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Abstract

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SPECTRAL STUDY OF THE INTERACTION OF SOLID LAYERS OF PORPHIN DYES WITH HYDRAZINE VAPORS

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Hemin and hematin are substances that form complex compounds with many solvents, with characteristic absorption spectra. They have an affinity for nitrogen-containing bases. If a little hydrazine hydrate is added to a solution of hemin in 0.1 *N* NaOH, then, owing to the formation of the corresponding hemochrome, the solution becomes red ([1,2]). Under the action of vapors of anhydrous hydrazine (N_2H_4) on solid layers of dyes of certain classes, unstable molecular compounds are formed ([3–5]). Depending on the part of the dye molecule in which the interaction is localized, either a colored or a colorless compound ("quasi-leuco base") is formed. It is also known that solutions of chlorophyll in a number of organic bases—piperidine ([6]), benzylamine ([7]), pyridine, nicotine, phenylhydrazine ([8])—have an absorption maximum at 642 mμ. It was of interest to study the direct interaction of solid layers of a series of porphin dyes with N_2H_4 vapors.

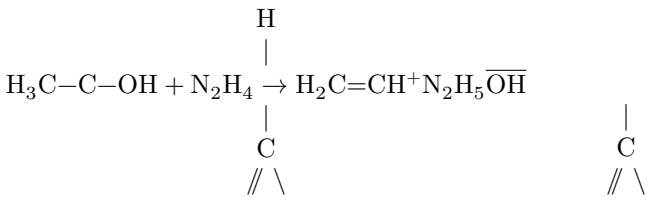
Experiments showed that, upon admission of N_2H_4 vapors, the spectrum of protoporphin does not change. From this important fact it follows that N_2H_4 molecules are localized neither at the vinyl groups of the pigment molecule nor at the nitrogen atoms of the pyrrole rings. Interaction with the carboxyl groups cannot affect the spectrum.

In contrast to the spectrum of protoporphyrin, the spectrum of the hematoporphyrin layer is very sensitive to N_2H_4 vapors. Curves 1 and 2 in Fig. 1 refer to a hematoporphyrin layer in vacuum and in N_2H_4 vapors, respectively. Under vacuum conditions the absorption maxima are at 502, 530, 560, and 600 mμ. After admitting the vapors, the new maxima are at 505, 536, 574, and 625 mμ, and their height increases regularly toward shorter wavelengths. The band at 405 mμ remains almost unchanged (curves 3 and 4). The same spectrum was observed for hematoporphyrin in 0.1 *N* KOH solution ([10]). It is noteworthy, however, that curve 2 is similar to curve 5, which belongs to a layer of protoporphyrin, except that it is shifted somewhat relative to curve 5 toward shorter

Fig. 1. Absorption spectra.

Figure 1: Fig. 1. Absorption spectra.

wavelengths. The similarity of the spectra is so complete that even the weak maximum at 270 mμ in the protoporphyrin spectrum is observed on curve 2. Since N_2H_4 molecules are localized neither at the nitrogen atoms of the pyrrole groups nor at the vinyl groups, and interaction with other parts of the 18-membered conjugated cyclic system is excluded—because in that case a hypsochromic effect would be observed, similar to that which occurs, for example, with cobalt phthalocyanine upon formation of its leuco form ([11])—it remains to assume that the molecules are localized at the secondary alcohol groups. If one takes into account that protoporphyrin can be obtained from hematoporphyrin by removal of two water molecules, and that in vacuum hematoporphyrin already loses two water molecules at 105°, then it may be assumed that the action of N_2H_4 vapors ultimately amounts to the abstraction from the hematoporphyrin molecule of two OH groups and two H atoms. In the limiting case the reaction may be written as follows:



Such an action of the vapors is manifested spectrally in the appearance of the spectrum of protoporphyrin. Pumping off the vapors at room temperature leads only to partial regeneration of the pigment.

If, in vacuum, vapors of N_2H_4 (3 mm) are admitted onto a hemin layer, the layer immediately becomes red. After prolonged pumping off of the vapors at room temperature the layer acquires its initial color. The spectrum of the hemin layer consists of broad bands with maxima at 270 (a strongly broadened band),

Fig. 1. Absorption spectra. 1 —hematoporphyrin in vacuum (thick layer); 2 — the same in N_2H_4 vapors; 3 —hematoporphyrin in vacuum (thin layer); 4 —the same in N_2H_4 vapors; 5 —protoporphyrin in N_2H_4 vapors; 6 —hemin in vacuum; 7 —the same in N_2H_4 vapors; 8 —hematin in vacuum; 9 —the same in N_2H_4 vapors

400, 515, 545 and 650 mμ (Fig. 1, 6). Under the influence of N_2H_4 vapors the band with a maximum at 650 mμ disappears and a band with a maximum at 325 mμ appears (Fig. 1, 7). In addition, relatively narrow bands are observed at 270, 410, 525 and 555 mμ. This spectrum coincides with the spectrum of the above-mentioned hemin solution to which hydrazine hydrate has been added. Since N_2H_4 vapors do not affect the spectrum of protoporphyrin, it is evident

Fig. 2. Absorption spectra. 1 —pheophytin a in vacuum; 2 —the same in N_2H_4 vapors; 3 —chlorophyll a in vacuum; 4 —the same in N_2H_4 vapors; 5 —methyl chlorophyllide a in vacuum; 6 —the same in N_2H_4 vapors

Figure 2: Fig. 2. Absorption spectra. 1 —pheophytin a in vacuum; 2 —the same in N_2H_4 vapors; 3 —chlorophyll a in vacuum; 4 —the same in N_2H_4 vapors; 5 —methyl chlorophyllide a in vacuum; 6 —the same in N_2H_4 vapors

that the N_2H_4 molecules, in their interaction with hemin, are localized at the iron atom. This is also confirmed by magnetic measurements [12]. In addition, as our measurements of absorption spectra in the visible and ultraviolet regions have shown, N_2H_4 vapors do not act on a layer of metal-free phthalocyanine, but do act, although weakly, on layers of magnesium and iron phthalocyanines. This agrees with the results of a study of the action of N_2H_4 vapors on infrared-

spectra of phthalocyanine layers ([13]). Thus, also in the direct interaction of N_2H_4 vapors with a hemin layer, the spectrum is transformed into the spectrum of the corresponding hemochrome, in which the band maxima at 410, 525, and 555 mμ prove to be shifted relative to the hemin maxima by 10 mμ toward longer wavelengths. As for the band at 325 mμ, it is apparently the result of the splitting of the band at 400 mμ into two independent bands as a consequence of the above-noted narrowing of the bands upon formation of the hemochrome (Fig. 1, 7-9).

Fig. 2. Absorption spectra. **1** —pheophytin *a* in vacuum; **2** —the same in N_2H_4 vapors; **3** —chlorophyll *a* in vacuum; **4** —the same in N_2H_4 vapors; **5** —methyl chlorophyllide *a* in vacuum; **6** —the same in N_2H_4 vapors.

The results of measuring the spectra of hematin layers in vacuum (Fig. 1, 8) and in N_2H_4 vapors (Fig. 1, 9)* lead to the same conclusions.

N_2H_4 vapors also interact with layers of green-leaf pigments related to blood pigments. Upon interaction of layers of pheophytin *a*, chlorophyll *a*, and methyl chlorophyllide *a* (Fig. 2) with N_2H_4 vapors, the main red maxima shift toward shorter wavelengths, while absorption in the region 405-420 mμ increases noticeably. Pheophytin is characterized by disappearance of the band at 320 mμ and enhancement of absorption at 504 mμ (Fig. 2, 1 and 2). If the spectrum of protoporphyrin is compared with the spectra of hematoporphyrin and pheophytin in N_2H_4 vapors, then in the region shorter than 550 mμ a similarity is observed between the spectra. For such a similarity to be observed, localization of the N_2H_4 molecule at the oxygen atom in the five-membered

* Through an unfortunate misunderstanding, in papers ([14,15]) for hematin we gave results relating to another compound.

ring of the pheophytin molecule, leading to the reaction:

structural fragment of pheophytin with HC−C=O + N_2H_4 ⟶ corresponding fragment with C−O^- and

Since a shift of the main red band is also observed for pheophytin*, it is obvious that it cannot be due to the interaction of the N_2H_4 molecule with the central magnesium atom in the chlorophyll molecule. The appearance in the spectrum of a chlorophyll layer exposed to N_2H_4 vapors of a band with a maximum at 640 m (Fig. 2, 4) is apparently connected with the above reaction. The band at 640 m in the spectrum of chlorophyll *a* solutions in organic bases may have the same origin. According to Krasnovskii and Brin ([8]), the shift may be associated with ionization of the "acidic" groups of the pigment molecule.

The difference between the frequencies of the maxima of chlorophyll *a* (624 and 672 m) and methyl chlorophyllide *a* (627 and 676 m), equal to 1150 cm^{-1}, decreases after admission of N_2H_4 vapors to 1050 cm^{-1}. This value is close to the difference between the frequencies of the absorption maxima of hemin and hematin in N_2H_4 vapors (525 and 555 m), which is equal to 1030 cm^{-1}. The possibility is not excluded that chlorophyll may interact weakly with the N_2H_4 molecule also by the hemochrome type.

Bilirubin—the coloring substance of bile—is also in close genetic relationship to hemin. The length of the chain of conjugated bonds in its molecule is approximately half that in the molecules considered above. The spectrum of a bilirubin layer consists of a main band (apparently double) in the region of 460 m and weak bands with maxima at 240, 285, and 320 m . In the presence of N_2H_4 vapors, the maximum of the main band shifts to 440 m , its height decreases by approximately 25%, and the half-width of the band increases by 20%. As was to be expected, N_2H_4 vapors do not exert a very strong effect on the general form of the spectrum, since N_2H_4 molecules can be localized at OH groups at the site of chain rupture. Interaction with OH groups was previously shown by us for aurin, fluorescein, and gallein ([5]).

Thus, analysis of the absorption spectra of layers of the pigments studied, kept in vacuum and in hydrazine vapors, makes it possible in each individual case to indicate the site of direct interaction of pigment and hydrazine molecules and to establish its nature.

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* In work ([8]), no such shift was found.

Note: Figure translations are in progress. See original paper for figures.