TABLE III

"Y" CONFIGURATION COMPOUNDS OTHER THAN ESTERS

| Ester family | Family peak, μ | Compound | Family peak, μ |
|---|---|---|---|
| Fumarates | 12.84–12.94 | Fumaric acid | 12.88 |
| | | Fumaryl chloride | 12.62, 12.59 |
| | | Fumaryl benzylamide | 12.4[a] |
| Crotonates | 11.9–12.0 | Crotonic acid | 11.88 |
| | | *trans*-1-Ethyl-2-nitroethylene[b] | 11.85 |
| Acrylates | 12.34–12.38 | Acrylic acid | 12.24, 12.29 |
| | | *t*-Butylacrylamide | 12.34 |
| | | Nitroethylene | 12.48 |
| | | Acrylamide | 12.22 (12.30 cryst.) |
| Methacrylates | 12.26–12.29 | Methacrylic acid | 12.24–12.39 |
| Acetylenedicarboxylates | 13.36–13.42 | Acetylenedicarboxylic acid | 13.42 |
| Cinnamates | 13.00–13.10 | Cinnamic acid | 13.04 |
| | | *trans*-β-Nitrostyrene | 13.00 |

[a] R. W. Stafford, R. J. Francel and J. F. Shay, *Anal. Chem.*, 21, 1455 (1949). [b] This is a near comparison, in the light of both compounds being *trans*-1,2-disubstituted ethylenes.

**Polarized Spectra of Other Esters.**—Cyclohexyl maleate was examined in oriented crystal film; it showed rather high dichroic ratios for the C–O stretching absorptions. Further, those absorptions were strong at the same setting of polarizer that gave strong absorption by the C=C stretching vibration. Thus, a high degree of coplanarity for the ester group and the ethylene exists. Significantly, the absorption at 8.2 μ which was shown to be characteristic of maleates[1] was absent. Thus, this molecule is preferentially oriented in crystallization and could be substantially unlike *normal* maleates. Another point of abnormality recorded in the earlier paper is that the melting point of the maleate is in this case higher than the fumarate: 86° *vs.* 35–36°.

Methyl cinnamate was studied; it yielded only moderate dichroic ratios in any assigned peaks. However, such ratios as were noted were again qualitatively in accord with expectations based on our generalization.

**Tabulated Spectral Data.**—Table II shows the wave lengths of the family-constant peak for the various ester families. Other peaks of interest also are shown.

Several ester families were examined to see if constant peaks were present in the 10–15 μ region and none were found. These included succinates, acetates and oxalates. However, the propionates have a peak that is remarkably constant at 12.4 μ as had been noted by Tompson and Torkington in 1945.[2] This absorption is very near the wave length of the peaks of the acrylates and the methacrylates. It is, however, much less prominent than that in the unsaturated esters. Possibly the explanation of this absorption could be that a low degree of double bond character exists at the C–C bond between the carbonyl and α-carbons and that some portion of the molecules at a given instant assume the conformation that most nearly matches that of the acrylates.

**Types Other Than Esters.**—It was noted early in this investigation that the corresponding acids have peaks which match the family-constant ester peak. As the study progressed more compounds of related types were examined and confidence increased in the greater breadth of the generalization. Table III lists other types in comparison with the esters.

**Acknowledgments.**—The authors are grateful to Drs. R. S. McDonald, J. F. Brown, Jr., and A. Pozefsky for helpful comments on the theoretical aspects of this work. They are also grateful to Dr. J. Rosenberg for valuable help in obtaining many of the compounds used in this study and to Dr. J. F. Brown, Jr., for the data on the nitroethylene spectra.

SCHENECTADY, NEW YORK

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN]

# Light Induced Spectral Shift of the Thiazine Dyes in the Bound State[1]

BY NEIL WOTHERSPOON AND GERALD OSTER

RECEIVED MARCH 8, 1957

We have found that thiazine dyes in the presence of polymethacrylic acid or polyacrylic acid undergo a spectral shift when irradiated with visible light. A combined recording spectrophotometer and irradiation apparatus was used to follow the reaction either at a fixed wave length or by scanning the spectrum repetitively. Two photo products are formed, one in the absence of oxygen and the second by the photoöxidation of the first. The rate of formation of the second product is retarded by small amounts of paraphenylenediamine or by potassium iodide suggesting that long-lived excited states are involved. The final photoproduct is the same for all the thiazine dyes studied and is identical to thionine. The reaction requires a polyacrylic acid but does not take place in the presence of other high polymeric acids or of dibasic acids.

## Introduction

During the course of our studies on the photochemical properties of methylene blue, we observed that a purple species having a bright red fluorescence was formed when a solution of the dye containing polymethacrylic acid was illuminated. It is the purpose of the present study to investigate the nature of this photochemical reaction. In particular, we are concerned with determining which dye and high polymer combinations exhibit this phenomenon.

The photochemical properties of many dyes

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[1] (a) This paper represents a part of the dissertation to be submitted by Neil Wotherspoon to the faculty of the Graduate School of the Polytechnic Institute of Brooklyn in partial fulfillment of the requirements for the degree of Doctor of Philosophy. (b) This research was supported by the United States Air Force through the Air Force Office of Scientific Research of the Air Research and Development Command under Contract No. AF 18(600)1182.

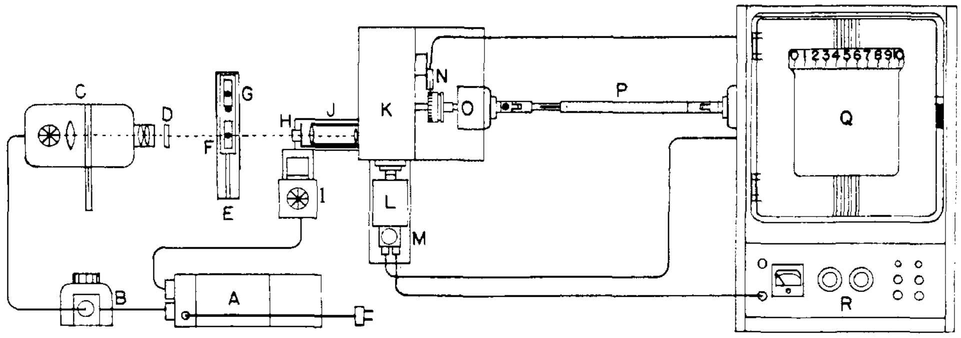

Fig. 1.—Combined recording spectrophotometer and irradiation apparatus: A, constant voltage transformer; B, Variac; C, Kodak Master projector; D, filter; E, slide transport for cells; F, blank (solvent) cell; G, reaction cell; H, fluorescence cell; I, light source and filter; J, collimating lens; K, monochromator; L, absorption cell compartment; M, multiplier phototube; N, microswitch for wave length marker; O, gear box; P, mechanical coupling; Q, Speedomax recorder; R, high voltage power supply.

bound to high polymer substrates are different from those of the free dyes.[2,3] The present work differs from previous studies of the photochemistry of bound dyes, however, in that only one class of dyes, namely, the thiazines, exhibits the light induced spectral shift. Furthermore, the high polymeric substrate for the reaction is highly specific and is apparently confined only to polyacrylic acid and polymethacrylic acid.

## Experimental

Materials.—Methylene blue, Merck, zinc free, was used without further purification. All other thiazine dyes were histological grade from National Aniline Division of Allied Chemical and Dye Corp.

Polymethacrylic acid (PMA) was prepared by polymerizing 10 g. of distilled methacrylic acid in 200 ml. of water containing 0.2 g. of potassium persulfate at 90° for 30 minutes. The mixture was previously degassed by bubbling with nitrogen. The polymer solution was dialyzed against distilled water for three days, and then freeze-dried.

Apparatus.—The equipment was conveniently arranged for both irradiating the dye solutions and simultaneously recording the absorption spectrum (Fig. 1).

The light source for the photochemical reactions was a 1000 watt Kodak Master slide projector. A Variac was used to reduce the input voltage to 65 volts in order to provide a convenient light intensity and to prolong the life of the lamp. Constancy of illumination was assured by a 500 VA Sola constant voltage transformer. Filters could be inserted in the projector slide carrier. The optical cells were made by cementing Pyrex plate glass disks (2.25 inch diam.) to a half inch section of 2.25 in. heavy wall Pyrex tubing in which two capillary tubes were sealed to permit bubbling with helium. Araldite epoxy resin was used as cement. The reaction cell and a blank cell filled with water were mounted on a transverse sliding block which allowed either cell to be irradiated.

The measurement of monochromatic radiant flux incident upon the cell was carried out with a calibrated Eppley thermopile whose output was read on a Leeds and Northrup Microvolt Amplifier No. 9835-A. This is a chopper stabilized amplifier inherently capable of high precision and freedom from zero drift.

The spectrophotometer consisted of a Bausch and Lomb 250 mm. grating monochromator in conjunction with a Leeds and Northrup Speedomax recorder. The recorder was modified by the inclusion of a special high impedance amplifier for use with an RCA 1P22 multiplier phototube. The recorder was adapted to permit direct mechanical coupling *via* gears and a pair of universal joints to the monochromator grating screws. In this way a linear relationship between the chart paper movement and wave length was obtained. A microswitch provided marker pulses to operate a marker pen at the right-hand side of the chart at intervals of 100 mμ. By reversing the chart motor and rescanning, a series of curves could be superimposed on the same chart for comparison. The recorder contained controls for dark current suppression and for sensitivity which could be made as high as $10^{-10}$ amp. per mm. A Scientific Specialties Corp. Regulated High Voltage Power Supply, Model PS-22M, was used to provide from 500 to 1000 volts for the phototube. The stability was such that phototube sensitivity drift was negligible over a period of an hour.

For kinetic studies the monochromator was set at the wave length of an absorption band of either a reactant or a product and the transmission of the solution recorded as a function of time of irradiation. Spectra of photosensitive systems were obtained by placing the solutions in the cell compartment between the monochromator and the phototube where the light intensity is very low.

Fluorescence spectra were recorded by rearranging the light source and optical cell. Either the mercury arc or the ribbon filament lamps supplied with the Bausch and Lomb Monochromator were used to illuminate a square optical cell which was placed near the entrance slit collimating lens. This lens formed an image of the fluorescence cell on the slit and thus excluded almost all of the scattered light. Only the front edge of the cell was imaged on the slit in order to avoid self absorption effects. The chart record of the fluorescence intensity *vs.* wave length had to be corrected for the unequal phototube sensitivity at each wave length. This was done by determining the phototube response to a ribbon filament lamp whose color temperature was measured by the National Bureau of Standards. From the Planck energy distribution equation and the phototube response, the absolute phototube sensitivity was calculated. This method eliminated the effect of anomalies in the transmission of the grafting monochromator.

Procedure.—The kinetic transmission curves were recorded at a given wave length by setting the blank transmission at or near the full scale of the recorder after adjusting the dark current to zero. The initial rate of disappearance of dye was used to calculate the quantum yield. In some experiments atmospheric oxygen was permitted to participate in the reaction. In order to prevent air oxidation helium (Airco) was used to degas the solution.

In order to determine the quantum yield for the conversion of methylene blue, a solution of the dye and PMA was irradiated by the light from the 500 watt projector lamp filtered by a Baird Associates interference filter with a peak transmission of 63% at 665 mμ and a half band width of 10 mμ. The rate of disappearance of the methylene blue absorption band at 665 mμ was obtained from the initial slope of the transmission *vs.* time curve as plotted by the recorder. The slope was converted into a rate by the use of the absorbancy index of the dye and of the initial transmittance. From the measured radiant flux (659 μw./cm.$^2$) obtained by the use of the thermopile and the rate of dye conversion, the quantum yield was obtained.

## Results

Methylene blue at concentrations of the order of $10^{-5}$ $M$ is stable to visible light of intensities

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(2) G. Oster, *Trans. Faraday Soc.*, **47**, 660 (1951).

(3) G. Oster and J. S. Bellin, THIS JOURNAL, **79**, 294 (1947).

considered in the present work. In the presence of excess polymethacrylic acid (PMA) the dye undergoes a photochemical change to give a species having an absorption band at shorter wave lengths (Fig. 2). The quantum yield of the disappearance of methylene blue is 0.0035. If the irradiation is carried out with oxygen present, the dye (curve I) is converted to a purple species (curve III). Whereas methylene blue free or bound is only feebly fluorescent (quantum yield less than 1%), the light produced species exhibits a strong red fluorescence with a quantum yield greater than 10%. An intermediate species is produced because the initial rate of disappearance of species I is greater than the appearance of species III. This is more clearly seen by carrying out the reaction in the complete absence of oxygen. Under this condition, a substance with an absorption band (curve II) intermediate between the other two is produced and no purple species is formed. If now a solution containing species II is irradiated in the presence of oxygen the purple species (curve III) is produced. It appears, therefore, that methylene blue undergoes an anaerobic photochemical transformation to species II which, in turn, undergoes a photoöxidation to species III.

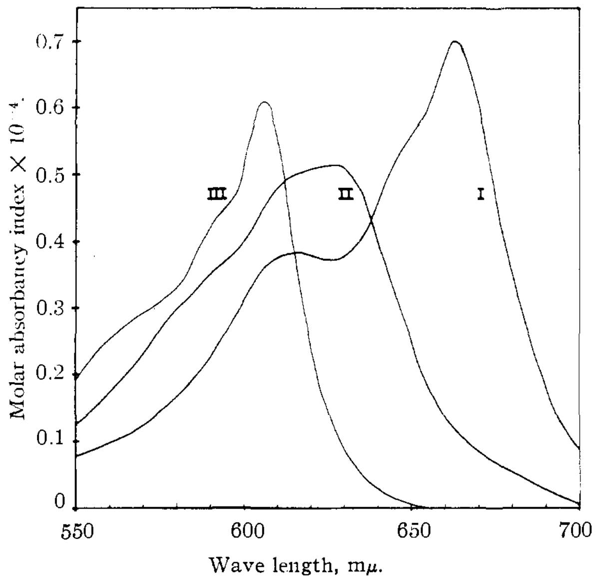

Fig. 2.—Light induced spectral shifts in methylene blue; dye concentration $10^{-5}$ $M$, PMA concentration $5 \times 10^{-4}$ $M$ (monomer basis), *p*H 7: I, unirradiated; II, irradiated in the absence of oxygen; III, irradiated in the presence of oxygen.

The rate of production of species III was reduced to about one-half its normal value in the presence of either $10^{-5}$ $M$ of KI or of *p*-phenyldiamine. The rate of production of species II, on the other hand, was not influenced by the addition of these substances up to a concentration of $10^{-2}$ $M$.

A number of high polymeric acids including desoxyribose nucleic acid (molecular weight about $5 \times 10^6$), alginic acid, hyaluronic acid, polyglutamic acid, hydrolyzed maleic anhydride–vinyl methyl ether copolymer, and agar were tested with methylene blue. None of these substances produced the light induced spectral shift and only polyacrylic and polymethacrylic acid were effective. A homologous series of dibasic acids (oxalic, malonic, succinic, glutaric, adipic, pimelic and suberic acids) were tested and found to be ineffectual.

Representative dyes (mainly cationic) from several classes (azo, triphenylmethane, xanthene, azine, etc.) were irradiated in the presence of PMA but no spectral shift occurred. On the other hand, all members of the thiazine class of dyes which we tested exhibited the phenomenon. In all cases, the absorption bands of the thiazine dyes were shifted to shorter wave lengths on irradiation in the presence of PMA and oxygen resulting in a strongly red fluorescent species (Table I). The absorption spectra of the photo-product is for all these cases identical with that of thionine. Similarly, the fluorescence spectrum of the photo-product is identical with that of thionine (Fig. 3). The photo-product (species III) is an acid–base indicator and changes reversibly from purple to red in basic solutions. Thionine behaves similarly but methylene blue does not show this reversible color change at high *p*H values.

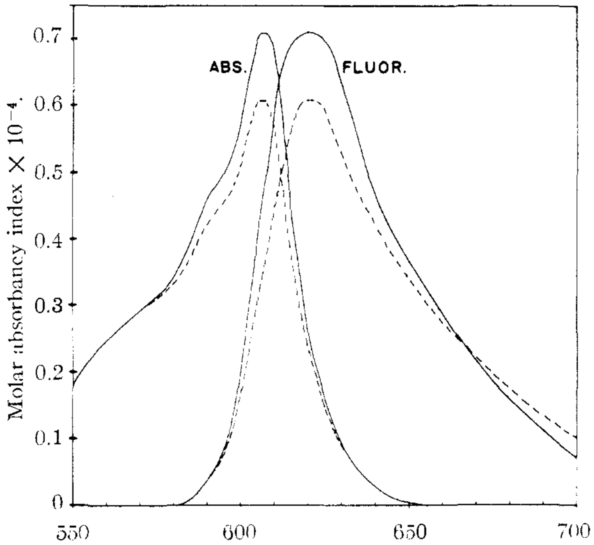

Fig. 3.—Absorption and fluorescence spectra of thionine and of the photoöxidation product: solid curves, thionine; dotted curves, photoöxidation products of methylene blue. Fluorescence curves are drawn to arbitrary ordinates.

## Discussion

The phenomenon of light induced spectral shift in dye–polymer mixtures has not heretofore been observed as far as we have been able to ascertain. It differs from light induced *cis–trans* conversions in that a specific polymer must be present and furthermore the reaction is irreversible.[4]

It is clear that in all cases the photo-product is identical with thionine. This is shown from the close resemblance between species III and thionine as regards their absorption spectra, fluorescence spectra and behavior as a *p*H indicator. This fact,

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(4) Compare, for example, G. M. Wyman, *Chem. Revs.*, **55**, 625 (1955).

together with the necessity for oxygen in the reaction, indicates that species III is a photoöxidation product of the thiazine dyes in which the methyl substituents were removed resulting in the formation of thionine. It is known that methylene blue may be demethylated by non-photochemical oxidation in alkali to give dyes with fewer methyl substituents.[5]

TABLE I

ABSORPTION MAXIMA OF PHOTO-PRODUCTS

| | Wave length of absorption max. | |
|---|---|---|
| Dye | Free dye | Photo-product with PMA |
| Thionine | 599 | 607 |
| Azure C (monomethylthionine) | 616 | 607 |
| Azure A (u-dimethylthionine) | 620 | 608 |
| Azure B (trimethylthionine) | 652 | 605 |
| Methylene blue (tetramethylthionine) | 665 | 606 |

The highly specific nature of the polymer required for the reaction implies that the polyacrylic acids serve as substrates for a very special type of dye binding. In the dye–polymer complex the transition from the ground to the first electronic excited state does not differ from that of the free dye since the absorption spectra are virtually identical. Transitions from the first electronically excited state to other states must, however, be profoundly affected by the complexation with the specific polymers. It appears that the spatial arrangement of the carboxyl groups on the polyacrylic acids is particularly suited to complex with the thiazines *via* their amino groups to distort the electron configuration of the excited dye molecules. This arrangement does not exist for any of the other polymeric acids which were tested nor for the dibasic acids studied.

There are two distinct photochemical processes involved in the light induced spectral shift of the complexed thiazine dyes. The first process to give species II does not require oxygen. It is possible that the absorption of light by the original dye imparts a certain electron configuration which enables the dye molecule to be bound to the substrate in a manner which allows it to be readily photoöxidized to give species III. This first process does not require oxygen and probably does not involve long-lived states. On the other hand, the second process involves long-lived states because very small amounts of KI or *p*-phenylenediamine retard the reaction. From the observed retardation we estimate, using the Stern–Vollmer expression and the calculated diffusion controlled encounter frequency in solution,[6] that the lifetime of the excited species which reacts with oxygen to be about $10^{-5}$ sec. By analogy with other dyes whose rate of photochemical conversion is influenced by $O_2$, KI and *p*-phenylenediamine, we propose that this long-lived excited state is a triplet state. It has been shown with triphenylmethane dyes[3] that binding can encourage triplet formation. Here, however, it appears that a specific substrate is required and that the binding takes place only after the dye is excited. The low quantum yield observed is probably accounted for in terms of internal conversion to the ground state which are competitive to transitions to the long-lived state.

(5) H. J. Conn, "Biological Stains," 5th Ed., Biotech Publications, Geneva, N. Y., 1946.

(6) G. Oster and A. H. Adelman, THIS JOURNAL, **78**, 913 (1956).

BROOKLYN 1, N. Y.

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[CONTRIBUTION FROM THE MELLON INSTITUTE AND DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PITTSBURGH]

# Diketene: Infrared Spectrum and Structure. II[1]

BY FOIL A. MILLER AND GERALD L. CARLSON

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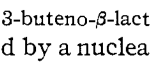
Earlier temperature-dependence measurements on the infrared spectrum of diketene vapor by Miller and Koch have been repeated, and have been found to be incorrect. There is no evidence from the vapor spectrum for an equilibrium between two forms. Furthermore the spectrum of the solid, the liquid and the vapor are essentially the same. It follows that diketene in all its states exists predominantly or entirely in a single form, which other X-ray and nuclear magnetic resonance spectra studies have shown to be the 3-buteno-β-lactone structure. It appears that the five strong bands in the double bond stretching region, long a stumbling block to understanding the structure, are due to two fundamentals and three combination tones.

## Introduction

Some time ago Miller and Koch[2] studied the infrared spectrum of diketene vapor as a function of temperature between 30 and 180°. They found reversible changes which were attributed to the existence of two forms of diketene in equilibrium. This conclusion helped explain the presence of five strong bands in the region 1650–1950 cm.$^{-1}$, and was attractive for understanding the welter of other conflicting evidence concerning the structure of diketene.

Since then an X-ray diffraction study by Katz and Lipscomb[3] has shown that crystalline diketene has the 3-buteno-β-lactone structure



3-buteno-β-lactone

This was confirmed by a nuclear magnetic resonance spectra study of crystalline diketene by Ford and

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(1) (a) From a thesis submitted by G. L. Carlson in partial fulfillment of the requirements for the degree of Master of Science at the University of Pittsburgh. (b) This work was supported in part by a grant from the National Science Foundation.

(2) F. A. Miller and S. D. Koch, Jr., THIS JOURNAL, **70**, 1890 (1948).

(3) L. Katz and W. N. Lipscomb, *Acta Cryst.*, **5**, 313 (1952).