimental conditions the formation of dimers can be eliminated and changes of absorption spectra in various

solvents can be attributed only to changes of physical properties of the medium wherein the chlorophyll molecule occurs. The general formula for the change of wavelength of absorption maxima depending on refraction index and dielectric constant of solvent is expressed by Seely and Jensen [12, 24] in the form

$$\Delta\lambda = \frac{e^2 f \lambda^3}{8\pi^2 m\, c^2 r^2}\left\{\frac{n^2-1}{2n^2+1}\right| + \frac{\lambda^2}{h\,c\,r^3}\left\{\frac{2n^2+1}{n^2+2}\right\}(\mu_e^2-\mu_g^2)\left|\frac{n^2-1}{n^2+2}\right\} + \\ + \frac{2\lambda^2}{h\,c\,r^3}\left\{\frac{2n^2+1}{n^2+2}\right\}\mu_g(\mu_e\cos\alpha-\mu_g)\left|\frac{D-1}{D+2}-\frac{n^2-1}{n^2+2}\right\} \qquad (1,2)$$

where e and m are the electronic charge and mass, f is the oscillator strength, μ_e and μ_g are the dipole moments of the excited and ground states of chlorophyll and α is the angle subtended between them, n and D are the refractive index and dielectric constant of the solvent, and r is the radius of the cavity containing the solute molecule. The position of absorption maxima depends much more on the refractive index than on the dielectric constant, and it may be concluded that for chlorophyll the general red shift is predominant.

2. Photochemical changes of chlorophyll *a* – experimental part

2. 1 PREPARATION OF PURE CHLOROPHYLL *a*; EXPERIMENTAL EQUIPMENT

Chromatographically pure chlorophyll *a* was prepared in the Department of Physics, Charles University in Prague by a method approximately corresponding to that of Hager [13]. The mixture of plant pigments obtained from acetone extract of fresh leaves of Urtica dioica (nettle) and Tradescantia was separated chromatographically on Whatman paper 3. System petrol, benzene, chloroform, acetone and isopropanol was used in the proportions 50 : 35 : 10 : 0,5 : 0,17. The dark green band corresponding to chlorophyll *a* was cut off and pigment eluted by acetone or petroleum ether. The extract obtained was further separated chromatografically on a thin layer of cellulose. A mixture of petroleum ether, acetone and *n*-propanol in the relations 90 : 10 : 0,45 was used as a developing system. All work was done in the dark and at low room temperatures. The chlorophyll prepared was kept in concentrated acetone solution at 0°C in sealed ampules or in a light — tight vacuum container. Chlorophyll *a* for measurements in other solvents than acetone was obtained from the stock solution by means of evaporation of acetone at room temperature in the vaccuum evaporator. The solvents acetone, benzene, cyclohexane and methanol were obtained from Lachema (R.P. grade) and used without further purification.

The concentration of chlorophyll was determined from the absorption spectra on the principle of Lambert — Beer law, from the relation

$$\varepsilon = \varepsilon_m \,.\, c \,.\, d \qquad (1,3)$$

where ε is an experimentally determined extinction coefficient of the red absorption maximum, ε_m is a molar extinction coefficient determined for the given solvent by

Seely and Jensen [12, 14], c is the concentration of chlorophyll in solution and d is the width of absorption layer. Absorption spectra were measured on UNICAM SP.800 and UV-VIS Zeiss spectrophotometers.

The illumination of samples resulting in photochemical changes of chlorophyll was made by projection lamps Tungsram 220 V, 300 W or 8 V, 50 W, respectively. The lamp was housed in a special cover and cooled by a stream of air. The lamp cover was equipped with a concave mirror placed behind the lamp. In front of the outlet aperture was placed a thermal water filter 3 cm thick, with flowing cold water. For the determination of the spectral region of illuminating light glass filters *BG* 12 (transmissible in region 325—500 nm), *VG* 6 (410—600 nm), *RG* 1 (for $\lambda > 600$ nm) and *RG* 5 (for $\lambda > 650$ nm) were used.

The intensity of illumination light was determined by means of a vacuum thermocouple of the type Kortüm *VTh* 8 with self-resistor 42.9 Ω, sensitive area 7 mm^2 and sensitivity 4 V/W. The thermoelectric voltage was measured by means of a loop galvanometer Zeiss, sensitivity $8.16.10^{-6}$ V/grade. By means of both this equippment and a set of grey filters the incident light energy up to the value 5.10^{-2} W/cm^2 was determined. Chlorophyll solutions were illuminated in a 20 mm glass cuvette, not completely filled with the solution.

2. 2. ABSORPTION SPECTRA REFLECTING PHOTOCHEMICAL CHANGES OF CHLOROPHYLL *a* INDUCED BY INTENESE ILLUMINATION WITH WHITE LIGHT

Samples of chlorophyll *a* solutions placed in a 20-mm glass cuvette were irradiated by a projection lamp without filters for periods of 5, 10, 15 and 20 minutes. The incident energy of light was superior to 5×10^{-2} W/cm^2. Concentrations of investigating chlorophyll solutions were within the limits 10^{-5} — 10^{-6} M/1. Measurements were taken at room temperature for the solution in acetone, benzene, cyclohexane and methanol.

Changes in absorption spectra of chlorophyll *a* caused by intense illumination can be, in all the solvents, characterized like this:

(1) A reduction of both absorption maxima of Soret's band and long-wave red bands, belonging to the first electronic transition takes place (Fig. 4), while the relation of extinction coefficients of Soret band (ε_s) and the main red band (ε) increased substantially with increasing illumination periods (Fig. 6).

(2) Absorption in the blue — green region (440—600 mm) increases with time of irradiation and in the region $\lambda > 700$ nm a slight increase of absorption can also be observed (Fig. 4, 5).

(3) Absorption spectra curves of the non-illuminated sample and of the illuminated solutions of chlorophyll exhibit three common intersections in the region 400—750 nm, the first being in the region 440—450 nm, the second around 600 nm and the third in the region 680—700 nm.

After a longer irradiation (15—20 min) a change in the colour of the solution was observed, from blue — green to brown — green.

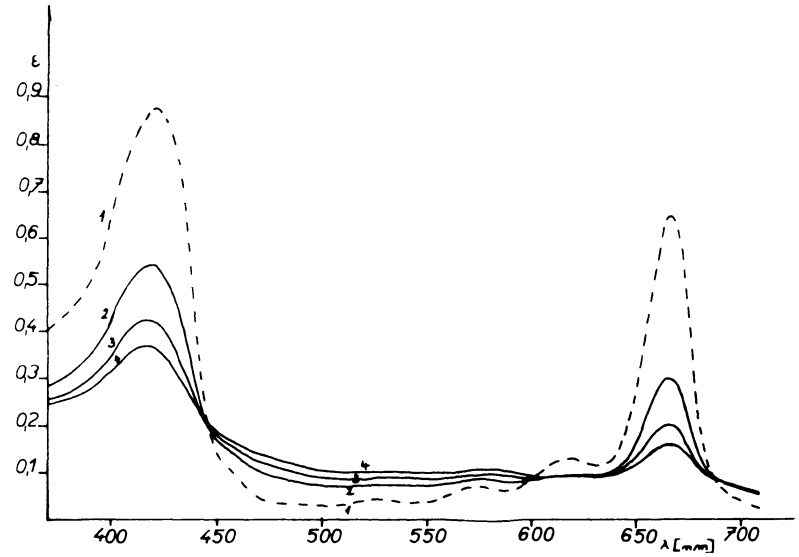

Fig. 4. Absorption spectrum of illuminated chlorophyll *a* in benzene (d = 10 mm).
1 ... non-illuminated sample ($c = 8.85 \times 10^{-6}$ M/l)
2 ... after 5 min 3 ... after 10 min
4 ... after 15 min illumination

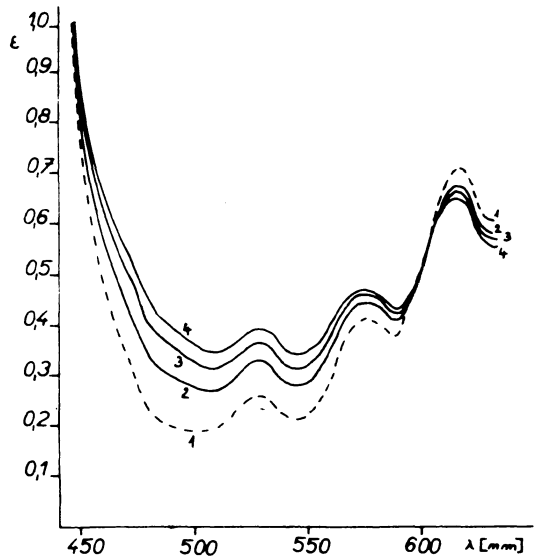

Fig. 5. Absorption spectrum of illuminated chlorophyll *a* in acetone (d = 10 mm).
1 ... non-illuminated chlorophyll ($c = 4.3 \times 10^{-6}$ M/l)
2 ... after 5 min 3 ... after 10 min
4 ... after 15 min illumination.

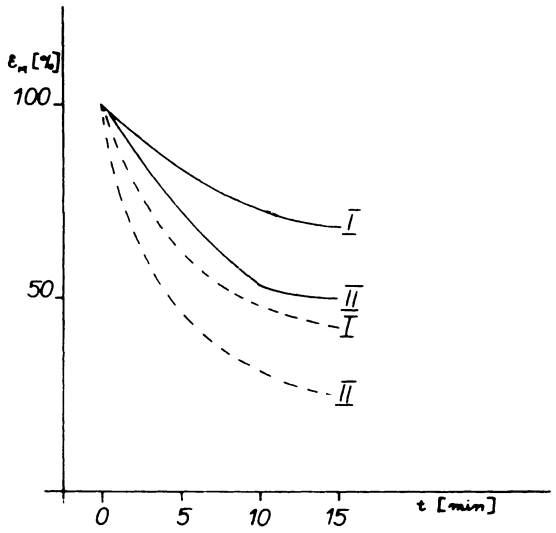

Fig. 6. Decrease of absorption maxima of Soret's (*I*) and red (*II*) bands in dependence upon the time of illumination for two starting concentrations of chlorophyll *a* in benzene.
$c = 8.8 \times 10^{-6}$ M/1 (—)
$c = 2.05 \times 10^{-5}$ M/1 (– – –).

2. 3. INFLUENCE OF SPECTRAL COMPOSITION AND INTENSITY OF LIGHT

The influence of spectral composition of illumination light became evident in absorption spectra through the dependence of the absorption decrease on the colour of light, at a constant incident energy of light. Samples were illuminated in the region of Soret's band (filter *BG* 12), in the green region (*IF* 545), and in the region of the main red band (filter *RG* 1). Relative change of absorption coefficients of Soret's band maxima and the main red maxima for different energie of red and blue light is demonstrated in Fig. 7. The rat of decrease of concentration of chlorophyll *a* in solution depends on the solvent. The reaction rates for chlorophyll *a* destruction in different solvents was determined bythe loss of intensity of the main red peak (Fig. 8).

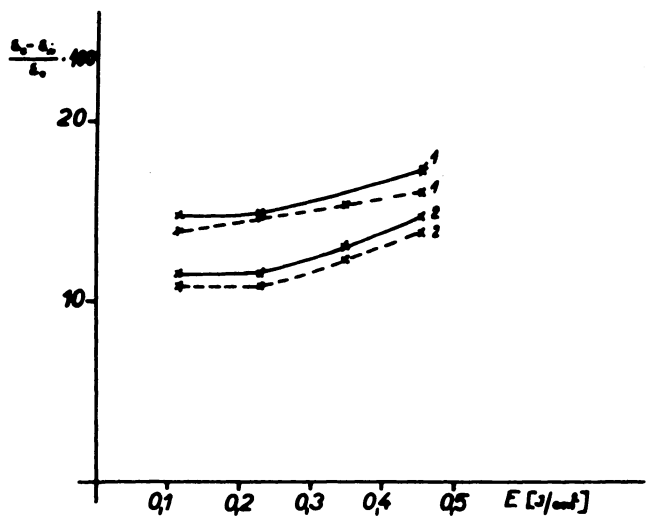

Fig. 7. Dependence of relative change ε_{max} of Soret's (– – –) and main red bands (————) of chlorophyl *a* in benzene on energies of blue (*1*) and red (*2*) lights.

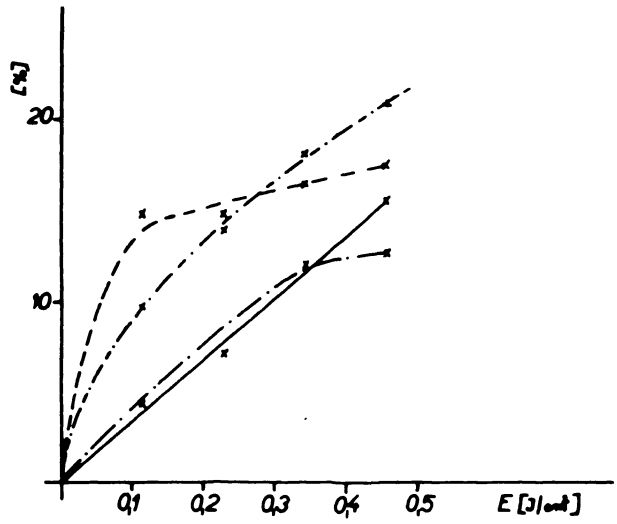

Fig. 8. Dependence of decrease of chlorophyll *a* on energies of incident light (325—500 nm) for these solvents: methanol (————), benzene (– – –), acetone (– –.– –), cyclohexan (–.–.–).

3. Photochemical changes of chlorophyll *a* – discussion

A number of authors paid attention to the influence of light on the stability of chlorophyll molecule in different organic solutions. As most important photochemical processes concerning chlorophyll can be considered photooxydation, photoreduction and the process of pheophytin formation in acid medium, speeded up under the influence of light.

Oxygen, or other oxydation agents being present, the photooxydation agent takes place during intense illumination. The problem of reversibility of this process has not been hitherto perfectly solved and even the final products of photooxydation have not been identified. Partially reversible photoproducts of oxydation of chlorophyll are described by Krasnovsky [15], where 6—8 % of original chlorophyll can be recovered by means of heating the oxydized solution to the boiling point, or by means of adding reduction agents (e. g. ascorbic acid).

The reversibility of oxydized chlorophyll has not been confirmed by other authors [16, 17, 18, 19, 20]. Karapetyan and Litvin investigated oxydation by means of differential spectrophotometry and found irreversible changes of absorption spectra of chlorophyll in ethanol solution even after 15-sec illumination.

The necessity of oxygen and light for irreversible bleaching of chlorophyll solutions was sufficiently confirmed by experiments. Chlorophyll solutions in acetone or ether may be kept in the dark in contact with air for a week at room temperature without change in absorbance, and similarly chlorophyll solutions prepared in high vacuum were stable to light [17, 19, 21]. Experimental conditions,

at which photochemical changes described in the present work took place, made photooxydation of chlorophyll by air oxygen possible, for, as shown by Dilung [17], samples of oxygen prepared in the air ($\sim 10^{-4} - 10^{-5}$ M/l) contain oxygen in concentrations exceeding considerably the concentration of pigment.

Photooxydation of chlorophyll runs over a number of intermediary stages. when the fully green colour of original solution gradually passes over light green, red-brown and pink to the yellow colour, characterizing the final product. The final yellow product can be obtained only by means of a prolonged intense illumination using a non-filtered light. The red intermediates are stable when kept in the dark and further illumination by red light does not result in any qualitative change in the absorption spectrum [17].

Photooxydation can be characterized as a second-order reaction. For the second-order reaction the relation

$$\frac{1}{c_t} = \frac{1}{c_0} + kt \tag{1,4}$$

can be used where c_0 is the starting concentration, c_t is the concentration in time t from the start of the reaction, and k is the reaction rate. As follows from Fig. 9, reciprocal value of concentration is a linear function of time. The values of reaction rates for different solvents are summed up in Table I.

Photochemical changes of chlorophyll investigated in the present work fall into the early stages of photodecomposition of chlorophyll *a*. In absorption spectra the

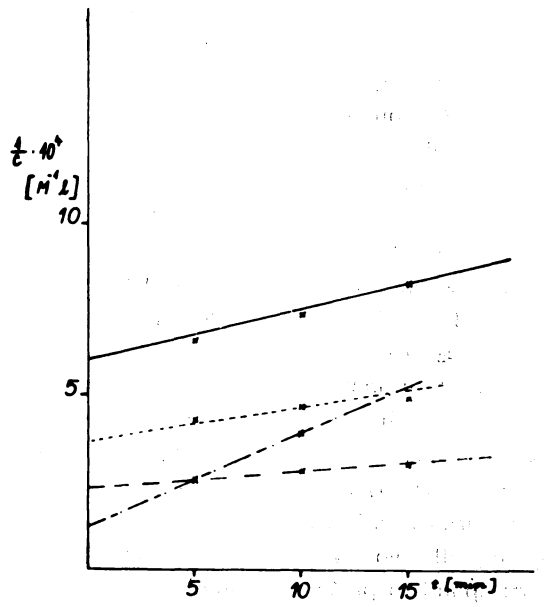

Fig. 9. Dependence of the reciprocal of the concentration of chlorophyll *a* in solution on the time of illumination for these solvents: methanol (————). acetone (– – –), benzene (– –.– –), cyclohexane (. . . . .)

photooxydation of chlorophyll becomes evident as an increase of absorption in the region 450—600 nm and above 700 nm, and as a decrease of maxima of Soret's and red absorption bands (620 and 660 nm).

Tab. 1

Solvent	k ($M^{-1}hod^{-1}$ l)
acetone	0.28×10^5
benzene	1.44×10^5
cyclohexane	0.60×10^5
methanol	0.815×10^5

In the region of increasing absorption no new absorption bands appear during illumination because of the fact that the solutions of chlorophyll for this experiment were prepared in the air and by daylight and that, consequently, a sample marked "non — illuminated" had been to a certain measure, photooxydized.

In certain absorption spectra of chlorophyll in the given solutions a band appeared in the region 505 nm and a slight shoulder around 470 nm. With increasing illumination time a shift of the maximum of Soret's band was observed towards shorter wavelengths and of the red maximum to the long-wave region. If a number of acid admixtures in the solvents used is considered ($\sim 2 \times 10^{-3}$ %) and given changes in absorption spectra compared with pheophytine spectrum, it can be held for probable that the photooxydation of chlorophyll is accompanied by formation of pheophytine.

The rate of photooxydation destruction of chlorophyll is, besides other parameters, dependent upon the spectral composition of activating light. Jen and Mackinney [19] determined, using a set of interference filters, the action spectrum of photooxydation of chlorophyll and showed that the decrease in concentration of chlorophyll during oxydation by the same incident light is the highest for $\lambda = 450$ nm, and 1.64 times greater than at the illumination by red light ($\lambda = 650$ nm) and 4.45 greater than at the illumination in the green region of the visible spectrum ($\lambda = 550$ nm). Aronoff came to similar conclusions [18] by comparing values of rates of the second order reaction at irradiation with light of the wavelengths $\lambda_1 = 435.8$ nm, $\lambda_2 = 546$ nm and $\lambda_3 = 577 - 579$ nm, the relation of which is given by $k_1 : k_2 : k_3 = 1.82 : 0.73 : 1$.

As mentioned above, the photodestruction of chlorophyll takes place in several stages. The first stage, corresponding to photochemical changes described in this work, is characterized in the region 500—650 nm by three absorption bands (in several cases by four — band 505 nm). This structure is typical of the spectra of porphyrines with dehydrogenated pyrrole nucleus IV (Fig. 2 — carbons C_7, C_8 giving off two hydrogens) and therefore the product of photooxydation could correspond to protochlorophyll.

The next phase of the decomposition is, according to Dilung [17], the formation of pheophytine and the whole process can be expressed by the following sequence:

$$Chl \xrightarrow[\text{I.}]{h\nu,\ O_2} protochlorophyll \xrightarrow[\text{II.}]{H^+} protopheophytin \xrightarrow[III.]{} final\ products$$

It was proved by Jen and Mackinney that phytol chain remains in the final product of photooxydation. i. e. that no chlorophyllid is produced. The photooxydation evidently begins with an attack at the methine bridges of chlorophyll molecule, which can be proved by *NMR* spectra and a chemical analysis of the product (the formation of methyl ethyl maleimide at the destruction of porphine skeleton of chlorophyll). In disagreement with this statement are the data by Seely [22] about the presence of porphyrine in the yellow product of oxydation. Similar experiments by Yevstignejev with illumination of Mg-phthalocyanine in the presence of oxydation reagents indicate a potential participation of delocalized electrons of a system of conjugated bonds at photooxydation of chlorophyll.

The identification of photoproducts of oxydation of chlorophyll continues to be an open question. The existence of intermediate points to a number of partial reactions of decomposition molecule of chlorophyll, the rate of destruction depending on the solvent, the quantity of the oxydation agent and the energy of the incident light.

The experiments presented in this work have shown that the first stage of photodecomposition of chlorophyll is a second-order reaction with reaction rate $\sim 10^5\ M^{-1}hod^{-1}$ l. A two-component character of the reaction is evidently linked with the existence of common intersections of absorption spectra of illuminated and non-illuminated solutions. This suggestion seems to be confirmed also by fluorescence studies of photochemical changes of chlorophyll [25].

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