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The Photoreduction of Porphyrins: Structure of the Products[1]

BY D. MAUZERALL

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Structures are proposed for the di- and tetrahydroporphyrins formed on photoreduction or chemical reduction of porphyrins. These structures are based on the results of oxidative and acid–base titrations of the reduced porphyrins and on the thermal disproportionation of the dihydroporphyrin into equimolar amounts of porphyrin and tetrahydroporphyrin. Water-soluble porphyrins are readily photoreduced with high quantum yield by such mild reducing agents as bis-tertiary amines at *p*H 6, the rate increasing with decreasing *p*H. Porphyrins are also readily reduced by dithionite in the dark at *p*H 6 or in the light at more alkaline *p*H. In acid solution, titanous or chromous ions readily reduce porphyrins to the tetrahydro stage in the dark, the rate increasing in the presence of light.

Introduction

Because of its relation to photosynthesis, the action of light on chlorophyll has long been of interest. The investigations of the photochemistry of this complex molecule have been well summarized by Rabinowitch.[2] The photoreduction of chlorophyll has been extensively studied by Krasnovskii and his co-workers,[3] but no definite conclusion as to structure or reduction level of the photoproducts has been reached. The photochemistry of the basic ring system, the porphyrin, has received less attention. The most commonly observed reactions have been photoöxidations.[4] The products of these oxidations have absorption bands near 650 mμ and have occasionally been confused with chlorins. The structures of the oxidation products are still uncertain. Studies on the photoreduction of porphyrins are less common. Krasnovskii and co-workers described the photoreduction of hematoporphyrin in pyridine–ascorbic acid[5] and the acid–base spectral shift of one of the products.[6]

The investigations of Oster and his students[7] have shown that certain classes of dyes, *e.g.*, thiazines, flavines and xanthenes, are readily photoreduced by mild reducing agents such as ascorbic acid, allylthiourea and tertiary amines. Ethylenediaminetetraacetic acid (EDTA) is particularly effective.[8]

The reduction level of dyes photoreduced by tertiary amines can be determined without isolating the leuco dye since an oxidant can be chosen which will oxidize the leuco dye, but not the tertiary amine. Moreover, a considerable fraction of the amino groups of a carboxylated bis-tertiary amine exist in the reactive unprotonated form near neutral *p*H. The combination of this amine and a water-soluble porphyrin allows the accurate control of *p*H and ion concentrations which are of critical importance in their photoreactions.

In this paper the formation and properties of two distinct levels of reduction of porphyrins will be described. Evidence will be presented for the formulation of the dihydroporphyrin as a phlorin, recently described by Woodward and co-workers as an intermediate in their masterful synthesis of chlorophyll,[9] and of the tetrahydroporphyrin as a porphomethene, previously found in a study of the porphyrin-sensitized photoöxidation of porphyrinogens.[10]

Experimental

Materials.—The porphyrins, obtained from a wide variety of sources, were purified by partition between organic phase and aqueous acid, and by column chromatography on alumina and crystallization of the methyl esters. Criteria of purity were paper chromatography and quantitative absorption spectroscopy. The molar extinction coefficients of uroporphyrin[11] and of other porphyrins[12] have been published previously. The uroporphyrin (URO) mixture of isomers III and IV used in most of this work was the generous gift of Dr. S. F. MacDonald of Ottawa, Canada, as were samples of uroporphyrin I, II and IV. Uroporphyrin III was obtained from Turaco bird feathers.[11] Uroporphyrin I and coproporphyrin I were purchased from Dr. T. K. With of Svendborg, Denmark. Coproporphyrin III was obtained from a fraction which was a by-product of diphtheria toxoid production, the gift of Dr. F. H. Clarke of Lederle Laboratories. Hematoporphyrin was prepared from protoporphyrin IX,[13] as was 2,4-bis-(1,2-dihydroxyethyl)-deuteroporphyrin (IX).[12]

Ethylenediaminetetraacetic acid (EDTA) was purified by crystallization of both the disodium salt and the free acid (from hot water). Other amines were fractionally distilled and crystallized as the hydrochlorides. Distilled water was redistilled through a glass column. Other materials, except titanous and chromous chlorides, were of reagent grade.

Methods.—Spectra were recorded from 250 to 1400 mμ with a Cary model 14 MR spectrophotometer. A modified cell compartment and suitable cells allowed illumination, measurement of potential, addition of reagents and measurement of spectra under an inert atmosphere. The instrument was modified to permit the use of the near infrared detectors in both the normal and reversed light path modes. The visible and near-infrared spectra could thus be measured with simultaneous high (photochemical) light intensities, or with minimal (monochromatic) light intensities, independently of the intense intermittent photochemical illuminations. Absorbancies were reproducible to ±0.2% and the spectral band width was less than $^1/_{20}$th that of the absorption bands.

(1) This research was supported by a grant from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service, No. R.G. 4922. Preliminary reports of this work have appeared: (a) D. Mauzerall, *J. Am. Chem. Soc.*, **82**, 1832 (1960); (b) Vth International Congress of Biochemistry, Moscow, August, 1961; Abstracts, p. 450, No. 22–31.

(2) E. I. Rabinowitch, "Photosynthesis," Vol. I, Chapt. 18, Vol. II, Pt. 2, Chapt. 35, Interscience Publishers, Inc., New York, N. Y., 1945, 1956.

(3) A. A. Krasnovskii, *J. chim. Phys.*, **55**, 968 (1958); A. A. Krasnovskii, *Ann. Rev. Plant Physiol.*, **11**, 363 (1960).

(4) H. Hellström, *Arkiv Kemi, Mineral. Geol.*, **11B**, no. 11, 1 (1933).

(5) A. A. Krasnovskii and K. K. Voinovskaya, *Doklady Akad. Nauk S.S.S.R.*, **96**, 1209 (1954).

(6) A. A. Krasnovskii and E. V. Pakshina, *ibid.*, **120**, 581 (1958).

(7) G. Oster and N. Wotherspoon, *J. Am. Chem. Soc.*, **79**, 4836 (1957); F. Millich and G. Oster, *ibid.*, **81**, 1357 (1959).

(8) J. R. Merkel and W. J. Nickerson, *Biochim. Biophys. Acta*, **14**, 303 (1954).

(9) R. B. Woodward, *et al.*, *J. Am. Chem. Soc.*, **82**, 3800 (1960); R. B. Woodward, *Angew. Chem.*, **72**, 651 (1960).

(10) D. Mauzerall and S. Granick, *J. Biol. Chem.*, **232**, 1141 (1958).

(11) D. Mauzerall, *J. Am. Chem. Soc.*, **82**, 2601 (1960).

(12) F. Sparatore and D. Mauzerall, *J. Org. Chem.*, **25**, 1073 (1960).

(13) S. Granick, L. Bogorad and H. Jaffe, *J. Biol. Chem.*, **202**, 801 (1953).

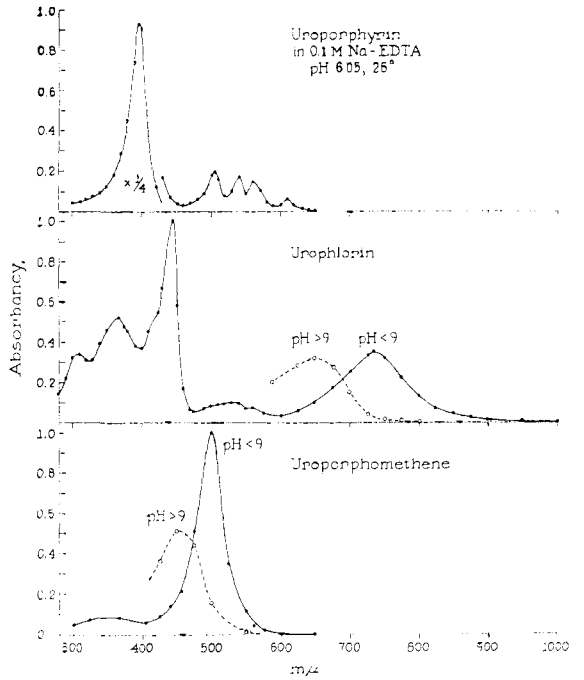

Fig. 1.—The absorption spectra of uroporphyrin and of its dihydro and tetrahydro derivatives formed by photo- or chemical reduction. The spectra were recorded continuously. The value of 1.0 on the absorbancy scale corresponds to the following molar extinction coefficients: uroporphyrin, 6.0×10^4; urophlorin, 6.2×10^4; uroporphomethene, 5.6×10^4.

In one procedure for the removal of oxygen, pre-humidified helium was led into the solution with a Teflon capillary for 0.5 hour or more, although 5 minutes was sufficient to eliminate any detectable effect on the rate of photoreduction of thionin. In a second procedure that was equally effective the oxygen was removed by two or three cycles of freezing and thawing under vacuum (<0.02 mm.) with intermittent flushing with "prepurified" nitrogen or with helium. With this procedure absorption cells provided with suitable side bulbs to allow shaking of the solution and addition of reagents were used.

The *p*H of the solutions was measured in the cuvette with a Beckman model G *p*H meter which was standardized at *p*H 4, 7 and 10 before and after each titration. Titrants were delivered from calibrated syringe-type microburets through Teflon capillaries inserted in slots in the Teflon combination *p*H electrode holder in the cell. The oxidants were previously deoxygenated and were standardized in the same apparatus using ferricyanide as a primary standard and a freshly prepared solution of ascorbic acid as a secondary standard. An accuracy of 0.5% was achieved.

Monochromatic light was obtained from a 500 watt tungsten projection lamp with interference filters of an average half-bandwidth of 5 mμ and suitable blocking filters. A voltage regulator (Sola) stabilized the lamp, and the intensity was controlled with a power-transformer (Variac) or neutral density filters. Infrared absorbing glass and a water cell were placed in the light beam. A system of lenses, a shutter and mirrors were used to project the light into the cell compartment at right angles to the spectrophotometric beam. The intensity of light throughout the cell volume varied by less than 10% and this variation was reduced by stirring. The light intensity was routinely measured with a calibrated photographic light meter, and the absolute light intensity was determined with a thermopile (Eppley) and a microvoltmeter (Hewlett–Packard no. 425A) or by ferric oxalate actinometry at the shorter wave lengths.[14]

(14) C. G. Hatchard and C. A. Parker, *Proc. Roy. Soc.* (London), **235A**, 518 (1956).

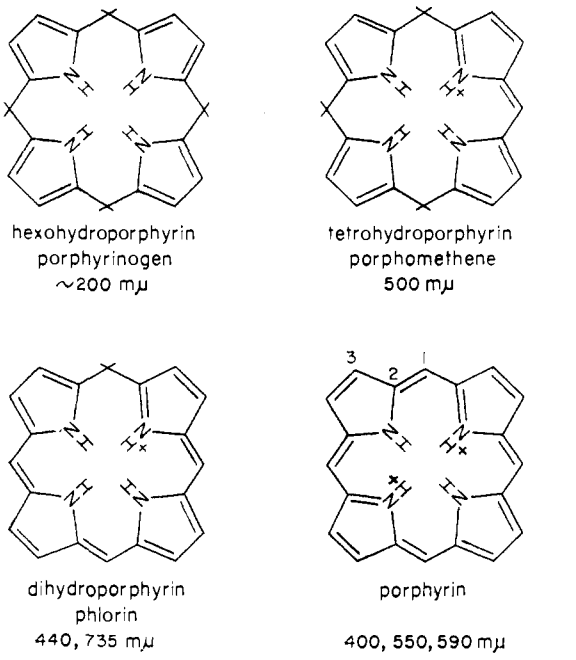

Fig. 2.—Structures of chemical or photoreduced porphyrins. Alkyl substituents on the ring carbons and hydrogens on the methine bridges are omitted.

The room temperature was controlled to within 1° during an experiment. Heating experiments were carried out with sealed cells in complete darkness. The cells were placed in pipes in an oven (100 ± 0.5°) or in an oil-bath (200 ± 2°). Before being placed in the spectrophotometer, the cells were cooled in water.

The hand spectroscope was invaluable for many preliminary and exploratory experiments on the photoreactions.

Results

General.—Both the photoreduction and the chemical reduction of porphyrins proceed through two distinct stages. The spectrum of the porphyrin used in most of the quantitative experiments is shown in Fig. 1, together with the spectra of the two stages of reduction: a dihydro- and a tetrahydro-porphyrin. A third stage, reached only with certain reducing agents, is the completely colorless hexahydroporphyrin or porphyrinogen. The spectra of other alkyl-substituted porphyrins and of their reduction products are very similar to those of uroporphyrin (Table I). Reduced uroporphyrin isomers I and IV and coproporphyrin isomer I have the same spectra as have the isomers of type III. The structures of the reduced porphyrins which best fit the data to be presented are shown in Fig. 2.

TABLE I

ABSORPTION BANDS OF PORPHYRINS PHOTOREDUCED IN EDTA SOLUTION, *p*H 6[a]

Name	2,4-Substituents[b]	Dihydro		Tetrahydro
Uro III	$-CH_2CH_2CO_2H$	737	440	500
Copro III	$-CH_2CH_2CO_2H$	722	436	500
Di-glycol IX	$-CHOHCH_2OH$	708	432	500
Hemato IX	$-CHOHCH_3$	705	432	502

[a] The maxima are listed in mμ. [b] The other substituents on the porphyrin rings are: 1,3,5,8-tetramethyl-6,7-di-(β-carboxy)-ethyl, except URO which is 1,3,5,8-tetracarboxymethyl-6-7-di-(β-carboxy)-ethyl.

The following compounds easily reduced various porphyrins in the dark: titanous and chromous chlorides (> 0.01 *M*) in acid solution to the tetrahydro level; sodium dithionite at *p*H 6–7 through the dihydro level to a colorless derivative; and sodium amalgam in alkaline solution cleanly through the di- and tetrahydroporphyrins to the porphyrinogen level of reduction.

Uroporphyrin and coproporphyrin were reduced on illumination in anaerobic solution through the dihydro level to the tetrahydro level, and no further, by the following compounds: ethylenediaminetetraacetic acid (*p*H <8), N,N,N′,N′-tetramethylethylenediamine (*p*H 6), sparteine (*p*H 6), δ-aminolevulinic acid (*p*H >7), glutathione (*p*H 7), sodium bisulfite (*p*H 5), sodium dithionite (*p*H 8–10) and dilute titanous chloride (*p*H∼0). Following oxidation, the porphyrin was recovered in high yield. Ascorbic acid (*p*H 7) caused reduction beyond the second stage to a colorless compound (porphyrinogen?); the recovery of porphyrin following oxidation with iodine was poor. The following conditions were inactive for the photoreduction of porphyrins: EDTA (*p*H 0, *p*H >8), thiourea (*p*H 7), hydroquinone (*p*H 7), potassium ferrocyanide (*p*H 6) and sodium borohydride (*p*H∼8).

Irradiation with monochromatic light of various wave lengths, or with white light, produced the same sequence of first and second stages of reduction, the rates of conversion increasing with increasing light intensity. The relative quantum yields for the formation of the first (dihydro) level of reduction from uroporphyrin in 0.1 *M* EDTA at *p*H 5.8, 22°, were about the same (0.35 ± 0.07) at 398 (Soret band), 502 and 605 mμ ("*x* and *y*" visible bands[15]). Under the same conditions the quantum yield of formation of the second level of reduction, calculated on the basis of light absorbed by the dihydroporphyrin, is much lower (<0.003).

The rate of photoreduction increased with EDTA concentration from 0.01 to 0.5 *M*. The usual concentration used (0.1 *M*) gave a near maximum rate and acted as a convenient buffer in the range of interest.

The *p*H was of critical importance for the photoreduction of porphyrin. No reaction was observed with tertiary amines in either alkaline solution or very acidic solution. With uroporphyrin the rate (*i.e.*, quantum yield) was appreciable (>10^{-4}) only below *p*H 9. The photoreduction of simpler dyes having no acid–base equilibria in the *p*H region of 9 to 2 shows that the rate depends on the concentration of unprotonated amine.[1a,7] Thus, the bis-*tert*-ethylenediamines were the most favorable amine reducing agents for the porphyrins. A quantitative study of these *p*H effects is now in progress. In this paper only the properties of the di- and tetrahydroporphyrins relating to their structure will be discussed.

Photoreduction. 1. Spectra.—In order to calculate the concentrations of both the di- and tetrahydroporphyrins it was necessary to determine their extinction coefficients at various wave lengths. The quantitative spectra shown in Fig. 1 were obtained by a graphical method discussed below, and by finding experimental conditions which gave quantitative conversion of porphyrin to either reduced from. The experimental conditions for uroporphyrin are: (1) dihydro level, 0.1 *M* EDTA, *p*H 6.0, short exposure to intense white or monochromatic light; (2) tetrahydro level, dilute titanous chloride in 0.5 *M* HCl and light. In most instances, however, considerable overlap of the two levels of reduction occurred. The resulting complex spectral changes were analyzed by solving the simultaneous equations relating the concentrations of each of the three components to their absorptions at three wave lengths and developing various linear equations to test the data. The calculation may be simplified by choosing one of these wave lengths in the far red region where only the dihydroporphyrin absorbs. Two examples of the data are shown in Fig. 3, and the equation used is given below. The

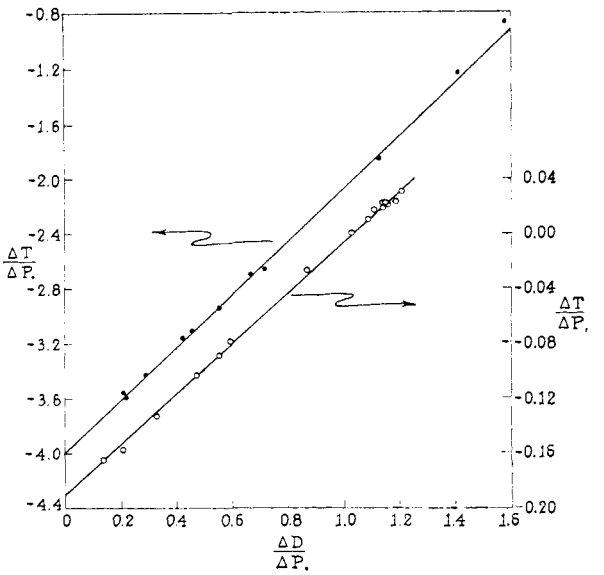

Fig. 3.—Plots of the ratios of absorbancy changes at various wave lengths according to eq. 1. The points refer to the left hand ordinate where $\Delta T/\Delta P$ represents the ratio of the changes of absorbancy at 500 and 550 mμ; the abscissa, $\Delta D/\Delta P$, the ratio of changes at 735 and 550 mμ. Uroporphyrin (2.3 × 10^{-5} *M*) in 0.33 *M* HCl and ∽10^{-4} *M* $TiCl_3$ was illuminated with monochromatic light of various wave lengths. The concentration of the dihydroporphyrin varied from 2 to 34% and that of the tetrahydroporphyrin from 0 to 46% of the original porphyrin concentration. The intercept and slope of the line are, respectively, −4.01 and 1.95; calculated from the extinction coefficients: −4.0 and 2.0. The circles refer to the right-hand ordinate where $\Delta T/\Delta P$ represents the ratio of the changes of absorbancy at 500 and 398 mμ; the abscissa, $\Delta D/\Delta P$, the ratio of changes at 735 and 398 mμ (multiplied by 10). Uroporphyrin (7.6 × 10^{-6} *M*) in 0.1 *M* EDTA, *p*H 5.8, was illuminated with monochromatic light (398 mμ). The concentration of dihydroporphyrin varied from 3 to 92% and that of the tetrahydroporphyrin from 0 to 74% of the original porphyrin concentration. The intercept and slope of the line are, respectively, −0.192 and 1.85; calculated from the extinction coefficients −0.20 and 1.95.

(15) J. R. Platt, "Radiation Biology," A. Hollaender, ed. Vol. III, McGraw-Hill Book Co., Inc., New York, N. Y., 1956, p. 100.

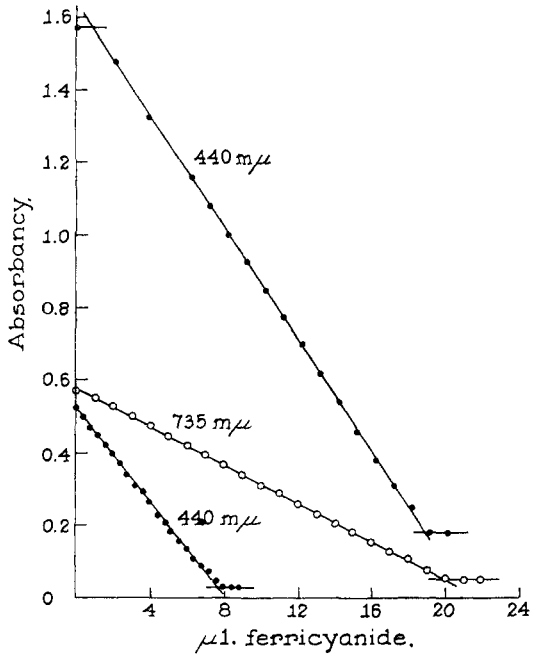

Fig. 4.—Oxidative titration of urophlorin with 1.03×10^{-3} M ferricyanide. The urophlorin was prepared by photoreduction in 0.1 M EDTA solution (4.1 ml.) at pH 6 with monochromatic light of various wave lengths. Measuring either absorption band of the phlorin, or changing its concentration, gave the same result: 2 ± 0.2 electrons per porphyrin ring.

agreement of the slope and intercept of the straight line with the predicted values shows that within the experimental error of 5%: (a) the reaction mixture contains only three components having the spectra of Fig. 1, (b) the reactions are nearly quantitative, and (c) the spectra of the two stages of reduction are the same at both pH 0 and 6 and are independent of the reductant. Similar equations and tests were developed to show that all of the bands of the complex spectrum of the first stage of reduction belong to a single component. The quantitative fit of the data and visual examination of the photoreactions with the hand spectroscope verified that no intermediate with very different spectrum and of lifetime greater than five seconds existed.

$$a\Delta A^{1}_{P} - b\Delta A^{2}_{T} = (bc - ad)\Delta A^{3}_{D} \quad (1)$$

$$a = \epsilon^{2}_{T} - \epsilon^{2}_{P};\ b = \epsilon^{1}_{T} - \epsilon^{1}_{P}$$

$$c = (\epsilon^{2}_{D} - \epsilon^{2}_{P})/\epsilon^{3}_{D};\ d = (\epsilon^{1}_{D} - \epsilon^{1}_{P})/\epsilon^{3}_{D}$$

ΔA^{1}_{P} = difference of absorbancy between init. time and time of measurement at wave length 1, due chiefly to porphyrin (P)

ΔA^{1}_{T2} = difference of absorbancy between init. time and time of measurement at wave length 2, due chiefly to tetrahydroporphyrin (T)

ΔA^{3}_{D} = difference of absorbancy between init. time and time of measurement at wave length 3, due only to dihydroporphyrin (D)

ϵ^{1}_{P}, etc. = molar extinction coefficient of porphyrin (P) at wave length 1, etc.

A complication arose in the shift of the spectrum of the first stage of photoreduction from shorter wave lengths (2–20 mμ) to the spectrum shown in Fig. 1 on standing in the dark. The difficulty

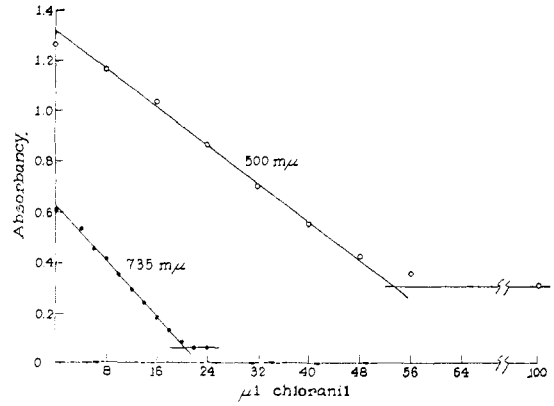

Fig. 5.—Oxidative titration of urophlorin and uroporphomethene with chloranil. The porphyrin was photoreduced in EDTA solution (5.1 ml.) and titrated with 4.9×10^{-3} M chloranil. Since the oxidation of the porphomethene was slow and followed second-order kinetics, the absorption at 500 mμ was followed after each addition of titrant until the change with time was small (10–30 min.).

in the quantitative calculation could be avoided by waiting until the spectral shifts were completed, or by using the isosbestic point of this change in the far red (735 mμ), and neglecting the small changes at the other wave lengths. Several possible explanations of the spectral shifts suggest themselves. The easiest possibility to eliminate was that of isomeric phlorin structures (Fig. 2) in which hydrogens are added to various methine bridge carbons. These carbons differ in the unsymmetrical type III isomers, but do not differ in the symmetrical type I isomer. The symmetrical uroporphyrin I and coproporphyrin I gave the same shifts, thus ruling out the possibility of isomeric phlorins with hydrogens on differing methene bridges. Photoisomerization alone cannot account for the spectral shifts since running spectra "backwards" (*i.e.*, toward longer wave length) or under constant, intense illumination or photoreducing with widely differing wave lengths of monochromatic light gave similar shifts. These spectral shifts were, however, definitely more rapid in the presence of light than in the dark. The most likely possibility—aggregation of these highly polarizable molecules—is diminished, but is not excluded, by the fact that the shifts were observed on dilution to about 10^{-6} M with roughly the same time constant (~30 min.) as at 10^{-5} M. This absorption on the short wave length side of the first reduction product (*i.e.* at 430 and 700 mμ) could be caused by an isomeric dihydroporphyrin and, in fact, it reacts preferentially with traces of oxygen or other oxidants.

During the reductions isosbestic points were seldom observed because of the overlapping spectra of the three components, and because of the band shifts in the first stage of reduction mentioned above. Under carefully controlled conditions, however, it was possible to see all *eight* isosbestic points between the spectra of the porphyrin and dihydroporphyrin. No shifts of the absorption band of the second stage of reduction greater than

1 mμ were observed. The two isosbestic points between this band and that of the first stage of reduction (458 and 547 mμ) were often seen.

2. Oxidative Titrations.—Since the first stage of reduction of porphyrins has a half-life of about 5 min. in air-saturated solution, the method of titration *in situ* and in an inert atmosphere described in the Experimental section was developed. The following compounds oxidize this reduced porphyrin immediately: iodine, chloranil, *p*-benzoquinone, ceric ion and ferricyanide ion. Oxygen and persulfate ion react more slowly. Quantitative spectrophotometric titrations were carried out with both ferricyanide ion and chloranil. Examples of the data are given in Figs. 4 and 5. The average of 6 titrations with ferricyanide ion gave 2 ± 0.2 electrons per porphyrin ring. The recovery of porphyrin in individual titrations was nearly quantitative. Five sequential photoreductions and titrations on a single sample yielded 97.8 ± 0.2% of porphyrin, or 99.6 ± 0.2% per titration. The photoreduction was only slightly inhibited by the ferrocyanide. No reaction of the ferricyanide with EDTA was observed over periods twenty times as long as was necessary for a titration. The same reduction level was obtained on titration with chloranil (Fig. 5).

The unlikely possibility that the products of the oxidation of the tertiary amine reacted with the oxidants was ruled out by titrating the leuco thionin formed by photoreduction in EDTA solution. The expected value of two electrons per mole was found at both pH 6 and pH 10.

The second stage of reduction is oxidized rapidly by iodine, more slowly by 2,3-dichloro-5,6-dicyanobenzoquinone or chloranil and very slowly by oxygen or ferricyanide ion. The oxidants of higher potential react too rapidly with the EDTA, particularly on exposure to light,[15] to be useful. Titration with chloranil demonstrated that the hydroporphyrin at the second stage of reduction contained 4.5 ± 0.5 electrons. An example of the data is shown in Fig. 5. The recovery averaged 90 ± 5%. Titrations with 2,3-dichloro-5,6-dicyanobenzoquinone gave values of 4 ± 1 electrons per ring. However, this quinone is unstable and reacts with the EDTA. Eisner and Linstead[16] used this quinone to determine the reduction level of chlorins. Repetitive titrations cannot be performed because the photoreduction is strongly inhibited by hydroquinones.

3. Disproportionation.—The dihydroporphyrin disproportionates into equimolar amounts of porphyrin and tetrahydroporphyrin in the absence of oxygen and light. A typical experiment is shown in Fig. 6. The dihydroporphyrin, twice formed by photoreduction and sequentially disproportionated by heating, yielded the expected one-quarter mole of porphyrin and three-quarter mole of tetrahydroporphyrin. The slow decomposition of the tetrahydroporphyrin prevented accurate measurement beyond two cycles. The half-time of the disproportionation is about a day at room temperature. The activation energy is roughly 10 kcal. per mole. The half-lives, however, varied between experiments by 50%. Decreasing the initial dihydroporphyrin concentration fifteen times gave about the same half-life, showing that the reaction is roughly first order. These facts argue in favor of a free radical mechanism for the disproportionation. The thermal disproportionation is far too slow to account for the reduction of the dihydro- to the tetrahydroporphyrin in the presence of intense light.

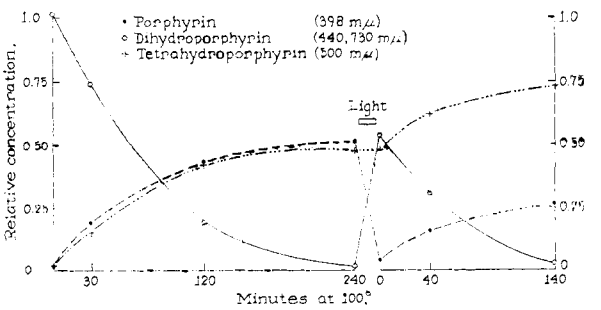

Fig. 6.—Disproportionation of the dihydrouroporphyrin. Solutions of the porphyrin (3×10^{-5} *M*) in 0.5 *M* EDTA, *p*H 5.6, were deoxygenated as described in the Experimental section and sealed into absorption cells. The dihydroporphyrin was formed by illuminating with monochromatic or white light. After heating in the dark the spectrum was measured at intervals at 25° with precautions to exclude excess light. When the disproportionation was complete, reillumination formed more dihydroporphyrin which again disproportionated when heated. Concentrations were calculated from the known extinction coefficients (Fig. 1), and suitable equations. The half-life of the tetrahydroporphyrin is about 20 hours at 100°, and a correction was made for its decomposition; it came to 15% of the concentration of this compound for the longest time shown. The points are averages of two separate experiments.

If the photoreduction of porphyrins by EDTA is a thermodynamically disfavored dark reaction which is driven by the energy of photons, it may be possible to reverse the reaction by heat or by a suitable catalyst. The thermal disproportionation of the dihydroporphyrin was observed while attempting to reverse its photoreduction. Heating the tetrahydroporphyrin for extended periods led only to low yields of porphyrin. No definite evidence for a thermal back reaction was thus obtained, but neither was evidence for a forward (reductive) thermal reaction found. Since both the dihydro- and the tetrahydroporphyrins are unstable at elevated temperatures, thermal reduction would manifest itself by a more or less rapid loss of porphyrin. In fact, heating deoxygenated solutions of uroporphyrin in 0.5 *M* EDTA near *p*H 6 (20°) for 31 hours at 200° or for 7 months at 100° led to 75 and 90% recovery of coproporphyrin. Most of the porphyrin loss occurred during the early stage of heating as the uroporphyrin decarboxylated to coproporphyrin. There is, therefore, no definite evidence for the thermodynamic balance of the reaction between porphyrins and EDTA.

4. Acid–Base Equilibria.—The color of the two reduced porphyrins changes in alkaline solution. The yellow-green dihydroporphyrin becomes green, and the orange tetrahydroporphyrin becomes yellow. The shifts of the longer wave

(16) U. Eisner and R. P. Linstead, *J. Chem. Soc.*, 1655 (1956).

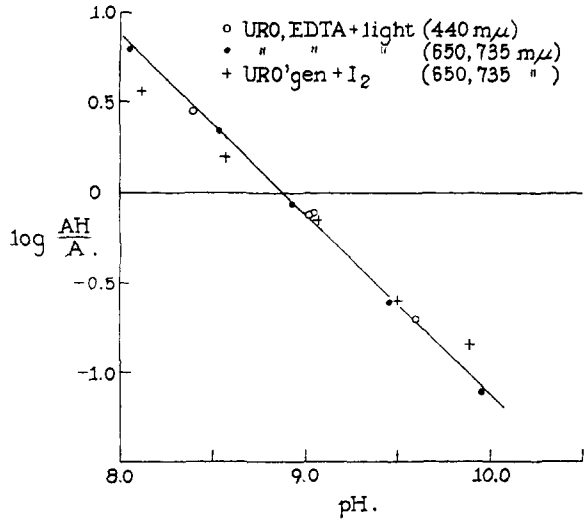

Fig. 7.—pK of urophlorin. The dihydroporphyrin, formed by photoreduction of porphyrin in 0.01 M EDTA or by partial oxidation of porphyrinogen with iodine, was titrated as described in the Experimental section. The logarithm of the ratio of acidic to basic forms was calculated from the absorption bands indicated on the figure. The straight line has slope -1 indicating a monoprotonic equilibria. The intercept of this line with the horizontal axis gives the pK 8.9 under the conditions 0.01 M EDTA + 0.15 M NaCl, 23°.

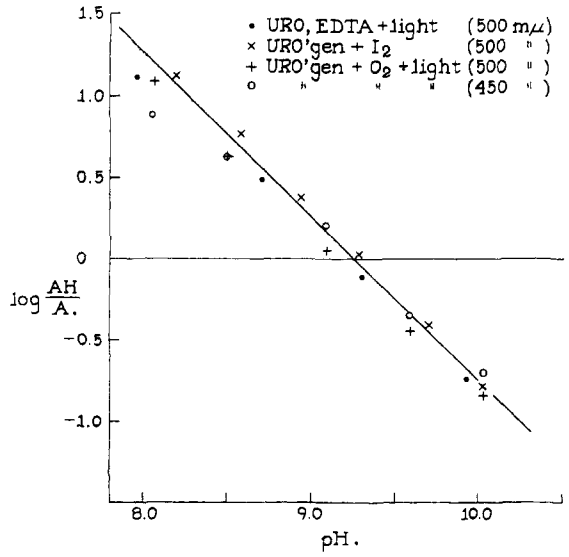

Fig. 8.—pK of uroporphomethene. The tetrahydroporphyrin was formed by photoreduction of porphyrin in 0.01 M EDTA solution, or by partial oxidation of porphyrinogen with iodine or with limited amounts of oxygen and light. The latter solution contained no EDTA. The plot is the same as in Fig. 7.

length bands are shown in Fig. 1. The dihydroporphyrin also has absorption bands in the near ultraviolet. Acid–base titrations of the reduced porphyrins showed that these spectral changes were the result of monoprotonic equilibria with pK 9.2 ± 0.05 for the dihydroporphyrin (Fig. 7) and 9.3 ± 0.05 for the tetrahydroporphyrin (Fig. 8) in 0.01 M EDTA, 23°. The values obtained were independent of the method of formation of the reduced porphyrins: photoreduction with EDTA or partial oxidation of the porphyrinogen with iodine or oxygen plus light. The pK's, however, were sensitive to ionic strength. The addition of about 0.15 M NaCl (in 0.01 M EDTA) caused the pK of the dihydroporphyrin to decrease 0.3 unit (Fig. 7). Thus the reduced uroporphyrins behave like dicarboxylic amino acids such as aspartic acid or glutamic acid, the pK's of which decrease with increasing ionic strength. The apparent "pK" of the 500 mμ absorption band of uroporphyrin itself shifts from 6.1 in 0.01 M EDTA to 5.6 in 0.1 M EDTA. In order to verify the monomolecularity of the acid–base equilibria of the reduced porphyrins the concentration of the dihydroporphyrin was varied over a 20-fold range and that of the tetrahydroporphyrin over a 5-fold range. The pK's did not change by more than could be expected from concomitant changes in salt concentrations. In the best experiments less than 2% of the reduced porphyrin was oxidized during the titration. In the very dilute solutions ($2 \times 10^{-6} M$), however, up to one-quarter of the dihydroporphyrin was oxidized by the end of the titration. Since the yield of porphyrin is very high (>90%), its absorption was used to correct the concentrations of the reduced porphyrin at each point of the titration curve. The pK values so determined were in good agreement with those derived from data where very little oxidation had occurred.

In concentrated sodium hydroxide the spectra of both reduced porphyrins reverted to spectra resembling those at pH <9 (see Table II). The difficulty of freeing strong sodium hydroxide solutions from air and peroxide prevented quantitative measurement, but reversal to the more acid forms along with oxidation could be demonstrated.

The acid form of the spectra of both the dihydro- and the tetrahydroporphyrins were unchanged up to an acidity of 1 M hydrochloric acid.

5. Reaction of the Tetrahydroporphyrin with Sulfite and Dithionite Ions.—The tetrahydroporphyrin was decolorized by sulfite and dithionite ions near neutral pH. This reaction may be a reduction to the dipyrrylmethane structure, *i.e.*, the porphyrinogen, or an addition of the sulfite ion to the *meso*-carbon of the dipyrrylmethene. At pH 5.8, with sulfite ion at 10^{-4} to $10^{-3} M$ and tetrahydroporphyrin at $10^{-5} M$, the data were compatible with a 1:1 complex having a dissociation constant of about $7 \times 10^{-6} M$. Measurement with more dilute sulfite ion, and attempted study of the pH dependence of this complex failed, at least in part, because of oxidation of the sulfite ion and tetrahydroporphyrin by traces of oxygen.

Chemical Reduction.—Alkylporphyrins are quantitatively reduced to porphyrinogens by shaking with sodium amalgam. If the porphyrin is reduced slowly in a closed tube, the spectral changes due to the formation of the alkaline forms of dihydro- (650 mμ) and tetrahydroporphyrins (460 mμ) are clearly seen. The stepwise reoxidation of the porphyrinogen also produces the same partially reduced porphyrins, the amount formed depending on the oxidant and its rate of addition. Bogorad[15]

observed these absorption bands at 440 and 500 mμ during oxidation of uroporphyrinogen with iodine.

The reduction of porphyrins in acid solution by titanous and chromous ions is remarkably specific. Reduction of uro- and coproporphyrin with titanous chloride in acid solution in the dark or light proceeds through the dihydro stage and stops at the tetrahydro stage. Simple alkyldipyrrylmethenes are not reduced under conditions where porphyrin and phlorin react rapidly.

The reduction of porphyrins by dithionite ion is complex. With uroporphyrin, the dark reaction is rapid at *p*H 6–7 (Fig. 9, curve 3) and very slow (<0.01 of the rate at *p*H 7) at *p*H greater than 8.4 (Fig. 9, curve 1). Illumination of the alkaline solution with white or monochromatic light causes rapid reduction to occur (Fig. 9, curve 2). The intermediate was identified as the dihydroporphyrin by its spectrum and by shifts of this spectrum at various *p*H's: the *pK* was near 9. The reduction of the dihydroporphyrin by dithionite ion proceeds in the dark, the rate decreasing with increasing *p*H. This rate was not increased by light absorbed by the band at 650 mμ. The dihydroporphyrin can also be reduced by acid sulfite ion in the dark at *p*H 6.2.

During the preparation of this paper a similar observation of the photoreduction of xanthene dyes by dithionite ion was made by Pietsch and Tuchnitz.[18]

Discussion

The assignment of structures to the reduced porphyrins is based on the comparison of spectra with those of model compounds, on the change of spectra with *p*H and on the chemical reactions of the hydroporphyrins. These data support the structures shown in Fig. 2.

Although the spectrum of the phlorin structure cannot be predicted in detail, arguments based on analogy with simple cyanine dyes,[19] with polypyrryl-polymethine dyes[20] and with the bile pigments[21] predict a long wave length band at 700–1000 mμ. The low extinction coefficient (*i.e.*, oscillator strength) of the band observed at 735 mμ may be caused by the *cis* structure of part of the conjugated system.[22] The band progression of the phlorin in the 400 mμ region with a spacing of 5000 cm.$^{-1}$ is also characteristic of the bile pigments. A linear trinuclear cyanine with a long wave length maximum at 615 mμ has a spectrum similar in form to that of the phlorins.[23] The spectrum of the tetrahydroporphyrin is very similar to that of a dipyrrylmethene.[10] The spectral properties of the reduced porphyrins and of a dipyrrylmethene are summarized in Table II.

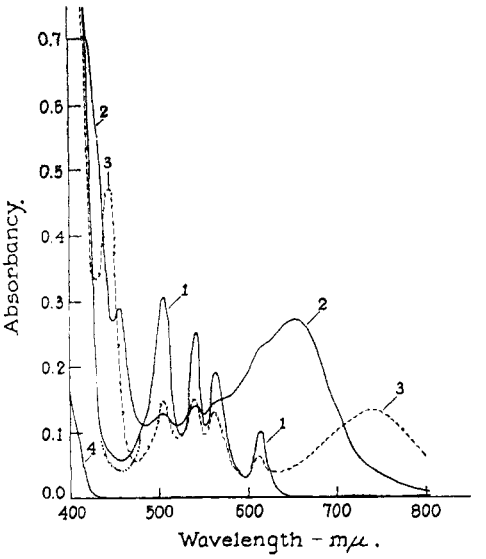

Fig. 9.—Chemical and photoreduction of uroporphyrin (1.4×10^{-5} *M*) by sodium dithionite (~0.02*M*): curve 1, *p*H 9.8, 175 min. in the dark; only a slight change near 450 mμ is seen; curve 2, as **1**, illuminated 1 min. with white light (10,000 foot candles); curve 3, *p*H 6.1, 1 minute after adding $Na_2S_2O_4$, in the dark; curve 4, as 3, 26 min. in the dark.

The *pK*'s and spectral changes of the porphomethene and of the dipyrrylmethene[10,24] are similar (Table II). Since the phlorin may be considered a vinylog of a dipyrrylmethene, its *pK* is also in the same region. In strong alkali the ionization of the hydrogen of the pyrrole of the dipyrrylmethene part of the porphomethene (or of the pyrrole adjacent to the methylene bridge in the phlorin) forms a symmetrical negative ion analogous to the positive ion which exists in acid solution (but not identical in the case of the phlorin). Similar symmetrical negative ions are formed with dipyrrylmethenes and with porphyrins.

The decolorization of the tetrahydroporphyrin by sulfite and dithionite ions quantitatively resembles that of the dipyrrylmethene.[10] The phlorin reacts more slowly with these ions, and at least a part of this reaction involves reduction of the ring. The chemistry of these reactions requires further study.

The disproportionation of the phlorin to porphyrin and porphomethene under gentle experimental conditions is similar to that observed with dihydropyridines.[25] The driving force for the reaction of phlorins may be the greater energy of the C—H bond over that of the N—H bond and the greater stability of the porphyrin and pyrrole ring structures as compared to the polypyrrylmethene structure of the phlorin.

The apparent specificity of many reductions of porphyrins to the tetrahydro level may be explained kinetically by the greater stability of the one-electron reduction products of the porphyrin

(17) L. Bogorad, *J. Biol. Chem.*, **233**, 501 (1958).

(18) H. Pietsch and J. Tuchnitz, *Z. physik. Chem.*, **216**, 372 (1961).

(19) K. Venkataraman, "The Chemistry of Synthetic Dyes," Academic Press, Inc., New York, N. Y., 1952, Vol. I, Chapt. 8; Vol. II, Chapt. 38.

(20) A. Treibs and W. Seifert, *Ann.*, **612**, 242 (1958).

(21) C. H. Gray, A. Kulczycka and D. C. Nicholson, *J. Chem. Soc.*, 2268 (1961).

(22) G. Scheibe, H. J. Friedrich and G. Hohlneicher, *Angew. Chem.*, **73**, 383 (1961).

(23) L. G. S. Brooker and L. A. Smith, *J. Am. Chem. Soc.*, **59**, 67 (1937).

(24) The *pK* of 3,3′,5,5′-tetramethyl-4,4′-di-(β-carboxyethyl)-2,2′-dipyrrylmethene is also about 8.5: C. H. Gray, A. Kulczycka and D. C. Nicholson, *J. Chem. Soc.*, 2276 (1961).

(25) F. Brody and P. R. Ruby in "Heterocyclic Compounds; Pyridine and Derivatives," ed. E. Klingsberg, Interscience Publishers, Inc., New York, N. Y., 1960, Pt. I, p. 229.

TABLE II

SPECTRAL CHARACTERISTICS OF UROPORPHYRIN, ITS REDUCTION PRODUCTS AND OF A DIPYRRYLMETHENE[a]

Compound	Condition	λ_{max}, mμ	ε, × 10⁴	Half-width, mμ	λ_{max}, mμ	ε, × 10⁴	Half-width, mμ	pK
Uroporphyrin	Acid	593	0.62	~16	406	51	10	
	Alkaline	612	0.45	~18	397	24	28	<7
	Very alk.	~590	>0.5	~17	~425	>30	19	
Urophlorine, dihydroporphyrin	Acid	735	2.2	120	440	6.2	36	
	Alkaline	650	2.0	120	..	..	..	9.3
	Very alk.	~760	..	..	~430	..	..	
Uroporphomethene, tetrahydroporphyrin	Acid	500	5.6	43	340	0.5	..	
	Alkaline	460	2.8	75	..	..	..	9.2
	Very alk.	~500	..	..	..	..	..	
3,3′,5,5′-Tetramethyl-2,2′-dipyrrylmethene	Acid	463	8.8	27	340	0.6	..	
	Alkaline	438	3.1	66	..	..	..	8.7
	Very alk.	~480	~9	26	..	..	..	

[a] Only two absorption bands are listed. The conditions are as follows: acid, 1 *M* HCl; alkaline, *p*H >9 often in the presence of 0.01 *M* EDTA; very alkaline, 10–15 *M* aqueous NaOH or sodium methoxide in dimethyl sulfoxide, both with a small amount of methanol.

and of the phlorin as compared to that of the dipyrrylmethene part of the tetrahydroporphyrin. Similarly, the greater stability of the one-electron oxidation product of the phlorin would explain why it is more rapidly oxidized than is the porphomethene.

The possible number of di- and tetrahydroporphyrins is quite large, even if only C–H bonds are considered. The protons on the nitrogens are at an equilibrium determined by the acidity of the solution. There are thirty dihydroporphyrins for which chemically reasonable structures may be written. These may be divided into three classes: The addition of two electrons and (a) no protons, (b) one proton and (c) two protons to the porphyrin ring. The single structure of class a, *i.e.*, the porphyrin structure of Fig. 2 with only ten double bonds and no charge, would be expected to have aromatic stability only if the unshared electron pairs on the nitrogens contribute to the conjugation. Although such a structure could have absorption at long wave lengths, it would resemble pyrrole in having *pK*'s at the extremes of the *p*H scale. Possibly this type of dihydroporphyrin is responsible for the spectral shifts which are observed on photoreduction of porphyrins to the dihydro level.

There are three structures of class b: the single proton may be on carbon 1 (bridge), 2 (internal) or 3 (ring) (see Fig. 2). The hydrogen on an internal carbon causes the ring to be non-planar and, as with the hydrogen on a ring carbon, decreases the length of the equivalent conjugated system. Thus, the usual considerations of stability in conjugated hydrocarbons[26] favor the phlorin structure.

Most of the numerous structures of class c, having two protons attached to carbon, are eliminated by the observed absorption of the dihydroporphyrin at 735 mμ. The remaining structures would be expected to have one or two *pK*'s in the acid region, whereas none were detected. The chlorins belong to this class of dihydroporphyrins and differ from the phlorin in both spectra and chemical reactivity. Octaalkylchlorins[27] have a band near 650 mμ which shifts to about 635 mμ in acid solution. They are reasonably stable in air and are slowly oxidized by chloranil at elevated temperatures.[28] Seely and Calvin have found that the reduction of zinc tetraphenylporphyrin with photoexcited benzoin leads to a chlorin.[28] The reactions leading to phlorins require different experimental conditions.

A very large number of tetrahydroporphyrin structures are possible, but again most are readily eliminated by the observed spectra and *pK*. In particular, the two bacteriochlorin structures with hydrogens on ring carbons of adjacent and opposite pyrrole rings[29] can be excluded. Most of the remaining structures possible are simply pyrrollenine forms of the porphomethene (Fig. 2). Since the dipyrrylmethane group does not interfere with the planarity of the dipyrrylmethene group, the pyrrole structure is favored.

The striking ease and exact stoichiometry with which two electrons and a proton are added to and withdrawn from the porphyrin ring resembles the analogous reactions of the phosphopyridine nucleotides. As with the latter compounds, hydrogen isotopes could be used to confirm the phlorin structure and to obtain information on the detailed mechanism of the photoreduction.

In general, protonation of the porphyrin ring will favor reduction because of increased electronegativity and especially because the one- (or two-) electron reduction products have better charge stabilization on the nitrogen atoms. Thus, the increased negative charge in the heterocyclic ring of zinc coproporphyrin may account for its lack of photoreduction with EDTA,[1a] since the bonding of the metal is largely ionic. The negatively charged ring would be expected to favor oxidation, and it has been shown that the one-electron oxidation products of the metal chelates of phthalocyanine are more stable than that of the free phthalocyanine.[30] The copper chelate of coproporphyrin is also photochemically inactive, but here the more complex binding between the

(26) G. W. Wheland, "Resonance in Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1955, Chapt. 3.

(27) H. Fischer and A. Stern, "Die Chemie des Pyrrols," Akademische Verlagsgesellschaft, Leipzig, 1940, Vol. II, pt. 2, p. 144 ff.

(28) G. R. Seely and M. Calvin, *J. Chem. Phys.*, **23**, 1068 (1955).

(29) G. R. Seely, *ibid.*, **27**, 125 (1957).

(30) P. George, D. J. E. Ingram and J. E. Bennett, *J. Am. Chem. Soc.*, **79**, 1870 (1957).

metal and the ring may allow rapid and reversible intramolecular charge transfer to occur.

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[CONTRIBUTION FROM THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY, CAMBRIDGE 38, MASS.]

Peresters. VII. *t*-Butylperoxyesters of *exo*- and *endo*-2-Norbornanecarboxylic Acids

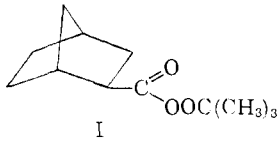
BY PAUL D. BARTLETT AND RICHARD E. PINCOCK

RECEIVED JANUARY 25, 1962

The relative rates of decomposition at 100° in chlorobenzene of the *t*-butylperoxyesters of cyclohexanecarboxylic acid, of *exo*- and of *endo*-2-norbornanecarboxylic acids are 6.0, 4.1 and 1.0, respectively. These peresters give 75, 76 and 58%, respectively, of a mole of carbon dioxide per mole of perester. Decomposition of the *exo*-perester in moist carbon tetrachloride in the presence of iodine decreased the yield of carbon dioxide to 56%. These results show that not more than 56% of the decomposition of the *exo*-perester is of the concerted two-bond type, and it is not certain whether any of these three peresters reacts by this mechanism. This, and the *exo/endo* rate difference of only fourfold, means that carbon 6 of the norbornane ring provides insufficient anchimeric assistance to make the formation of a 2-norbornyl radical comparable in ease to that of *t*-butyl, trichloromethyl or benzyl.

Introduction

Previous studies in this Laboratory[1] have shown that the rates of thermal decomposition of *t*-butylperoxyesters are related (1) to the stability of the alkyl radicals formed and (2) to substitution favoring the cationic character of these radicals in the transition state. Accordingly, the *t*-butylperoxy esters of *exo*- and *endo*-norbornane-2-carboxylic acids (I and II) possess a double interest.



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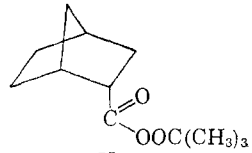

II

If the 2-norbornyl free radical were stabilized by a bridged structure like that of the norbornyl cation,[2] we might expect a large excess of k_{exo} over k_{endo} in any reaction in which the 2-norbornyl radical was formed in the rate-determining step. Even if, in accord with a number of indications, there is no favored bridged structure for the radical, it would be of interest to learn whether in the polar contributing structure III[1b] of the transition state anchimeric assistance of the known cationic type in the norbornyl group is able to facilitate homolytic bond fission. Free radical rearrangement of phenyl groups does not occur by direct participation of the migrating group as the original bond breaks, but by rearrangement of the group after initial formation of the radical.[3] This fact, together with the general lack of examples of free radical alkyl rearrangements,[4,5] suggests that neighboring group interaction with the radical center does not occur.

$$R^{+}CO_2\ ^{-}OC(CH_3)_3 \quad \text{(III)}$$

Results

The norbornyl peroxyesters were prepared by reaction of the *exo*- or *endo*-norbornanecarbonyl chlorides with *t*-butyl hydroperoxide in the presence of pyridine in pentane at 0°. The perester derived from cyclohexanecarboxylic acid was also prepared in order to compare it with the bicyclic peresters. The compounds obtained were oils, characterized by their infrared spectra, peroxide titers and elementary analyses.

The kinetic and product studies were carried out in chlorobenzene since relatively high temperatures (100–120°) were required to cause decomposition of these peresters at conveniently measurable rates. The rates were followed by the decline in intensity of the perester carbonyl band at about 5.63 μ. All the runs gave good first-order plots with some increase in rate constant with concentration in the case of the *endo*-norbornyl and of the cyclohexyl peresters. The results of the kinetic runs are shown in Table I and the products of decomposition are shown in Table II.

To investigate the possibility that only the peroxide bond of the *exo*-perester was breaking in the rate-determining step, an attempt was made to trap with iodine in moist carbon tetrachloride the acyloxy radicals which would then be formed.[6] Table III shows the results of decomposing *exo*-2-carbo-*t*-butylperoxynorbornane in carbon tetrachloride in the presence of iodine and water. A control run was carried out without the iodine present.

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(1) (a) P. D. Bartlett and R. R. Hiatt, *J. Am. Chem. Soc.*, **80**, 1398 (1958); (b) P. D. Bartlett and C. Rüchardt, *ibid.*, **82**, 1756 (1960); (c) P. D. Bartlett and D. M. Simons, *ibid.*, **82**, 1753 (1960).

(2) S. Winstein and D. Trifan, *ibid.*, **71**, 2953 (1949); **74**, 1147, 1154 (1952).

(3) (a) F. H. Seubold, *ibid.*, **75**, 2532 (1953); S. Winstein, R. Heck, S. Lapporte and R. Baird, *Experientia*, **12**, 138 (1956); J. Weinstock and S. N. Lewis, *J. Am. Chem. Soc.*, **79**, 6243 (1957); (b) C. G. Overberger and H. Gainer, *ibid.*, **80**, 4561 (1958).

(4) J. A. Berson, C. J. Olsen and J. S. Walia, *ibid.*, **82**, 5000 (1960); S. J. Cristol and G. D. Brindell, *ibid.*, **76**, 5699 (1954); S. J. Cristol and R. P. Arganbright, *ibid.*, **79**, 6039 (1957); J. A. Berson and W. M. Jones, *ibid.*, **78**, 6045 (1956).

(5) For a kinetic and product study of the effect of neighboring sulfur and of iodine see J. C. Martin and W. G. Bentrude, *Chemistry & Industry*, 192 (1959), and J. E. Leffler, R. D. Faulkner and C. C. Petropoulos, *J. Am. Chem. Soc.*, **80**, 5435 (1958).

(6) (a) G. S. Hammond and L. M. Soffer, *ibid.*, **72**, 4711 (1950); (b) H. J. Shine and D. M. Hoffmann, *ibid.*, **83**, 2783 (1961); (c) D. F. DeTar and R. C. Lamb, *ibid.*, **81**, 122 (1959).