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Some Types of Organic High Polymers and Permutoids Provided with Active Groups: Their Synthesis, Optical Properties, and Reactivity*

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In connection with our studies on organic high polymers provided with active groups and on permutoids we will report on permutoids with ionic active groups and on high polymers and permutoids with active groups of the chlorophyll and hemin series.

I. PERMUTOIDS WITH IONIC ACTIVE GROUPS

The development of ion-exchanging permutoids based on lignin[1] was stimulated by the permutoid structure of lignin: after the careful removal of the polysaccharides from lignified cells lignin preparations remain which in spite of a 75% loss in mass still show all details of the morphologic structure of the cell.[2] In spite of the loss of their morphologic structure, soluble lignin preparations are also adapted to the production of ion exchangers, since the active groups effecting the exchange of ions are favorably distributed over the entire molecule (*e.g.*, lignosulfonic acid) and since the preparations react like high molecular phenols with periodically recurring functional groups which are qualified for the introduction of ion-exchanging groups.

Permutoids with *SO_3H groups* are obtained with a maximum exchange capacity of 1.7–2 gram equivalents per 1000 g. of exchanger, if—as shown

TABLE I

CATION EXCHANGERS

Active group	Raw material	Oxidating agents	Cross-linking agents	Exchange capacity, Val/1000 g. exchanger	
				NaCl	Na acetate
—SO_3H	Lignosulfonic acid	—	CH_2O	1.7–1.9	1.9–2.2
(—COOH)		—	CrO_3		
—COOH	HCl or alkaline lignin	$O_2 + CO(OH)_3$; oxidation by pressure	CH_2O	—	1–2

* Presented at the International Congress of Pure and Applied Chemistry, September, 1951.

TABLE II

ANION EXCHANGERS

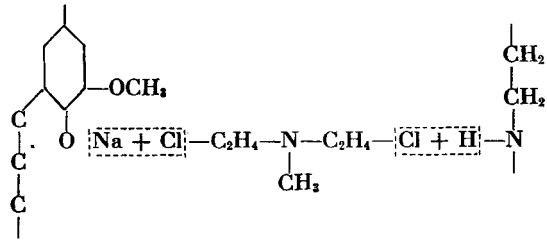
Active group	Raw material	Cross-linking agents	Exchanger contains: % lignin[a]	Exchanger contains: % N	Exchange capacity,[b] g./100 g. exchanger: H_2SO_4	Exchange capacity,[b] g./100 g. exchanger: Lignosulfonic acid	Capacity for splitting up neutral salts (for NaCl)[c] Val NaOH/-1000 g. of exchanger
—N=, —NH—	Polyethyleneimine	C_2H_4Cl / H_3CN / C_2H_4Cl	0	24.8	72.3	14.2	0.8
	Lignin + polyethyleneimine	"	14.4	22	63.7	75.9	0.02
			47.4	13.3	30.7	66.3	
			76.2	5.3	9.8	*100.5*	
	Alkaline lignin	"	84.5	3.2	5.1	82.3	
$\equiv N^{(+)}OH^{(-)}$	Polyethyleneimine + pyridine-epichlorohydrin adduct	Epichlorohydrin					*3.7*
	Polyethyleneimine + lignin + epichlorohydrin-pyridine adduct	CH_2O					1.4

[a] Calculated from the OCH_3 value of the exchanger preparation.
[b] Exchanger $N^{(+)}OH^{(-)}$ + lignin $SO_3^{(-)}H^{(+)} \rightleftharpoons$ exchanger $N^{(+)(-)}$ O_3S-lignin + H_2O.
[c] Exchanger $N^{(+)}OH^{(-)}$ + $Na^{(+)}Cl^{(-)} \rightleftharpoons$ exchanger $N^{(+)}Cl^{(-)}$ + $Na^{(+)}OH^{(-)}$.

in Table I—formaldehyde or chromic acid acts on concentrated lignosulfonic acid solutions, a two-step condensation being appropriate.

Ion exchangers provided with *carboxylic groups* and with an exchange capacity of 1–2 gram equivalents per 1000 g. of exchanger can be obtained by the oxidation of lignin preparations, the best way being the use of oxygen together with catalysts or of pressure with a subsequent cross-linking with formaldehyde.

Anion-exchangers with secondary or tertiary aliphatic nitrogen as ion-exchanging groups are obtained by cross-linking reactions of alkaline lignin preparations with polyamines, polyethyleneimines in particular, the proportion of condensation being optional. The conversion takes place according to the following scheme:

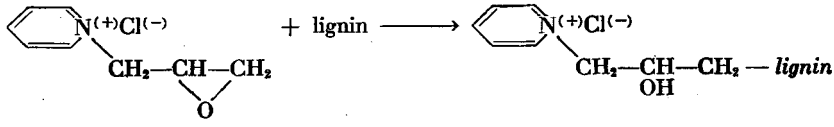


unit of lignin polyethyleneimine

FORMULA A

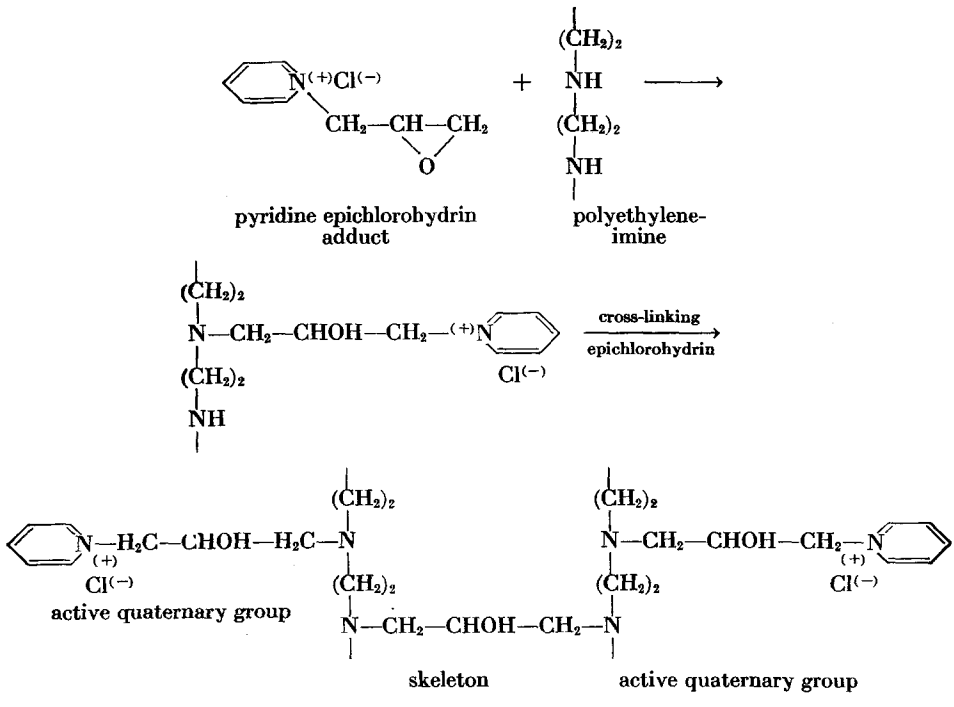
The exchangers containing lignin possess an excellent capacity of adsorbing lignosulfonic acid. Table II demonstrates that a small proportion of lignin in the ion exchanger (14.4%) will already increase the capacity of adsorbing lignosulfonic acid to a degree five to six times larger than that found in exchangers free from lignin; the maximal capacity of adsorption was observed in preparations three quarters of which consisted of lignin.

Anion exchangers with quaternary ammonium groups distinguished by their capacity for splitting up neutral salts can be obtained by converting lignin preparations by means of adducts from pyridine and epichlorohydrin:



FORMULA B

With polyethyleneimine *alone*, one obtains particularly effective, colorless resins giving up to 4 gram equivalents NaOH when splitting sodium chloride, and being more or less capable of swelling according to the degree of cross-linking. Their structure is as follows:



FORMULA C

II. Kinetics of the Decomposition of Diazoacetic Ester and of Nitrosomethylurea with Regard to Acid and Basic Active Groups of Ion Exchangers

In connection with our *comparative* studies on reactions *in solutions* and to *permutoids* and in connection with the examination of the catalytic efficiency of the ions of hydrogen contained in the SO_3H— and COOH— groups of the ion exchangers, we studied the acid active groups by means of the decomposition of diazoacetic ester observed for the first time by Bredig[3] for the determination of the concentration of ions of hydrogen; we characterized the OH ions in quaternary ammonium bases of anion exchangers by means of the decomposition of nitrosomethylurea.

Under the conditions as chosen for our purposes (the proportion of ionic active groups in gram equivalents to moles of diazoacetic ester being about 20–500:1—for measuring reasons) both decompositions are catalytic processes going on under the influence of H or OH ions, respectively.

Both reactions were examined by means of Warburg's manometric procedure in the presence of the ion exchangers transformed into the free acids or free bases, respectively. They behave according to the first order rate law. If the constants of the reaction velocity (ordinate) are entered against the concentration of hydrogen ions according to Bredig or against the gram equivalents of the used exchangers (abscissa) the result (*cf.* Fig. 1) is, for decomposition in solution, according to Bredig a straight line, whereas for

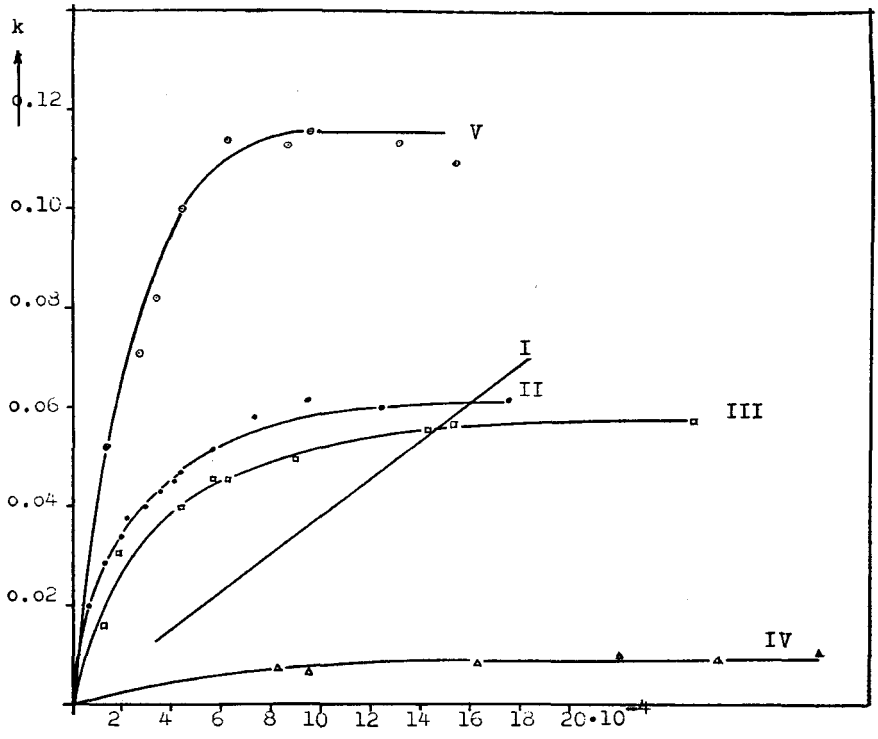


Fig. 1. Decomposition of diazoacetic ester and of nitrosomethylurea with regard to permutoids, *k* values dependent on: (I) concentration of hydrogen ions in the added acid in solution according to Bredig; (II) added quantity of the exchanger Amberlite; IR 100 (SO_3H); (III) added quantity of the exchanger Amberlite; IR 120 (SO_3H); (IV) added quantity of the exchanger Amberlite; IR C50 (COOH); (V) used quantity of the exchanger Amberlite; IR A 400 ($\equiv N^{(+)}OH^{(-)}$).

decomposition through ionic active groups of the exchangers the result is a curve, the first section of which shows a linearity between the quantities of ion exchangers expressed in gram equivalents; these quantities approximate asymptotically to a limit not altered even if the quantity of ion exchangers is further increased.

The asymptotic course of the curves showing the values of *k* must obviously be interpreted as follows: after the beginning linearity between the values for *k* and the number of active groups a further increase of the quantities of exchangers leads to an overlapping of the active spheres of the exchanger particles, so that after the limit has been reached there is evidently no alteration of the quantities of active groups in spite of no increase whatsoever of the quantity of exchangers. That finding is remarkable for reactions in *exchanger columns*, where the exchanger particles generally do not alter their respective position, conditions which apply more or less to the larger quantities of exchangers as examined by means of Warburg's manometric technique under the particular conditions mentioned above. That interpretation necessarily includes the observation that a very quick shaking of the measuring vessels, by which the different particles become independent of each other, will bring about an essentially higher, though not measurable, velocity of the decomposition.

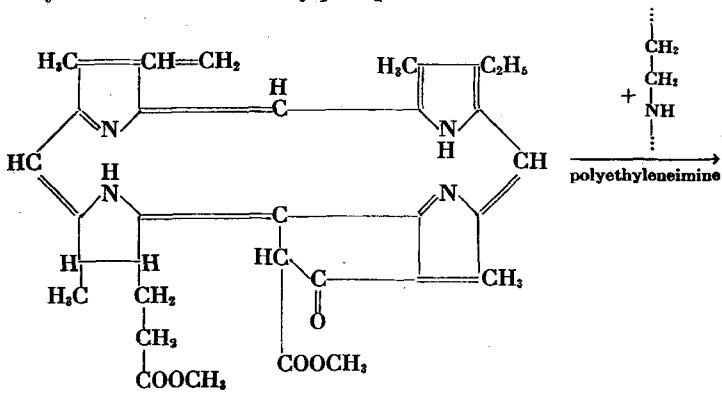
III. High Polymers and Permutoids with Active Groups of the Chlorophyll and Hemin Series

In connection with the studies on catalysts and enzyme models, we set ourselves the task of providing organic high polymeric compounds and permutoids obtained from them by cross-linkage with prosthetic groups of the hemin and chlorophyll series. With regard to the great biological importance of that class of bodies such model examinations are of a special interest.

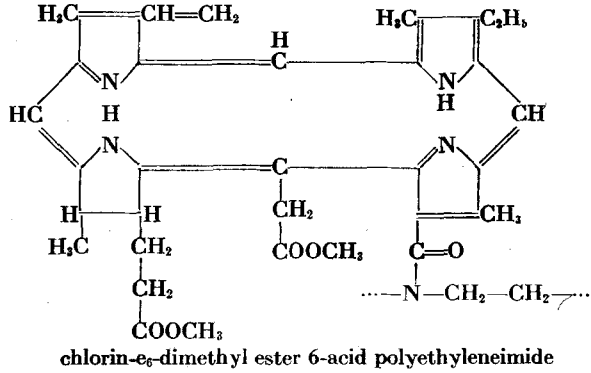
We reached our aim in the following ways: (*1*) by introducing the active groups in question into high polymer compounds; (2) through copolymerization; and (*3*) by including the prosthetic group in the polycondensation process.

(*1*) *Introduction of Active Groups of the Chlorophyll and Hemin Series into Polyethyleneimine*

In analogy to H. Fischer and S. Goebel's[4] observations that methylpheophorbide-a reacts with ammonia and amines by breaking up the isocyclic ring and producing the corresponding 6-acid amides we obtained chlorin-e_6-dimethyl ester 6-acid polyethyleneimide by reaction of polyethyleneimine with methylpheophorbide-a:



methylpheophorbide-a



chlorin-e_6-dimethyl ester 6-acid polyethyleneimide

FORMULA D

The compound thus obtained has the same properties with regard to solubility as polyethyleneimine; it can be dissolved in neutral, acid, and slightly alkaline aqueous media.

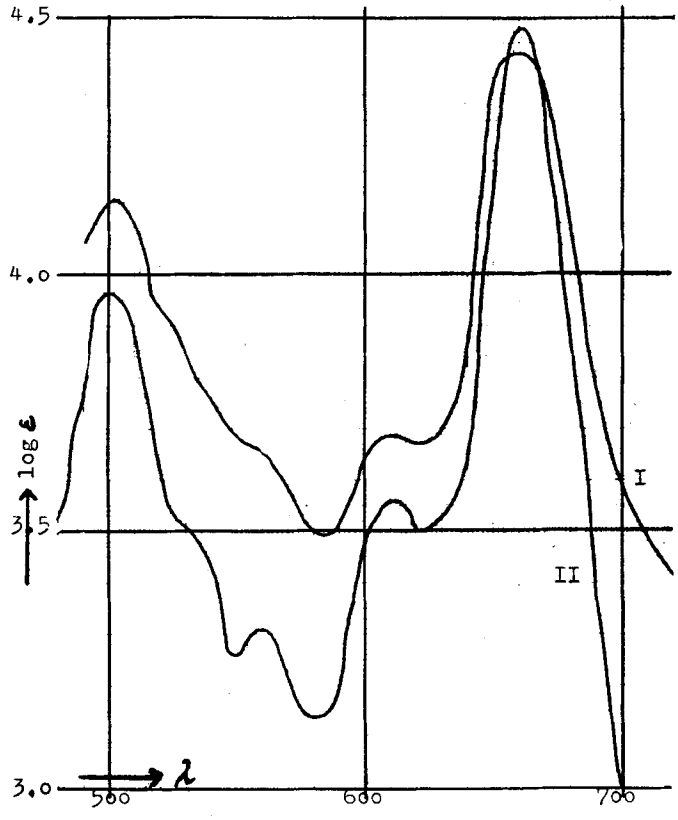


Fig. 2. Absorption spectrum of: (I) chlorin-e_6-dimethyl ester 6-acid ethylamide; (II) chlorin-e_6-dimethyl ester 6-acid polyethyleneimide.

The spectrum of the pigment linked to the macromolecule (*cf.* curve II, Fig. 2) equals in its visible portion chlorin-e_6 and is identical with chlorin-e_6-dimethyl ester 6-acid ethylamide (curve I) produced in an analogous way. The identical course of the curves is a constitutional proof for the linkage of the active group in the high polymer. The spectra were taken by means of the test arrangement shown in Figure 2a. The polychromatic light of the light source (1) is split up in the double monochromater (2–4). In the slide box (6) either the measuring cell or the comparison cell can be brought into the rays of the monochromatic light. The intensity of the passing light is measured by the photoelectron multiplier (7) and by the galvanometer (8).

By producing the spectra of the above-mentioned chlorin-e_6-dimethyl ester 6-acid ethylamide and polyethyleneimide at *different* pH values it was possible to attribute the different spectral types to those of the free base,

of the single and double charged cation, and to find the dissociation constant of the cation acid of the two compounds, the logarithms of which represent the *p*K values.[6] There were remarkable differences between the low-molecular compound and the compound when linked as an active group to the high polymer: the *p*K values of the single charged cation acid were in the first case 2.7, in the latter 3.75.

There were further differences between the low-molecular and the high-molecular compounds: the spectrum of the single charged cation acid of the chlorin-e_6-dimethyl ester 6-acid ethylamide (*cf.* curve I, Fig. 3) shows—if compared to the spectrum of the single charged cation acid of the chlorin-e_6-dimethyl ester 6-acid polyethyleneimide (curve II)—a shift toward the red of the long-wave band from 632 mμ to 644 mμ, whereas the spectra of the free base and of the double charged cation of the low- and high-molecular compounds (not shown here) are identical.

It is a known fact that in many cases the linkage of the prosthetic group to the apoenzymes is combined with a shift toward the red of the absorption spectrum.[7]

As a desmoenzyme model the chlorin-e_6-dimethyl ester 6-acid polyethyleneimide described above was subjected to a cross-linking reaction with epichlorohydrin. By using different molecular quantities of the single com-

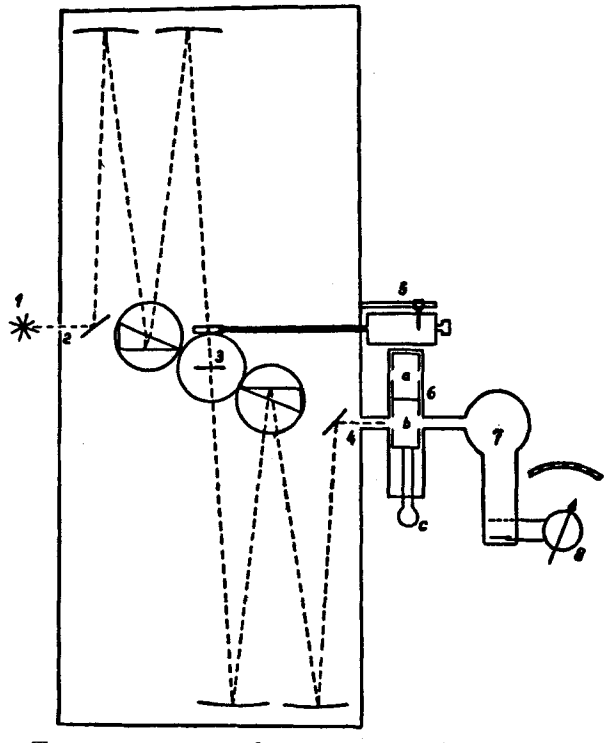
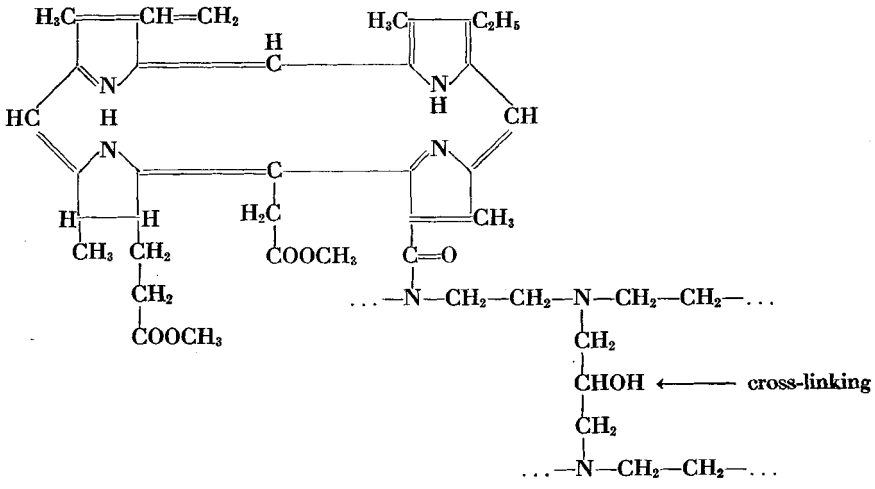


Fig. 2a. Test arrangement for measuring the absorption spectra:[5] (1) light source; (2 and 4) deflecting mirror; (3) central slit; (5) wave length drum; (6) sliding box for cells—*a*, empty cell, and *b*, measuring cell; (7) photoelectron multiplier; (8) galvanometer.

ponents the proportion of active groups (1 prosthetic group to 100, 1,000, or 10,000 units of polyethyleneimine), the degree of cross-linking and thus of swelling was subject to a wide range of variations. In structural scheme E of such a permutoid the cross-linking and the prosthetic group are marked.



FORMULA E

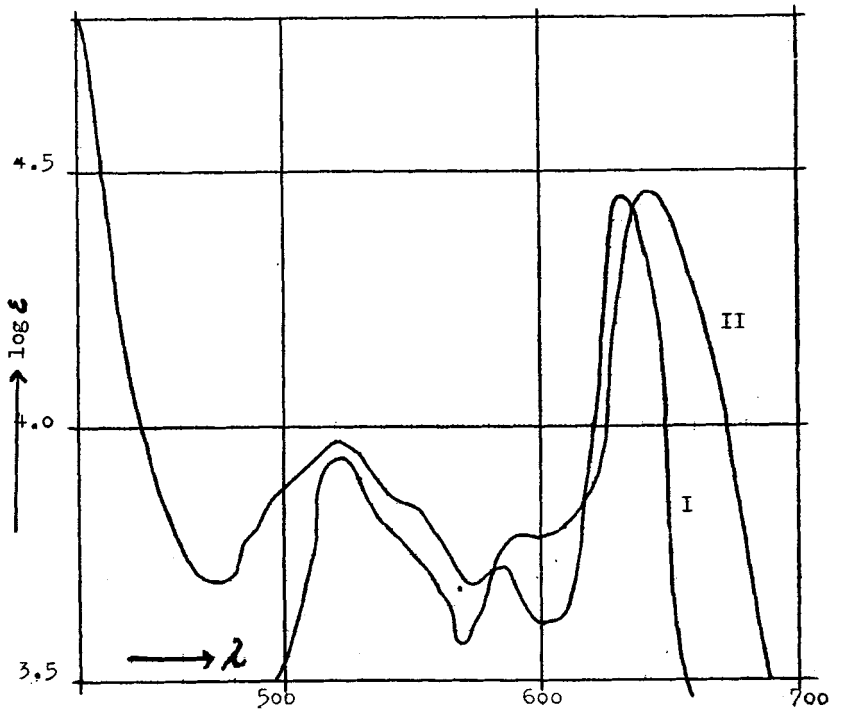


Fig. 3. Absorption spectra of: (I) cation acid$^{1(+)}$ of the chlorin-e_6-dimethyl ester 6-acid *ethylamide*, (II) cation acid$^{1(+)}$ of the chlorin-e_6-dimethyl ester 6-acid *polyethyleneimide*.

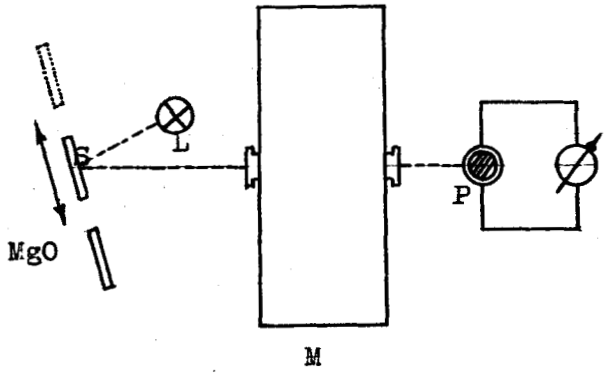


Fig. 4. Arrangement for making reflection spectra.

It is of course impossible to give the constitutional proof of the mode of linkage of the prosthetic group by making an absorption spectrum. However, that proof was made by determining the *reflection spectrum* by means of a highly sensitive apparatus, the design of which is shown in

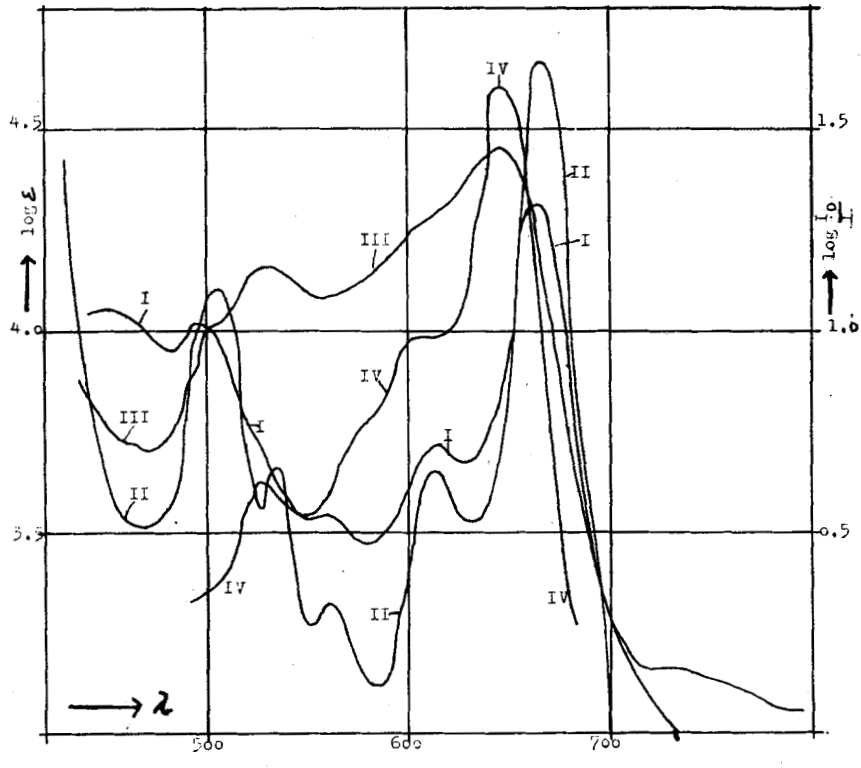


Fig. 5. Reflection and absorption spectra, respectively, of: (I) permutoid (cross-linked chlorin-e_6-dimethyl ester 6-acid polyethyleneimide); (II) chlorin-e_6-dimethyl ester 6-acid ethylamide in toluene–pyridine; (III) cation acid $2^{(+)}$ of I; (IV) cation acid $2^{(+)}$ of II.

Figure 4. The diffusely reflected light obtained by irradiation of polychromatic light (from the light source L) is split into a spectrum by the double chromator (M); the intensity of the light J of the wave length in question is measured by means of a secondary electron multiplying photocell (P). J_0 was obtained, at the same wave length, through reflection to MgO, which does not show a selective absorption.

In Figure 5, curve I shows the reflection spectrum of the permutoid in a neutral desiccated state, curve II the absorption spectrum of the chlorin-e_6-dimethyl ester 6-acid ethylamide in a toluene–pyridine solution.

Apart from a slight shift toward the red of the band maxima I and V the spectra are identical in the permutoid, which establishes the mode of linkage of the prosthetic group. Even linked to the permutoid, the active group is able to take up protons and to form, at the same time, the corresponding cation acid, which can be recognized by the equality of the spectra

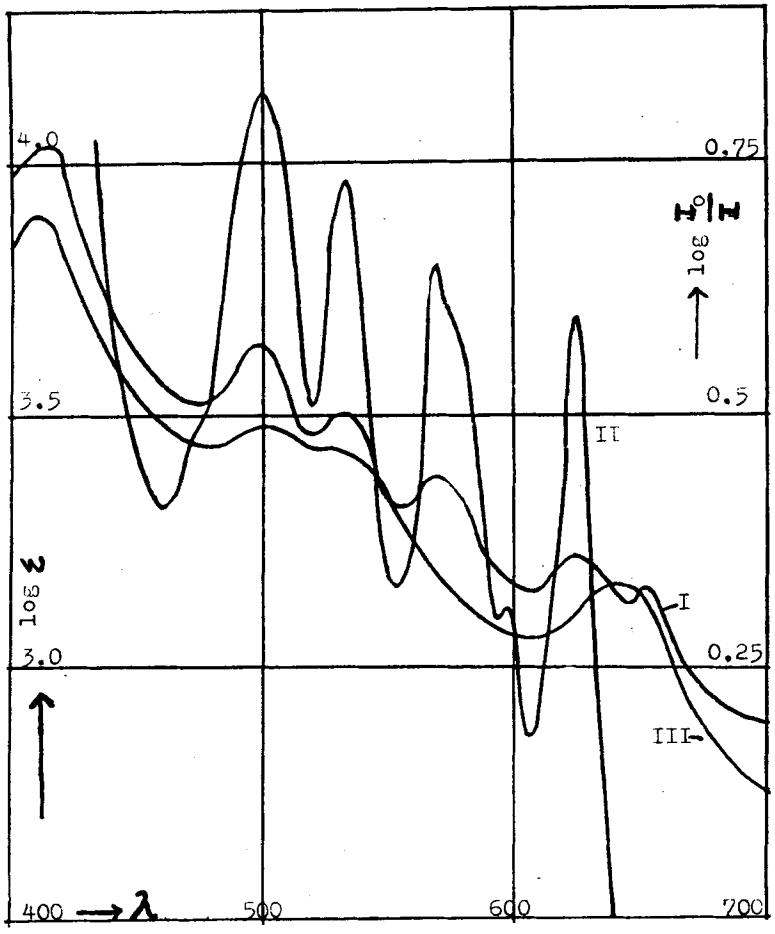
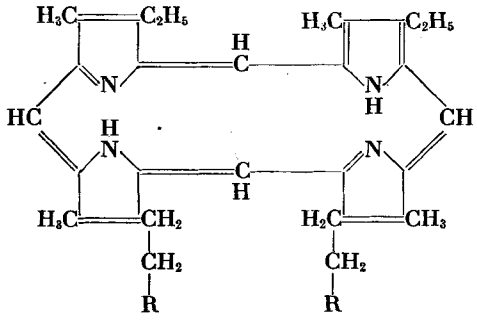


Fig. 6. Reflection and absorption spectra: (I) reflection spectrum of the permutoid; (II) absorption spectrum of the urea derivative (*cf.* formula F.IV); (III) reflection spectrum of the ferric complex salt of I.

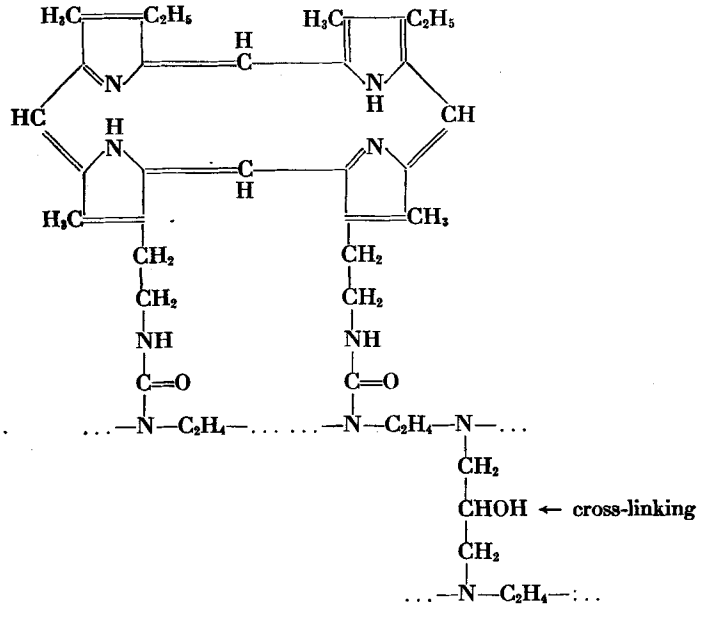
of the double loaded cation acid of the permutoid (curve III; reflection spectrum) and of the low-molecular, dispersedly dissolved amide (curve IV; absorption spectrum) both being obtained by shifting the *p*H.

For the synthesis of another enzyme model with a prosthetic group of the *blood pigment series*, we prepared the mesoporphyrin-IX-diazide using H. Fischer and E. Haarer's method described for pyrroporphyrin-XV.[8] The mesoporphyrin-dimethyl ester (*cf.* formula F.I) was transferred to the acid dihydrazide (II), and this by the reaction with nitrous acid to the mesoporphyrin-IX diazide (1,3,5,8-tetramethyl-2,4-diethylporphyrin-6,7-di-(propionic azide) (III).



(I) R = —$COOCH_3$: mesoporphyrin-IX-dimethyl ester
(II) R = —$CONH_2$: mesoporphyrin-IX-dihydrazide
(III) R = —CON_3: mesoporphyrin-IX-diazide
(IV) R = —NH—CO—N $(C_2H_5)_2$ (see text)

FORMULA F



FORMULA G

By reaction of the diazide, which is difficult to dissolve, with polyethyleneimine in carefully cleared dioxane the prosthetic group can be introduced into the polyethyleneimine chain, and through subsequent cross-linking with epichlorohydrin a permutoid of the structure G is obtained. Varying according to the proportion of active groups, such a permutoid is of a pale-pink to dark-red color (1 prosthetic group to 1,000 or 100 units of ethyleneimine, respectively). The prosthetic group cannot be split off, either by organic solvents or by dilute acids or alkalis.

The constitutional proof for the mode of linkage of the prosthetic group is brought about by a comparison of the band maxima of the reflection spectrum of the neutral, desiccated permutoid (curve I, Fig. 6) and of the absorption spectrum of the low-molecular urea derivative (curve II: 1,3,5,8-tetramethyl-2,4-diethylporphyrin-6,7-di(ethyl-ω-diethylurea); *cf.* formula F.IV).

The prosthetic group of the permutoid compound is able to take up heavy metal ions, *e.g.*, iron, copper, silver, and cobalt cations, in complex linkage out of aqueous solutions of the salts or out of polar, organic solvents

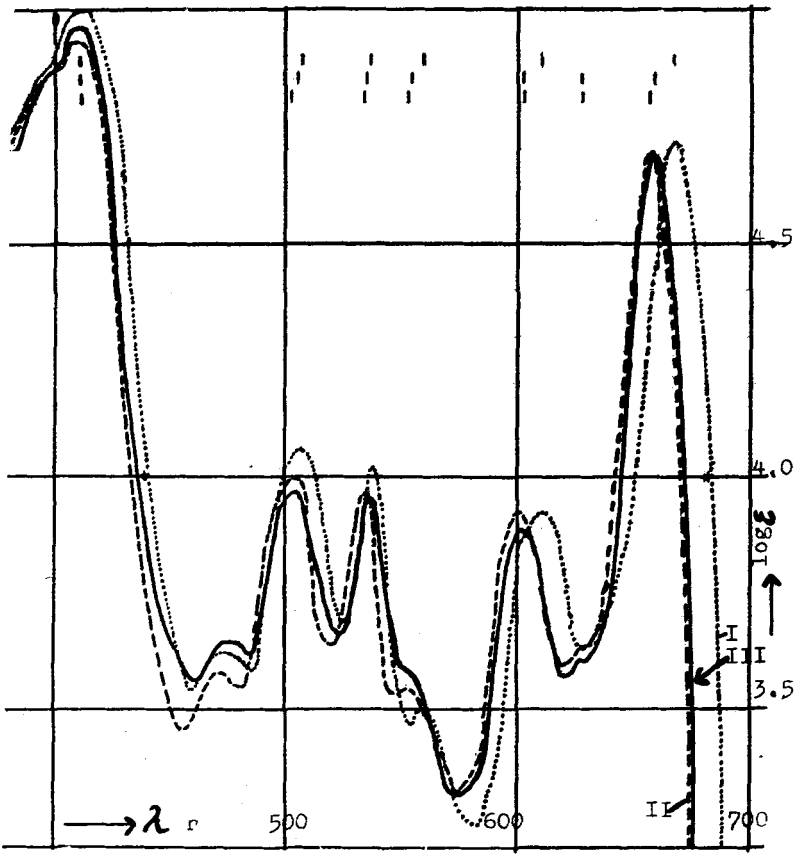


Fig. 7. Absorption spectra of: (I) methylpheophorbide-a; (II) mesomethylpheophorbide-a; (III) polystyrene with the prosthetic group.

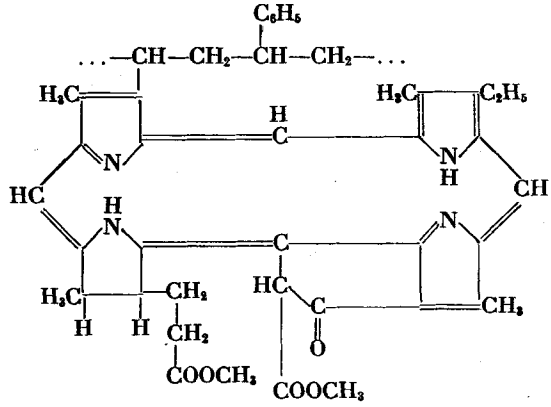
such as acetic acid.[9] With ferrous salts and a slight admixture of NaCl a change of color indicates the formation of a ferric complex salt, the reflection spectrum of which is shown in curve III.

(2) *Introduction of the Active Group through Copolymerization*

Through interpolymerization we succeeded in introducing active groups into high polymers by means of the hitherto unobserved capacity of the vinyl group to polymerize or interpolymerize in 2- or in 2- and 4-position of derivatives of chlorophyll or hemin.

If a mixture of styrene and methylpheophorbide-a or protoporphyrin-dibenzyl ester[10] in the molecular proportion 10^3 to 10^5:1 is subjected to thermal copolymerization, resins result that are soluble in toluene, but which cannot be freed of the pigment part, not even by concentrated hydrochloric acid.

The absorption spectrum of the active groups that are statistically distributed over the polystyrene chain, belongs—starting from *methylpheophorbide-a*—to the type of the mesopheophorbide-a, *i.e.*, of that compound resulting from the hydrogenation of the vinyl groups to the ethyl group. The shift toward the blue can be observed as a rule after the elimination of the vinyl chromophore has taken place, as can be inferred from a comparison of the band maxima of the absorption curve of the methylpheophorbide-a (curve I, Fig. 7), of the mesomethylpheophorbide-a (curve II), and of the prosthetic group linked to the macromolecule of the polystyrene (curve III). The insignificant shift toward the red of the band maxima of the coloring matter linked to the macromolecule compared to the mesopheophorbide-a is remarkable and characteristic of the linkage of the prosthetic group to the macromolecule (*cf.* formula H).



FORMULA H

Analogous observations were made with compounds obtained by interpolymerization of *protoporphyrin-dibenzyl ester* in the polystyrene chain: compared with the protoporphyrin dibenzyl ester (curve I, Fig. 8) the band maxima of the absorption spectra of the polymerized prosthetic

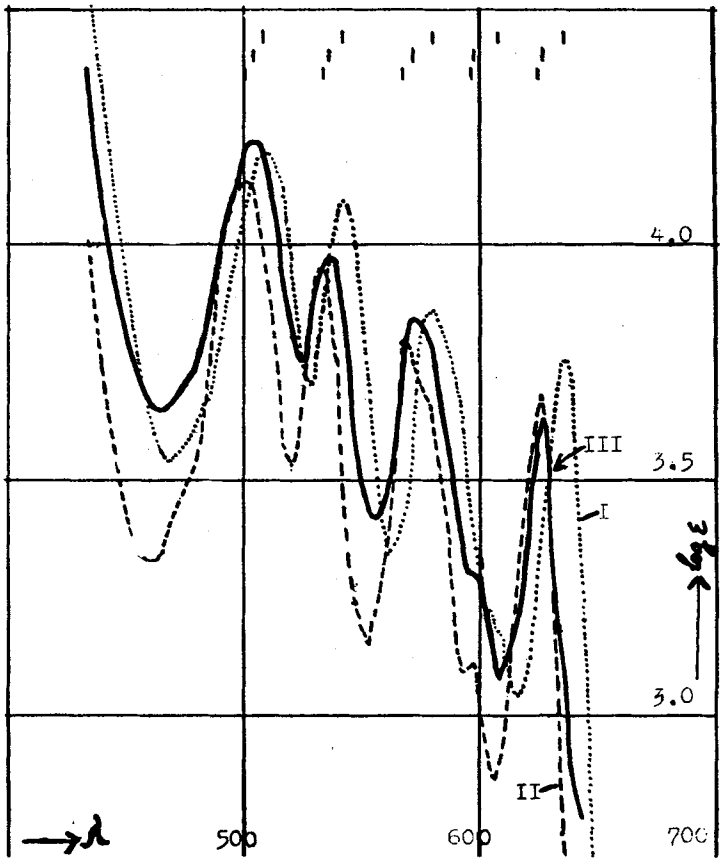
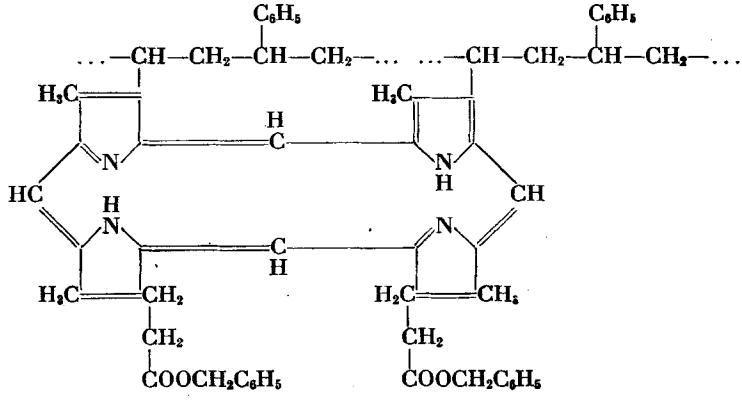


Fig. 8. Absorption spectra of: (I) protoporphyrin-dibenzyl ester; (II) mesoporphyrin-IX-dibenzyl ester; (III) prosthetic group in the polystyrene.

group (curve III) show the expected shift toward the blue. Compared with the hydrogenated compound, the mesoporphyrin-IX (curve II), a

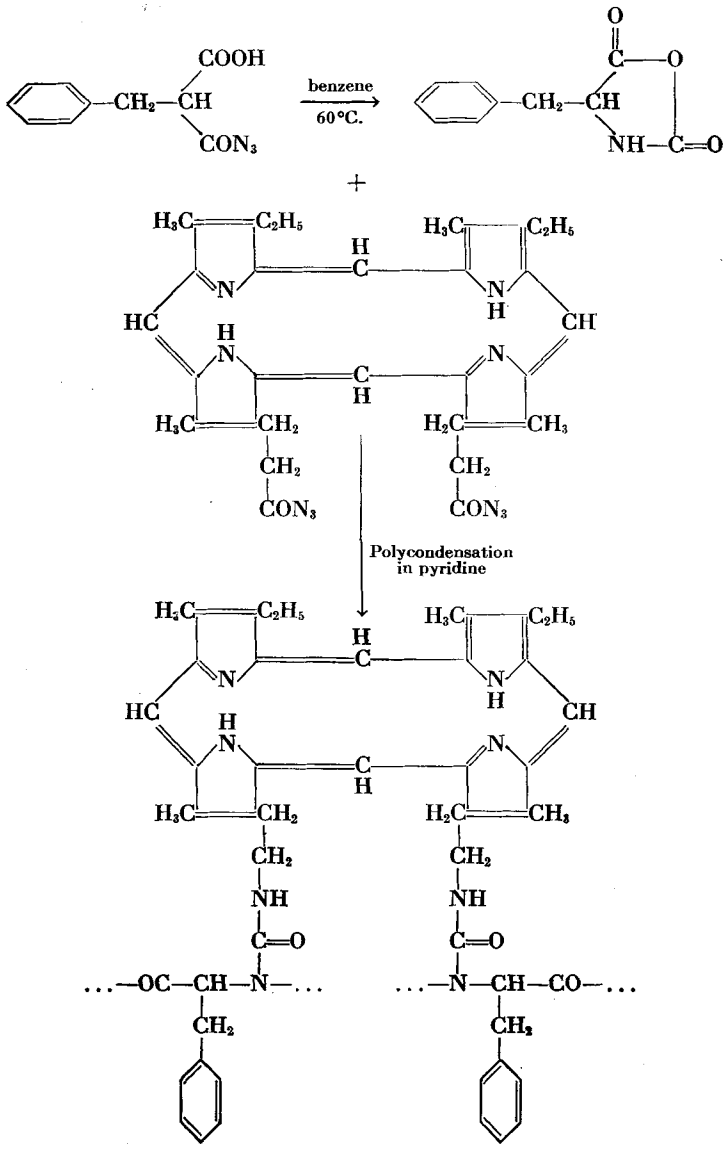


FORMULA J

minimal, but distinct shift toward the red was observed. The prosthetic group is therefore included in the polystyrene chains as in formula J.

(3) Introduction of the Prosthetic Group by Polycondensation

Compounds, the prosthetic group of which is linked to a polypeptide, should have a greater biological interest as enzyme models. Moreover, it



FORMULA K

Synthesis of a polyphenylalanine with mesoporphyrin-IX active group

seemed to be of interest to build up such compounds on the third possible synthetic way, *viz.*, by introducing the active group through inclusion in the polycondensation process.

Taking into consideration Curtius'[11] studies on the obtaining of *N*-carbonic acid anhydrides out of the semiazide of substituted malonic acids as well as the treatises of Leuchs,[12] Wessely,[13] K. H. Meyer,[14] Astbury,[15] Woodward and Schramm,[16] and Bailey,[17] and starting from benzylmalonic azide acid treated in benzene at 60°C., we obtained the 4-benzyl-2,5-dioxo-oxazolidine, which in the presence of the above-mentioned mesoporphyrin-IX diazide was polycondensed in pyridine in a changing proportion of moles. See formula K.

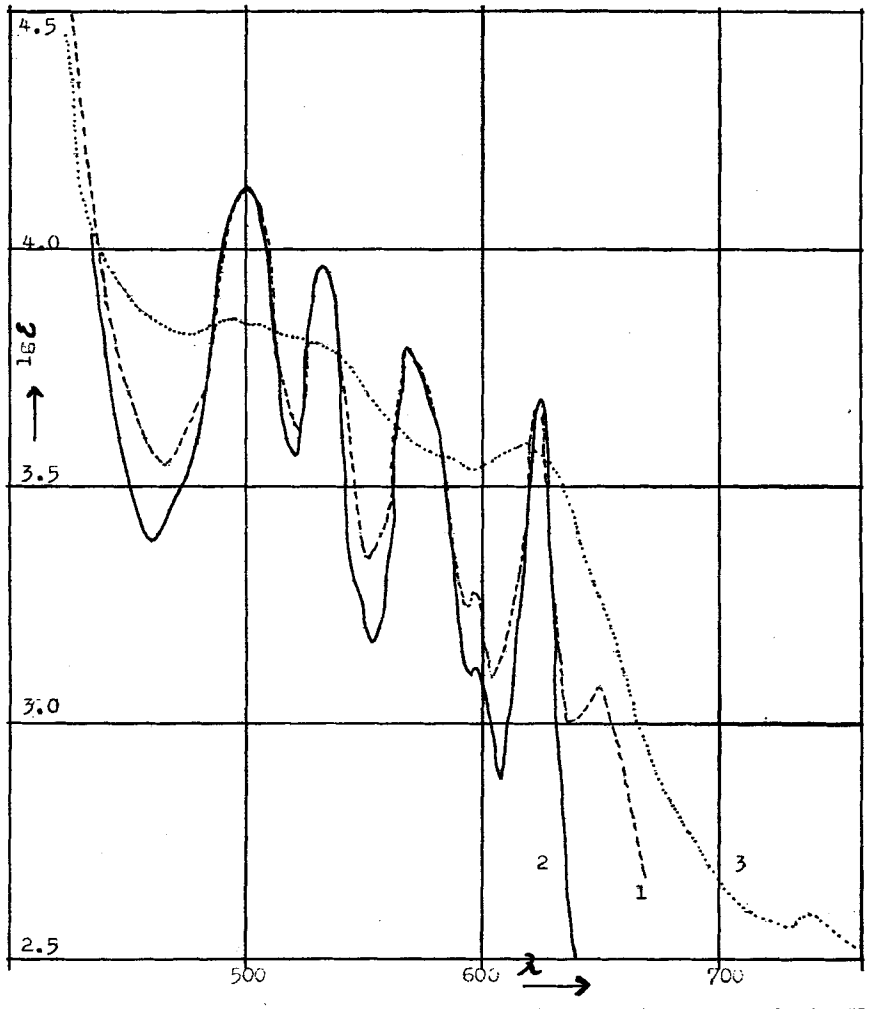


Fig. 9. Absorption spectra of: (1) polyphenylalanine with mesoporphyrin-IX active group in pyridine; (2) mesoporphyrin-IX urea derivative (1,3,5,8-tetramethyl-2,4-di-ethylporphyrin-6,7-di(ethyl-ω-diethylurea), in pyridine; (3) ferric complex salt of the polyphenylalanine with mesoporphyrin-IX active group in *m*-cresol.

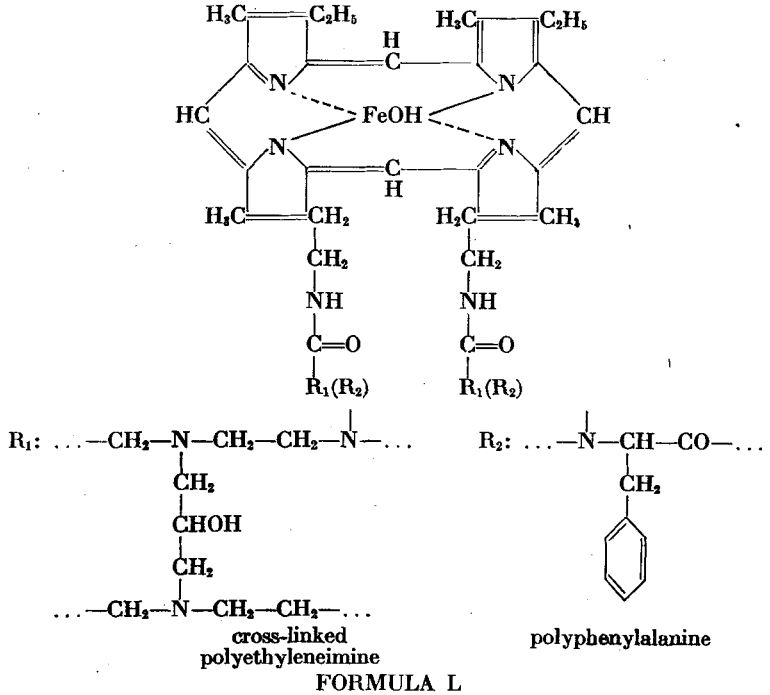
The product of the polycondensation process, obtained from the solution of pyridine by precipitation with petroleum ether, is a powder of a pale-pink to dark-red color according to the quantity of condensed pigment. It is, on the whole, as soluble as polyphenylalanine. Curve 1, Figure 9, shows the absorption spectrum of a preparation with 1 active group to about 150 polyphenylalanine units in pyridine–toluene.

A comparison with the spectrum of the above-mentioned mesoporphyrin-IX urea derivative (1,3,5,8-tetramethyl-2,4-diethylporphyrin-6,7-di(ethyl-ω-diethylurea) which is built up in analogy to the active group, shows that the prosthetic group as a urea derivative is linked to the polypeptide.

Through the introduction of iron, the prosthetic group was transmitted into the ferric complex; the absorption spectrum taken in *m*-cresol is shown in curve 3.

IV. Catalytic Effect of High Polymers with Active Groups of the Hemin Series

The discussed ferric complex salts with the mesoporphyrin-IX active group, fixed one time to the cross-linked polyethyleneimine (*cf.* formula L, R_1) and another time to polyphenylalanine (*cf.* formula L, R_2), were examined as to their catalytic effect in connection with the transmission of oxygen to cysteine—use being made of the reaction of the transmission of oxygen in the presence of ferrous ions (explained by O. Warburg[18] and of D. C. Harrison's[19] and H. A. Kreb's[20] studies with hematin on the same system.



FORMULA L

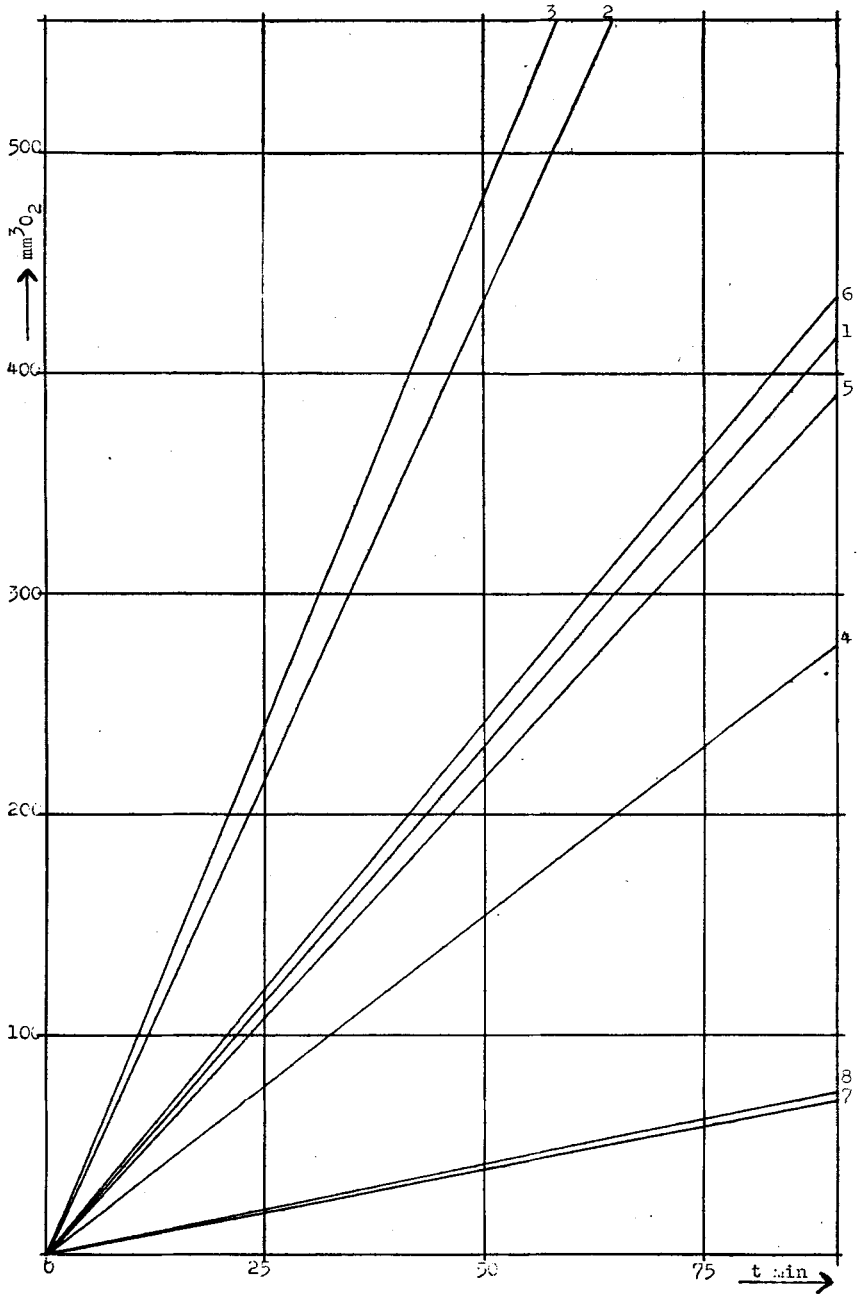


Fig. 10. The catalytic efficiency of: (1) polyphenylalanine with mesohemin active groups, $\sim$ 15 γ Fe + 1 γ Fe^{++}; (2) polyphenylalanine with mesohemin active groups, $\sim$ 15 γ Fe + 1 γ Fe^{++} + pyridine; (3) cross-linked polyethyleneimine with mesohemin active groups, 15 γ Fe + 1 γ Fe^{++} + pyridine; (4) 1 γ Fe^{++}; (5) cross-linked polyethyleneimine with mesohemin active groups, $\sim$ 15 γ Fe + 1 γ Fe^{++} + pyridine + *CO*, (6) hematin-IX, $\sim$ 10 γ Fe; (7 and 8) the used cysteine without any further additions measured at 20.00 °C. and *p*H 7.3 with 50 mg. of cysteine in each case.

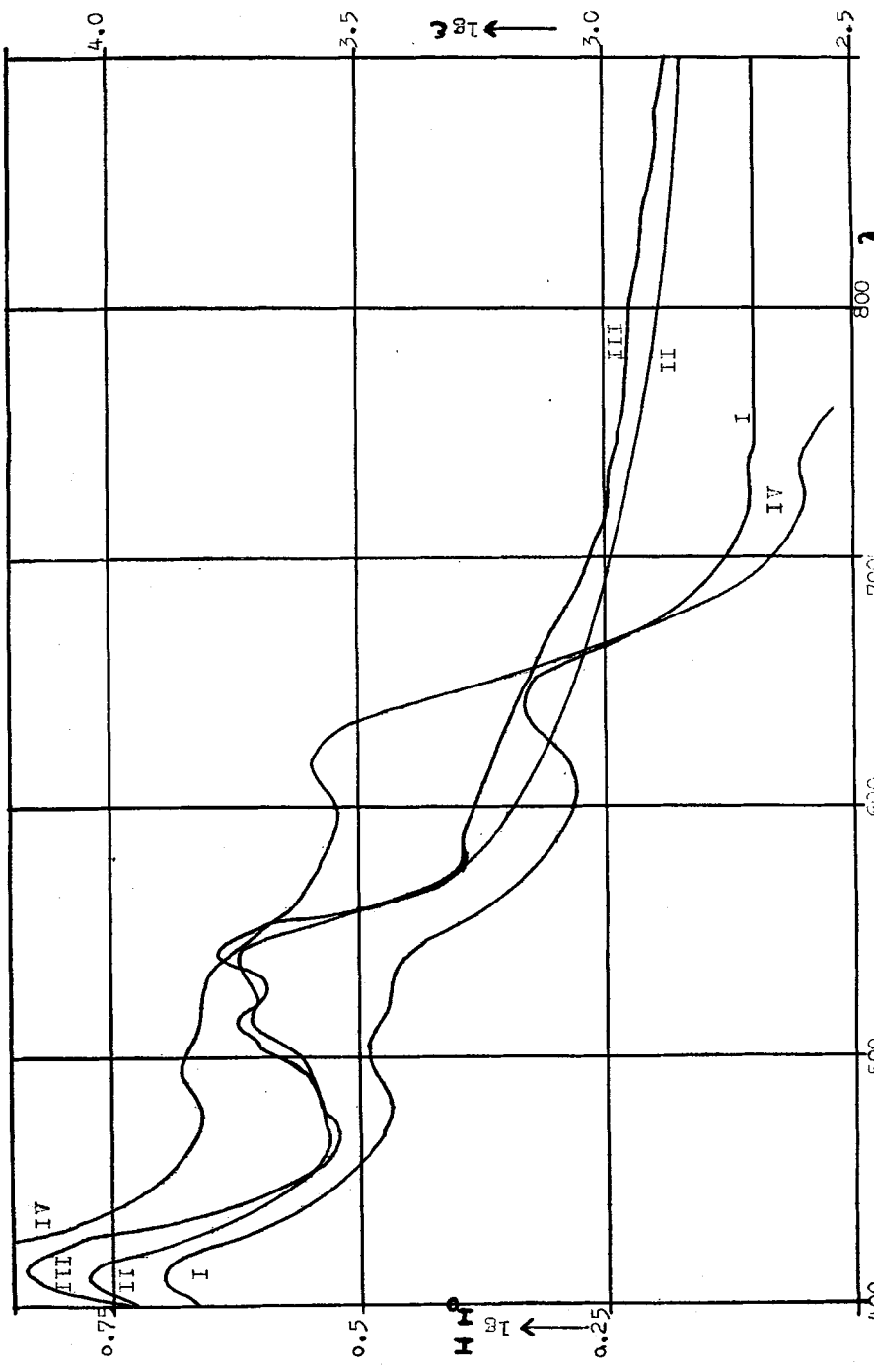


Fig. 11. Reflection and absorption spectra, respectively, of: (I) cross-linked polyethyleneimine with mesohemin active groups, desiccated, ferric complex; (II) as I, treated with pyridine (hemochromogen); (III) as I, treated with pyridine *and* hydrazine (hemochromogen); (IV) polyphenylalanine with mesohemin active group (ferric complex).

The velocities of the transmission of oxygen were measured in the Warburg apparatus by means of quartz vessels; the cysteine used for the measurements and the buffer solutions were carefully freed from traces of heavy metal by a preliminary treatment with a cation exchanger. The velocities of the transmission of oxygen through the prosthetic group were examined in both cases and compared with each other and with the velocity through ferrous ions and hematin in solution. The inhibition by carbon monoxide was also examined.

The result of these examinations is demonstrated in Figure 10. The curves 7 and 8 show the blind values for the measurement of the preparations resulting from traces of still existing heavy metal cations; curve 4 the acceleration of the transmission of oxygen through the addition of 1 γ Fe^{++}; curve 1 the catalytic effect of polyphenylalanine with mesohemin as an active group in the presence of the same quantity of ferrous ions which corresponds to curve 4. The addition of pyridine brings about another large acceleration of the velocity of the transmission, as is demonstrated in curve 2. The same result was obtained, with regard to quantity as well, when the mesohemin active group is attached to the cross-linked polyethyleneimine, as can be seen from curve 3. A comparison between curves 3 and 5 shows that, just as with Warburg's iron oxygenase (cytochrome oxidase), there is an inhibition through carbon monoxide. The homogeneous catalysis in the presence of hematin-IX is found in curve 6.

According to Warburg,[21] the catalysis in the presence of ferrous ions takes place via the stage of ferrous cysteine; the heterogeneous catalysis through polyphenylalanine with the mesohemin active group is therefore to be formulated as follows:

$O_2 \longrightarrow$ polyphenylalanine with mesohemin active group $\longrightarrow$ ferrocysteine $\longrightarrow$ cysteine

The result of these examinations is this: the mesohemin active group fixed to the cross-linked polyethyleneimine or to polyphenylalanine, respectively, catalyzes the transmission of oxygen to cysteine. That catalysis can be accelerated by pyridine and inhibited by carbon monoxide. With regard to the transmitting effect the enzyme models react in the same way as the hemin model.

The addition of pyridine resulted in remarkable differences with regard to the valency of the complex-linked iron of hematin in *solution* and of the mesohemin active group *linked to high polymers:* whereas the iron in the dissolved hematin mainly occurs in the trivalent form, the iron complex linked in the prosthetic group mainly shows divalency, to be recognized by the immediate red coloring after the addition of pyridine. As is shown in Figure 11, the addition of pyridine to mesohemin, no matter whether it is fixed to the cross-linked polyethyleneimine or to polyphenylalanine, will in any case generate hemochromogen (curve II); its spectrum is identical with the spectrum of hemochromogen obtained by the addition of pyridine *and* hydrazine, as is demonstrated by a comparison of curves II and III.

A comparison of the spectrum of the mesohemin fixed to the cross-linked polyethyleneimine with that of the mesohemin fixed to polyphenylalanine reveals a far-reaching identity of the band maxima, apart from the red band, the maximum of which shows a considerable shift of about 20 mμ.

References

(1) W. Lautsch, "Ionen-Austauscher auf Lignin-Basis," *Chemie*, **57**, 149 (1944).

(2) K. Freudenberg, *Tannin, Cellulose, Lignin*, Springer, Berlin, 1933, p. 140.

(3) G. Bredig and W. Fraenkel, *Z. Elektrochemie*, **1905**, 525. W. Fraenkel, *Z. physik. Chem.*, **60**, 202 (1907).

(4) H. Fischer and S. Goebel, *Liebig's Ann. Chem.*, **524**, 269 (1936).

(5) W. Broser and R. Havemann, *Z. Naturf.*, **5a**, 385 (1950).

(6) W. Broser and W. Lautsch, *Naturw.*, **38**, 209 (1951). W. Broser and W. Lautsch, *Naturw.*, **38**, 209/10 (1951).

(7) O. Warburg and W. Christian, *Naturw.*, **20**, 980 (1932). A. Seybold and K. Egle, *Biol. Arch.*, **41**, 578 (1940); see also A. Pirson, "Stoffwechsel der Pflanzen," in FIAT Review for German Science, *Biologie*, Vol. 52, pp. 55/56.

(8) H. Fischer and E. Haarer, *Z. physiol. Chem.*, **229**, 63 (1934).

(9) The tests were made by Mr. H. Klaus.

(10) After the phosgene method of H. Fischer and W. Schmidt, *Liebig's Ann. Chem.*, **519**, 244 (1935).

(11) T. Curtius and W. Sieber, *Ber.*, **55**, 1543 (1922).

(12) H. Leuchs and W. Geiger, *Ber.*, **41**, 1721 (1908).

(13) F. Wessely and co-workers, *Z. physiol. Chem.*, **146**, 72 (1925); **157**, 91 (1926); **159**, 102 (1926).

(14) K. H. Meyer and co-workers, *Helv. Chim. Acta*, **17**, 1448 (1934).

(15) W. T. Astbury and co-workers, *Nature*, **162**, 596 (1948).

(16) R. B. Woodward and C. H. Schramm, *J. Am. Chem. Soc.*, **69**, 1551 (1947).

(17) J. M. Bailey, *Nature*, **164**, 889 (1949).

(18) O. Warburg and S. Sakuma, *Pflüger's Arch. ges. Physiol. Menschen, Tiere*, **200**, 203–6 (1923). S. Sakuma, *Biochem. Z.*, **142**, 68 (1923). See also O. Warburg, *Schwermetalle als Wirkungsgruppen von Fermenten*, Berlin, 1946.

(19) D. C. Harrison, *Biochem. J.*, **18**, 1009 (1924).

(20) H. A. Krebs, *Biochem. Z.*, **204**, 322 (1929).

(21) O. Warburg, *Schwermetalle als Wirkungsgruppen von Fermenten*, Berlin, 1946.

Summary

The synthesis and the properties of permutoids with ionic active groups and the kinetics of the decomposition of diazoacetic ester and of nitrosomethylurea through the ionic active groups are dealt with. A discussion follows of the synthesis of high polymers and permutoids with active groups of the chlorophyll and hemin series obtained by the introduction of the corresponding active groups into high-polymeric compounds, by copolymerization, and by inclusion of the prosthetic group in the polycondensation process. A report is added on the optical properties of the high-molecular compounds provided with active groups and on remarkable differences between low-molecular compounds in a dissolved state and as active groups linked to high polymers. The apparatuses developed for taking absorption and reflection spectra are described. Finally, the catalytic effect of the high polymers provided with active groups of the hemin series is examined.

Résumé

La synthèse et les propriétés des permutoïdes avec des groupes ioniques actifs sont communiquées, de même que la cinétique de la décomposition de ester diazo-acétique et

de la nitrosométhylurée par ces groupes ioniques actifs. Une discussion de la synthèse de hauts polymères et de permutoïdes est ensuite présentée, caractérisés par des groupes actifs de la série des hémines et chlorophylle, dont l'introduction dans les dérivés polymériques est assurée par copolymérisation et par inclusion du groupe prosthétique dans le processus de polycondensation. Un rapport est ensuite ajouté concernant les propriétés optiques des composés macromoléculaires, pourvus de groupes actifs, et l'attention est attirée sur les différences notoires entre des composés de poids moléculaire faible soit à l'état dissout soit liés à des hauts polymères comme groupes actifs. L'appareil mis au point pour mesurer les spectres d'absorption et de réflection est décrit. Finalement l'effet catalytique de hauts polymères, pourvus de groupes actifs de la série des hémines a été examiné.

Zusammenfassung

Die Synthese und die Eigenschaften von Permutoiden mit ionisch aktiven Gruppen und die Kinetik der Zersetzung von Diazo-Essigester und von Nitroso-Methylharnstoff durch die ionisch aktiven Gruppen wurde behandelt. Es folgte eine Diskussion der Synthese von Hochpolymeren und Permutoiden mit aktiven Gruppen der Chlorophyll- und Hämin-Reihen, welche durch Einführung der entsprechenden aktiven Gruppen in die Hochpolymerverbindung durch Copolymerisierung und durch Einschliessung der prosthetischen Gruppe in den Polykondensationsprozess erhalten worden waren. Es wurde ein Bericht über die optischen Eigenschaften der hochmolekularen Verbindungen mit aktiven Gruppen beigefügt, und über die bemerkenswerten Unterschiede zwischen Verbindungen mit niedrigem Molekulargewicht im gelösten Zustand und als aktive, mit Hochpolymeren verbundenene Gruppen. Der zur Bestimmung von Absorption- und Reflektionsspektren entworfene Apparat wurde beschrieben. Endlich wurde die katalytische Wirkung der Hochpolymeren mit aktiven Gruppen der Häminreihe untersucht.

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