Absorption and emission spectral studies of two vinylogous series of cyanine dyes
by Nancy Jane Land Roth

A thesis submitted to. the Graduate Faculty in partial fulfillment of the requirements for the degree
DOCTOR OF PHILOSOPHY in Chemistry
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The absorption and emission spectra in methanol of the dyes of the 3,3'-diethylthiacyanine iodide series and of the 3.3.'-diethyloxacyanine iodide series (except for the first member which was the triiodide) were determined. The integrated intensities and oscillator strengths were calculated. In each series these quantities increase as the methylene chain length increases. From the absorption and emission spectra, the radiative lifetimes were calculated by the formula derived by Strickler and Berg as modified by Birks and Dyson: The lifetimes were of the order of three nanoseconds for the thiacyanine series and of the order of two nanoseconds for the oxacyanine series. The fluorescence efficiencies were determined.. They increase by a factor of ten as the chain length, increases except for the. first member of the sulfur series which is lower by a factor of 100 from the next member. The observable lifetimes were calculate from the radiative lifetimes and the fluorescence efficiencies. Since the rate of all decay processes is the inverse of the observable lifetime, a minimum may. be placed on the rate of energy transfer from the first excited state for these dyes when energy transfer processes take place.

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ABSORPTION AND EMISSION SPECTRAL STUDIES OF TWO VINYLOGOUS SERIES OF CYANINE DYES

by

NANCY JANE LAND ROTH

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

of

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ABSTRACT

The absorption and emission spectra in methanol of the dyes of the 3,3'-diethylthiacyanine iodide series and of the 3,3'-diethyloxacyanine iodide series (except for the first member which was the triiodide) were determined. The integrated intensities and oscillator strengths were calculated. In each series these quantities increase as the methylene chain length increases. From the absorption and emission spectra, the radiative lifetimes were calculated by the formula derived by Strickler and Berg as modified by Birks and Dyson:

$$\frac{1}{\tau_o} = 2.880 \times 10^{-9} \left(\frac{n_{uo \to l}}{n_{lo \to u}}\right)^3 \left\langle \bar{\nu}_f^{-3} \right\rangle_{av}^{-1} \frac{g_l}{g_u} \int \epsilon(\bar{\nu}_{lo \to u}) \, d \ln \bar{\nu}_{lo \to u}$$

The lifetimes were of the order of three nanoseconds for the thiacyanine series and of the order of two nanoseconds for the oxacyanine series. The fluorescence efficiencies were determined. They increase by a factor of ten as the chain length increases except for the first member of the sulfur series which is lower by a factor of 100 from the next member. The observable lifetimes were calculated from the radiative lifetimes and the fluorescence efficiencies. Since the rate of all decay processes is the inverse of the observable lifetime, a minimum may be placed on the rate of energy transfer from the first excited state for these dyes when energy transfer processes take place.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

A colorful, bright purple dye formed in small yield when A. W. Hofmann[1] in 1887 reacted 3-pentylbenzothiazolium iodide and 2-methyl-3-pentylbenzothiazolium iodide in the presence of ammonia. Like other dyes of the cyanine class, this compound was not stable enough for dyeing textiles. Previously in 1873, H. W. Vogel[2] had found that photographic emulsions could be made sensitive to colors other than blue by bathing the photographic plates in a dilute solution of dyes such as the cyanines. Since the cyanine dyes including that one first prepared by Hofmann were found to be useful for sensitizing photographic films, members of this family of dyes were synthesized and characterized.

Hofmann's dye was found by W. H. Mills[3] to have the structure shown in Figure 1 where Y = S and n = 1. The common name for this dye is 3,3'-diethylthiacarbocyanine iodide (Dye II) while the dye with Y = S and n = 0 is 3,3'-diethylthiacyanine iodide (Dye I) and the dye with Y = S and n = 2 is 3,3'-diethylthiadicarbocyanine iodide (Dye III).

There is a similar vinylogous series of dyes where Y = O. The first member where n = 0 is 3,3'-diethyloxa-

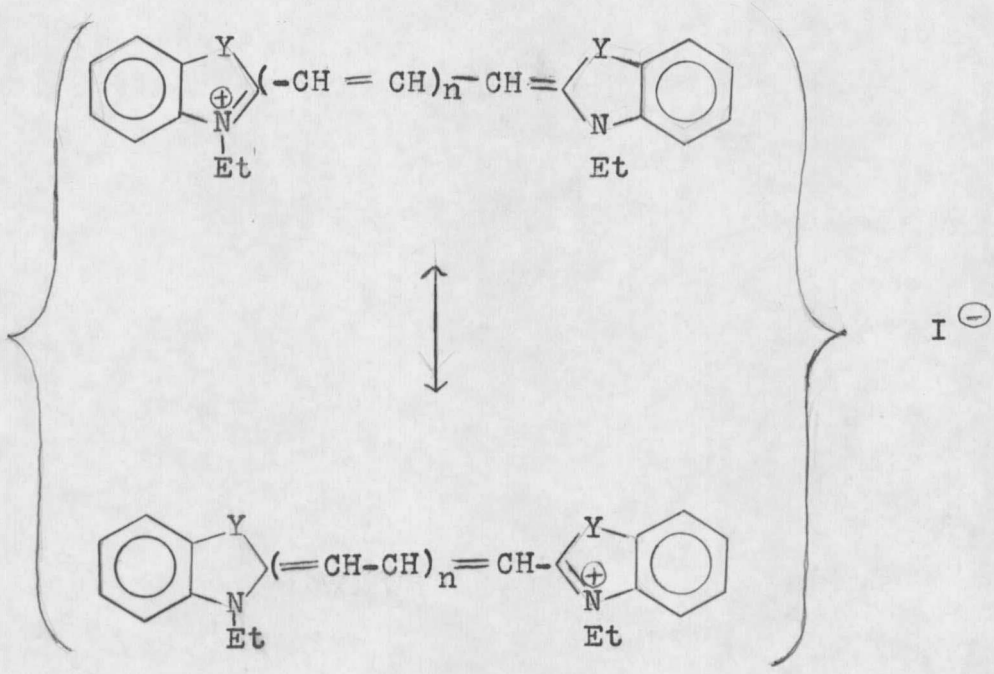


Figure 1

cyanine iodide (Dye IV when iodide is replaced by triiodide ion); the second where n = 1 is 3,3'-diethyloxacarbocyanine iodide (Dye V); and the third where n = 2 is 3,3'-diethyloxadicarbocyanine iodide (Dye VI).

A cyanine, such as that shown in Figure 1, is a mono-acid salt in which the nitrogen atoms of two heterocyclic rings are linked by a chain of alternating double and single bonds. Since this chain consists of an odd number of carbon atoms, the two structures

are resonance hybrids. Thus each nitrogen atom is probably best represented as something between tertiary and quaternary, and each bond as something between single and double.

A molecule with a conjugated system of this type might be expected to have a relatively small energy difference between the ground state and the first electronic excited state. This has been found to be true. Fisher and Hamer[4] reported wavelengths for the absorption maxima of the six dyes: Dye I, 422 mμ; Dye II, 557 mμ; Dye III, 650 mμ; Dye IV, 372 mμ; Dye V, 485 mμ; Dye VI, 580 mμ. They also gave the qualitative visual colors of the fluorescence for these dyes. Hofer, et al.[5] in 1950 studied the monomeric fluorescence of some of these dyes at room temperature in glycerol. The positions of the fluorescent maxima were determined visually from photographic plates. The maxima quoted were the following: Dye I, no observable fluorescence; Dye II, 586 mμ; Dye III, 691 mμ; Dye V, 512 mμ. Some work was done in a rigid medium of E.P.A. (ethanol-ether-isopentane 2 : 5 : 5) at 77° K by Mazzucato, et al.[6] in 1964. Their results were 575 mμ for the fluorescence of Dye II under these conditions and 498 mμ for Dye V.

To measure the absorption spectrum of a molecule in solution, the sample is contained in a cell of path length l (in cm.), at a concentration C (in g. moles per liter), and the ratio of the transmitted light intensity I to the incident light intensity I_o is determined. This ratio is given also by

$$\log \frac{I}{I_o} = \epsilon C l$$

where ϵ is called the extinction coefficient (units of liter moles^{-1} cm.$^{-1}$). The maximum value of ϵ (ϵ max) for an absorption band is a rough measure of the intensity of the band. However, absorption bands for most molecules are broad and different in shape. Thus a better measure of intensity is the area under the absorption curve, that is, the integrated intensity. Since the oscillator strength f is proportional to the integrated intensity, the oscillator strength is generally calculated to compare the intensities of absorption bands.[7]

The intensity of absorption for a transition from a state of energy E_n to a state E_m (where $E_m > E_n$) may be defined as the energy absorbed from the incident beam of 1 cm.^{2} cross-section per unit time. If $\bar{\nu}$ is

the wave number in cm.$^{-1}$ of the incident radiation and $\rho(\bar{\nu})$ is its radiation density, then

$$I_{abs.} = \rho(\bar{\nu})\, N_n\, B_{nm}\, h\, c\, \bar{\nu}\, dl \qquad (1)$$

where N_n is the number of molecules per cm.^{3} in the initial state, B_{nm} is Einstein's coefficient of absorption, which is a characteristic of the molecule, and dl is the thickness of the layer in cm. The quantity h is Planck's constant while c is the speed of light.

The intensity of the incident light, I, which is the energy falling on 1 cm.^{2} per second, can be given by

$$I = c \int \rho(\bar{\nu})\, d\bar{\nu} . \qquad (2)$$

If equation (1) is multiplied on both sides by $d\bar{\nu}$, and equation (2) is substituted into equation (1) then the following equation is obtained:

$$I_{abs.}\, d\bar{\nu} = I\, N_n\, B_{nm}\, h\, \bar{\nu}\, dl. \qquad (3)$$

When I passes through an absorbing layer of thickness dl, the part of I absorbed will be proportional to both I and dl such that

$$I_{abs.} = -\alpha I\, dl \qquad (4)$$

where α is the absorption coefficient. The absorption coefficient is related to the molar extinction coefficient in the following way:

$$\ln \frac{I_o}{I} = \alpha l = \log \frac{I_o}{I} \left(\ln 10 \right) \left(\frac{1}{22.41} \right) \epsilon l$$

so that $$\alpha = 0.1028 \epsilon \, . \qquad (5)$$

Substitution of the value of ϵ into equation (4) gives

$$I_{abs.} = -0.1028 \epsilon \, I \, d l \, . \qquad (6)$$

If equation (6) is multiplied on both sides by $d \bar{\nu}$ and subtracted from equation (3), the following will result

$$0.1028 \epsilon \, (\bar{\nu}) \, d\bar{\nu} = N_n \, B_{nm} \, h \bar{\nu} \, . \qquad (7)$$

Since the absorption band of a molecule is broad, the strength of a molecular electronic transition can be defined by the integral of the intensity over the whole band of the particular electronic transition. Then equation (7) can be written as

$$0.1028 \int \epsilon \, (\bar{\nu}) \, d\bar{\nu} = N_n \, B_{nm} \, h \bar{\nu} \qquad (8)$$

for molecular absorptions.

If an electron were considered to be a three-dimensional harmonic oscillator then the intensity of absorption would be

$$I_{abs.} = f_{nm} N_n \pi \frac{e^2}{m_e c} \rho(\bar{\nu}) \, d\,l \qquad (9)$$

where f_{nm}, the oscillator strength, is equal to one. In this equation m_e is the mass of the electron and e is the charge of an electron.

Then equation (9) can be equated to equation (1) to give

$$f_{nm} = \frac{h c^2 m_e}{\pi e^2} \bar{\nu} B_{nm} . \qquad (10)$$

When equation (10) is substituted into equation (8), the oscillator strength is given as

$$f_{nm} = \frac{0.1028 \, c^2 m_e}{N_n \pi e^2} \int \epsilon(\bar{\nu}) \, d\bar{\nu}$$

$$4.319 \times 10^{-9} \int \epsilon(\bar{\nu}) \, d\bar{\nu} . \qquad (11)$$

Thus the oscillator strength is proportional to the integrated absorption intensity.[8]

The mean lifetime for spontaneous emission from an excited state of a molecule is the inverse of A, the Einstein transition probability coefficient for spontaneous emission. The spontaneous emission probability was shown by Einstein to be directly proportional to the corresponding absorption probability and to the third power of the frequency of the transition.

If the molecule has two electronic states, a ground state l and an excited state u, the corresponding electronic wave functions may be called Θ_l and Θ_u. A series of vibrational states are associated with each electronic level. In the Born-Oppenheimer approximation, each vibronic state has a wave function ψ which can be written as a product of an electronic function Θ and a vibrational function ϕ, such that $\psi_{la} = \Theta_l \phi_{la}$ and $\psi_{ub} = \Theta_u \phi_{ub}$ where a and b are the quantum numbers describing the state of nuclear motion.

If a large number of these molecules, immersed in a nonabsorbing medium of refractive index n, are in thermal equilibrium within a cavity in some material at a temperature T, the radiation density within the medium is given by Planck's blackbody radiation law

$$\rho(\bar{\nu}) = (8\pi h \nu^3 n^3/c^3) \left[\exp(h\nu/kT)-1\right]^{-1} \quad (12)$$

where ν is the frequency in cycles/sec.

These molecules can be excited from state la to state ub by transitions induced by the radiation in the cavity. The rate of molecules undergoing this transition by absorption of radiation is

$$N_{la} B_{la \to ub} \rho(\nu_{la \to ub}) \qquad (13)$$

where N_{la} is the number of molecules in state la. Molecules in state ub can go to state la by spontaneous emission with probability $A_{ub \to la}$ or by induced emission with probability $B_{ub \to la} \rho(\nu_{ub \to la})$. The rate at which molecules undergo this transition downward is

$$N_{ub} \left[A_{ub \to la} + B_{ub \to la} \rho(\nu_{ub \to la}) \right] \qquad (14)$$

where $B_{ub \to la} = B_{la \to ub}$ and $\nu_{ub \to la} = \nu_{la \to ub}$. At equilibrium the two opposing rates must be equal, so if expressions (13) and (14) are equated, the resultant equation is

$$A_{ub \to la} / B_{ub \to la} = \left[(N_{la}/N_{ub}) - 1 \right] \rho(\nu_{ub \to la}). \qquad (15)$$

According to the Boltzmann distribution law, the number of molecules in each of the two states at equilibrium is related by

$$N_{ub}/N_{1a} = \exp\left[-(h\,\nu_{ub\rightarrow 1a}/k\,T)\right] . \qquad (16)$$

If equations (12) and (16) are substituted into equation (15), the resultant is Einstein's relation

$$A_{ub\rightarrow 1a} = 8\pi h\,\nu_{ub\rightarrow 1a}^{3}\,n_{ub\rightarrow 1a}^{3}\,c^{-3}\,B_{ub\rightarrow 1a}. \qquad (17)$$

In the usual absorption experiment the molar extinction coefficient ϵ can also be defined by

$$\rho(\nu,1)/\rho(\nu,0) = 10^{-\epsilon(\nu)Cl} = e^{-2.303\,\epsilon(\nu)Cl} \qquad (18)$$

if $\rho(\nu,1)$ is the radiation density in the light beam after it has passed a distance $\underline{l}$ through the sample and $\rho(\nu,0)$ is the initial radiation density. If a short distance $\underline{dl}$ is considered, the change in radiation density may be written as

$$-d\rho(\nu) = 2.303\,\epsilon(\nu)\,\rho(\nu,0)\,C\,dl \qquad (19)$$

All the molecules will be assumed to be in the ground vibronic state, ψ_{1o}, for ease of derivation since the result will not be materially different than if the true distribution of molecules in the vibronic states were used. When $\underline{N_{1o}}$ is the number of molecules in the lowest vibrational level per cm.^{2} cross-section and $\mathcal{N}$ is Avogadro's number, the following equality

can be made:

$$c\, d\, l = 1000\, N_{lo}\, \eta^{-1}. \qquad (20)$$

Furthermore $\Delta N(\nu)$, the number of molecules excited per second with energy $h\nu$, is given by

$$\Delta N(\nu) = -c\, d\, \rho(\nu)/(h\, \nu\, n). \qquad (21)$$

The combination of equations (19), (20) and (21) gives

$$\Delta N(\nu)/N_{lo} = \left[2303\, c\, \epsilon(\nu)/h\, \nu\, n\, \eta\right] \rho(\nu,0). \qquad (22)$$

Equation (22) gives the probability that a molecule in state lo will absorb a quantum of energy $h\nu$ and go to some excited state. To obtain the probability of going to state ub, equation (22) must be integrated over the finite range of frequencies for this occurrence. Then $\rho(\nu,0)$ can be assumed constant over this range and equal to $\rho(\nu_{lo\to ub})$. Thus the integrated equation is

$$\frac{\Delta N_{lo\to ub}}{N_{lo}} = \left[\frac{2303\, c}{h\, \eta\, n_{lo\to ub}} \int \frac{\epsilon(\nu_{lo\to ub})\, d\nu_{lo\to ub}}{\nu_{lo\to ub}}\right] \rho(\nu_{lo\to ub})$$

$$= \left[\frac{2303\ c}{h\ \eta\ n_{lo \to ub}} \int \epsilon(\nu_{lo \to ub})\ d \ln \nu_{lo \to ub} \right] \rho(\nu_{lo \to ub}) \qquad (23)$$

This equation shows for a molecule in state lo that the probability of undergoing a transition to state ub is proportional to $\rho(\nu_{lo \to ub})$, the constant of proportionality being the term in brackets. Expression (13) gave a similar relation for a molecule in state lo with the proportionality constant being given as $B_{lo \to ub}$. Therefore, identifying the constant $B_{lo \to ub}$ with the bracketed expression of equation (23) gives

$$B_{lo \to ub} = \frac{2303\ c}{h\ \eta\ n_{lo \to ub}} \int \epsilon(\nu_{lo \to ub})\ d \ln \nu_{lo \to ub}. \qquad (24)$$

Still assuming that all the molecules originate in the state lo, it is possible to sum expression (24) over all the vibrational levels of the excited electronic state to obtain a probability coefficient for all transitions to the electronic state u. This is given by

$$B_{lo \to u} = \sum_{b} B_{lo \to ub}$$

$$= \frac{2303\ c}{h\mathcal{N} n_{lo\rightarrow u}} \int \epsilon(\nu_{lo\rightarrow u})\ d \ln \nu_{lo\rightarrow u} \qquad (25)$$

where $n_{lo\rightarrow u}$ is the mean refractive index of the non-absorbing medium over the absorption band which is an approximation.

When a molecule goes from a lower electronic state to an upper one by absorption, it will often be to an excited vibrational level of the upper electronic state. However, if the molecule is in a condensed medium, it will usually lose energy by collision until it is in the lowest vibrational level of the state. In fluorescing, the molecule may then go to various vibrational levels of the ground electronic state. Thus in absorption the transitions observed are the $lo \rightarrow \sum_b ub$, while in fluorescence they are the $uo \rightarrow \sum_a la$. It is necessary, therefore, to find a relationship between $B_{lo\rightarrow u} = \sum_b B_{lo\rightarrow ub}$ and $A_{uo\rightarrow l} = \sum_a A_{uo\rightarrow la}$.

The wave functions of vibronic states, ψ, are functions of both the electronic coordinates $\underline{x}$ and the nuclear coordinates, which may be taken as the normal coordinates $\underline{Q}$. If $\underline{M(x)}$ is the electric dipole operator for the electrons, then the probability for induced absorption or emission between two states is

proportional to the square of the matrix element of $\underline{M(x)}$ between the two states; that is

$$B_{1a \to ub} = B_{ub \to 1a}$$

$$= K \left| \iint \psi_{1a}^{*}(x,Q)\, M(x)\, \psi_{ub}(x,Q)\, dx\, dQ \right|^2 \qquad (26)$$

where $\underline{K}$ is the proportionality constant.

The wave functions can be written as products of the electronic and vibrational parts, for example,

$$\psi_{1a}(x,Q) = \Theta_{1}(x,Q)\ \phi_{1a}(Q). \qquad (27)$$

So the integral in equation (26) may be expressed as

$$\iint \psi_{1a}^{*}(x,Q)\, M(x)\ \psi_{ub}(x,Q)\, dx\, dQ$$

$$= \int \phi_{1a}^{*}(Q)\, M_{1u}(Q)\, \phi_{ub}(Q)\, dQ \qquad (28)$$

where

$$M_{1u}(Q) = \int \Theta_{1}^{*}(x,Q)\, M(x)\, \Theta_{u}(x,Q)\, dx$$

is an electronic transition moment integral for the transition, assuming the nuclei to be fixed in a position $\underline{Q}$. It can be expanded in a power series in the normal coordinates of the molecule:

$$M_{lu}(Q) = M_{lu}(0) + \sum_r (\partial M_{lu}/\partial Q_r)_0 Q_r \quad \cdots . \qquad (29)$$

For strongly allowed transitions in a molecule, and for reasonably small displacements from the equilibrium nuclear configuration, the zeroth-order term in this expansion should be the dominant one. With the assumption that this is the case, equation (26) reduces to

$$B_{la \to ub} = B_{ub \to la}$$
$$= K \left| M_{lu}(0) \right|^2 \left| \int \phi_{la}^* \phi_{ub} \, dQ \right|^2 \qquad (30)$$

The quantity necessary for the calculation of the lifetime is $A_{uo \to l}$, the rate constant for emission from the lowest vibrational level of the excited electronic state to all the vibrational levels of the ground electronic state. From equations (17) and (30) this can be written as

$$A_{uo \to l} = \sum_a A_{uo \to la}$$
$$= (8\pi h n_{uo \to l}^3/c^3) K \left| M_{lu}(0) \right|^2 \sum_a \nu_{uo \to la}^3$$
$$\cdot \left| \int \phi_{la}^* \phi_{ub} \, dQ \right|^2 \qquad (31)$$

where $n_{uo \to l}$ is the mean refractive index of the non-absorbing medium over the fluorescence band, an approximation.

It is desirable to be able to evaluate the term $\sum_a \nu_{uo \to la}^3 \left| \int \phi_{la}^* \, \phi_{ub} \, dQ \right|^2$ experimentally. Since the fluorescence band is not narrow, a possible procedure can be derived by dividing the term by $\sum_a \left| \int \phi_{la}^* \, \phi_{uo} \, dQ \right|^2 = 1$. This sum is equal to unity since the ϕ_{la} comprise a complete orthonormal set in Q space. This gives the following expression:

$$\frac{\sum_a \nu_{uo \to la}^3 \left| \int \phi_{la}^* \, \phi_{uo} \, dQ \right|^2}{\sum_a \left| \int \phi_{la}^* \, \phi_{uo} \, dQ \right|^2}$$

Each term in the numerator of this expression is proportional to the intensity of one vibronic band in the fluorescence spectrum. Each term in the denominator is proportional to ν^{-3} times the intensity of one vibronic band. The sums over all the vibronic bands can be replaced by integrals over the fluorescence spectrum, so that the expression reduces to

$$\frac{\int I(\nu) \, d\nu}{\int \nu^{-3} \, I(\nu) \, d\nu} = \left\langle \nu_f^{-3} \right\rangle_{av}^{-1}, \qquad (33)$$

the reciprocal of the mean value of ν^{-3} in the fluorescence spectrum, where $I(\nu)$, the intensity in the spectrum, is measured in terms of relative numbers of quanta at each frequency.

From equation (30) when $B_{lo\to ub}$ is summed over b, the result is

$$B_{lo\to u} = \sum_b B_{lo\to ub}$$

$$= K \left| M_{lu}(0) \right|^2 \sum_b \left| \int \phi_{la}^{*} \, \phi_{ub} \, dQ \right|^2 ,$$

$$B_{lo\to u} = K \left| M_{lu}(0) \right|^2 , \qquad (34)$$

since the ϕ_{ub} comprise a complete orthonormal set in Q space. Thus from equations (34) and (25), the expressions for $B_{lo\to u}$ can be equated:

$$K \left| M_{lu}(0) \right|^2$$

$$= \frac{2303 \, c}{h \eta \, n_{lo\to u}} \int \epsilon(\nu_{lo\to u}) \, d \ln \nu_{lo\to u} . \qquad (35)$$

If equations (33) and (35) are substituted into equation (31), the result is

$$A_{uo\to l} = \frac{8 \times 2303\, \pi\, n_{uo\to l}^{3}}{c^{2}\, \eta\, n_{lo\to u}} \left\langle \nu_{f}^{-3} \right\rangle_{av}^{-1} \cdot \int \epsilon\,(\nu_{lo\to u})\, d \ln \nu_{lo\to u} . \qquad (36)$$

If either or both of the electronic states are degenerate, a factor of g_l/g_u is multiplied times the right-hand side of equation (36) to sum over these states.

It is more convenient to use this equation in terms of $\bar{\nu}$ instead of ν. The result of this conversion is

$$\frac{1}{\tau_o} = A_{uo\to l} = \frac{8 \times 2303\, \pi\, c\, n_{uo\to l}^{3}}{\eta\, n_{lo\to u}} \left\langle \bar{\nu}_{f}^{-3} \right\rangle_{av}^{-1} \cdot \frac{g_l}{g_u} \int \epsilon\,(\bar{\nu}_{lo\to u})\, d \ln \bar{\nu}_{lo\to u},$$

$$= 2.880 \times 10^{-9} \frac{n_{uo\to l}^{3}}{n_{lo\to u}} \left\langle \bar{\nu}_{f}^{-3} \right\rangle_{av}^{-1} \frac{g_l}{g_u} \cdot \int \epsilon\,(\bar{\nu}_{lo\to u})\, d \ln \bar{\nu}_{lo\to u}. \qquad (37)$$

This equation is applicable to strongly allowed transitions and to only reasonably small displacements of the atoms from the equilibrium nuclear configuration in both electronic states.[9]

Another quantity of interest which may be determined from the emission spectrum is the quantum efficiency of the fluorescence. To determine fluorescence efficiency directly it is necessary to compare the rate of absorption of exciting light with the total rate of emission of fluorescence of all wavelengths and in all directions. In principle this is simple but in practice it is a difficult experiment to perform with precision. An easier method by which fluorescence efficiency may be determined is comparison between a known "standard" substance and the material of interest.[10]

If a solution of the compound of interest is dilute enough that errors due to excessive absorption of exciting light or to self-absorption of the fluorescence are negligible, the total rate of emission of fluorescence is proportional to the product $I_o \varepsilon C l \phi_f$ where ϕ_f is the quantum efficiency. Now the integrated area under the corrected fluorescence spectrum is also proportional to the total rate of emission of fluorescence. Thus, if the fluorescence emission spectra of two solutions are measured with the same instrumental geometry and at the same intensity of exciting light, the ratio of the two observed fluorescence intensities is given by

$$\frac{Q_2}{Q_1} = \frac{\text{area}_2}{\text{area}_1} = \frac{I_o \epsilon_2 c_2 l \phi_2}{I_o \epsilon_1 c_1 l \phi_1} \quad . \qquad (38)$$

If the absolute fluorescence efficiency (ϕ_1) of one of the substances is known, that of the other is simply calculated.[11]

In practice most of the above conditions are not met so corrections must be made for them.

A change in refractive index (n) of the solution results in a variation in the angles of the rays emerging from a plane-cuvette-air interface. Thus if the two substances to be compared are dissolved in different solvents, the observed intensities must be corrected by multiplying by n^2.[12]

If the two substances are excited at two different wavelengths, then the intensity of the exciting light is probably different. To correct for this, each area can be divided by its peak height. Then the ratio of these two numbers can be multiplied by a ratio of the fluorescence peak heights when both substances are excited by light of the same wavelength, usually somewhere between the two original exciting wavelengths. The equation is [11]

$$\frac{\left(\frac{\text{area}_2}{\text{pk. ht.}_2}\right)_{\lambda_2} \cdot (\text{pk. ht.}_2)_{\lambda_3}}{\left(\frac{\text{area}_1}{\text{pk. ht.}_1}\right)_{\lambda_1} \cdot (\text{pk. ht.}_1)_{\lambda_3}} = \frac{I_o \epsilon_2 c_2 l \phi_2}{I_o \epsilon_1 c_1 l \phi_1} \quad (39)$$

The fluorescence spectra of the two substances, in general, will not be over the same wavelength region. So the areas must be corrected for these three wavelength dependent factors: the quantum efficiency of the photomultiplier, the band width of the monochromator and the transmission factor of the monochromator. If R_{SL} is the photomultiplier response, then

$$R_{SL} = (d Q/d \lambda)_{SL} (S_\lambda) \quad (40)$$

where $(d Q/d \lambda)_{SL}$ is the true intensity at wavelength λ and S_λ is the correction for the three wavelength dependent factors.[13]

If a series of compounds are available for which the corrected fluorescence emission spectrum has been precisely determined, measurement of the uncorrected spectrum of these compounds with the instrument to be calibrated permits the direct calculation of S_λ since $(d Q/d \lambda)_{SL}$ now represents the known spectral distribution of one of the standard compounds and R_{SL} the observed readings. The compound must be free from other fluorescent

material and the fluorescence must be measured under the same conditions of temperature, solvent, concentration, pH, etc. for which the standard fluorescence curve is provided. Then this calculated S_λ can be used to correct the intensities of the two substances used for the quantum efficiency determination.[14]

RESULTS AND DISCUSSION

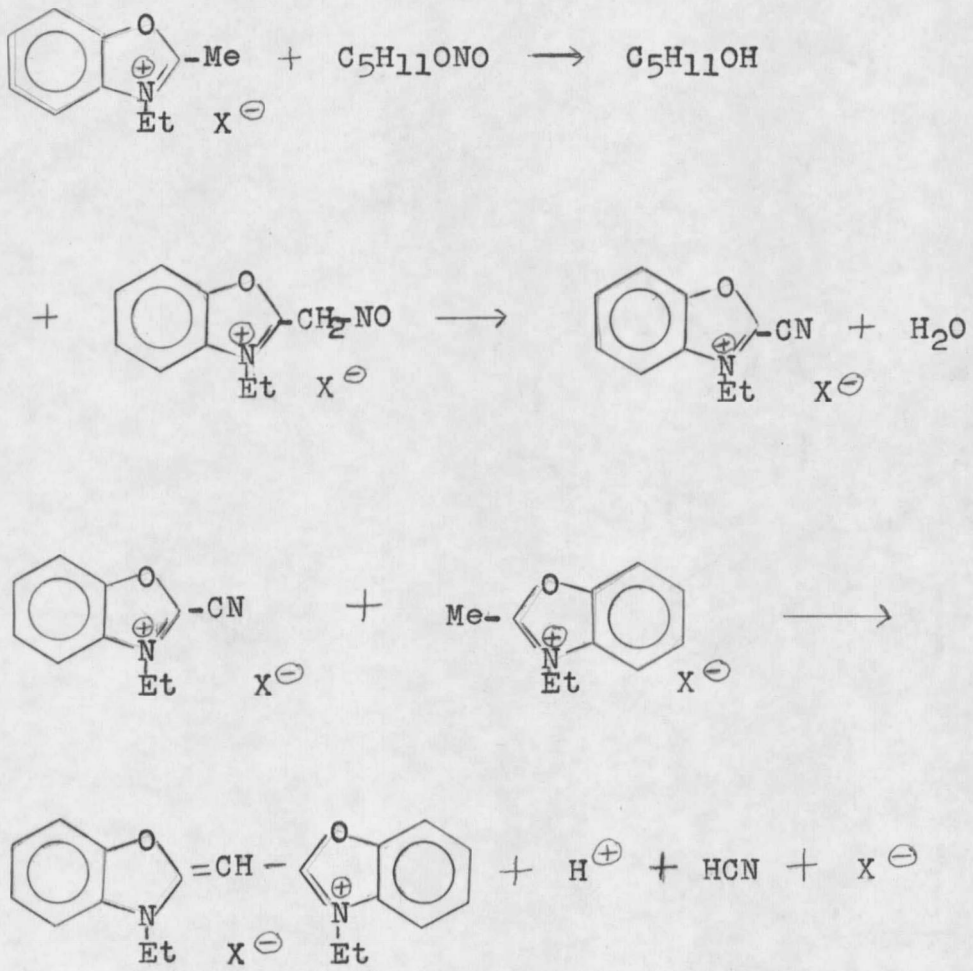
The cyanine dyes I through VI were selected for a study of their absorption and fluorescence spectra, the intrinsic lifetime of their first excited singlet state, the fluorescence efficiency from that state, and a calculation of the observable lifetime of the first excited singlet state. These particular dyes were chosen because they were the most readily available vinylogous series of dyes which had the unusual property that the bond order is the same between any two carbons in the methylene chain (See p. 2 for a more complete discussion of this property.). These dyes are also of interest because they can transfer energy from the first excited singlet state. One example of this ability is their use in sensitizing photographic films. The rate of the energy transfer must be at the least as great as the rate of emission from the first excited state in order to be competitive with the fluorescence. If the rate of this emission could be determined either by measurement or calculation, then this would be a lower limit on the rate of energy transfer. Even though there have been some reports of the absorption maxima and some rough estimates of the fluorescence maxima (See

p. 3 for details and references.), these data could not be used for further calculations because the solvent must be the same for both the absorption and the fluorescence and the integrated areas under the spectra must be known. Also no observable fluorescence has ever been reported for Dye I. Thus the purpose of this research was to determine either by measurement or calculation, the oscillator strengths of the absorbances; the corrected integrated areas of the fluorescences; the intrinsic lifetimes, the quantum efficiencies, and the observable lifetimes (the inverse of the sum of the rates for all decay processes) for the first excited singlet states of these dyes.

Each of the dyes was prepared by the method outlined in detail in the experimental section. After the initial reaction between 3-ethyl-2-methylbenzoxazolium iodide with isopentyl nitrite, the dye formed is the triiodide salt. To prepare the iodide in order to have the same anion as the other dyes, the triiodide was reduced by bubbling sulfur dioxide through a suspension of the dye. Then the reduced dye was recrystallized from methanol. Upon the second recrystallization, the dye apparently decomposed into a foamy yellow substance. Since the iodide was not pure after only one recrystallization

and the triiodide was found to be pure by elemental analysis, the triiodide of Dye IV was used for the spectroscopic studies.

A possible mechanism for the formation of Dye IV, similar to that proposed by Fisher and Hamer[15] for the thiacyanine Dye I, can be written as follows:



It is questionable whether the final coupling step of this mechanism, that of 2-cyano-3-ethylbenzoxazolium iodide with 3-ethyl-2-methylbenzoxazolium iodide, would occur.

The triiodide anion is formed by the oxidation of two iodide ions to iodine which then reacts with an iodide ion to form the complex ion, triiodide. The oxidizing agent in the reaction is the isopentyl nitrite which is probably reduced to nitric oxide and isopentyl alcohol.

Originally a third vinylogous series of dyes wase to have been studied also. The dye 1,1',3,3'-tetraethylbenzimidazolocarbocyanine iodide could be formed in only a small yield and very poor purity even under drastic reaction conditions such as the reaction of 1,3-diethyl-2-methylbenzimidazolium iodide in nitrobenzene with 3 M sodium ethoxide as a basic catalyst. The product of this reaction after five recrystallizations from methanol had an extinction coefficient of 51 at the 486 mμ maximum. The extinction coefficient at the maximum absorption for allowed transitions such as those in these dyes are usually in the region of 10^4. Therefore, the study of this series of dyes was abandoned.

In Figure 2 may be found the absorption spectra of the lowest electronic transition for Dyes I, II and III, while Figure 3 gives the absorption spectra for Dyes IV, V and VI. These spectra were examined in dilute solutions of about 10^{-5} M in methanol and are plotted by ϵ, the extinction coefficient, versus the wavelength in $m\mu$. All the spectra have a similar shape with a shoulder at shorter wavelength. In each series the extinction coefficient at the maximum absorption increases as the chain length increases. As calculated by equation (11), the oscillator strength similarly increases. The integration for the oscillator strength was performed by the method of the sum of the areas of small rectangles. The error in the reported oscillator strengths is estimated to be about three per cent. The absorption results are summarized in Table I.

Figure 4 gives the emission spectra at room temperature for dilute methanol solutions of Dyes I, II and III, while Figure 5 gives the emission spectra for Dyes IV, V and VI. The concentrations of the dyes were about 10^{-6} M for Dyes III and VI and about 10^{-5} M for all the others. These spectra are corrected for the quantum efficiency of the photomultiplier as given by the curve in Figure 6 for a typical tube of this type

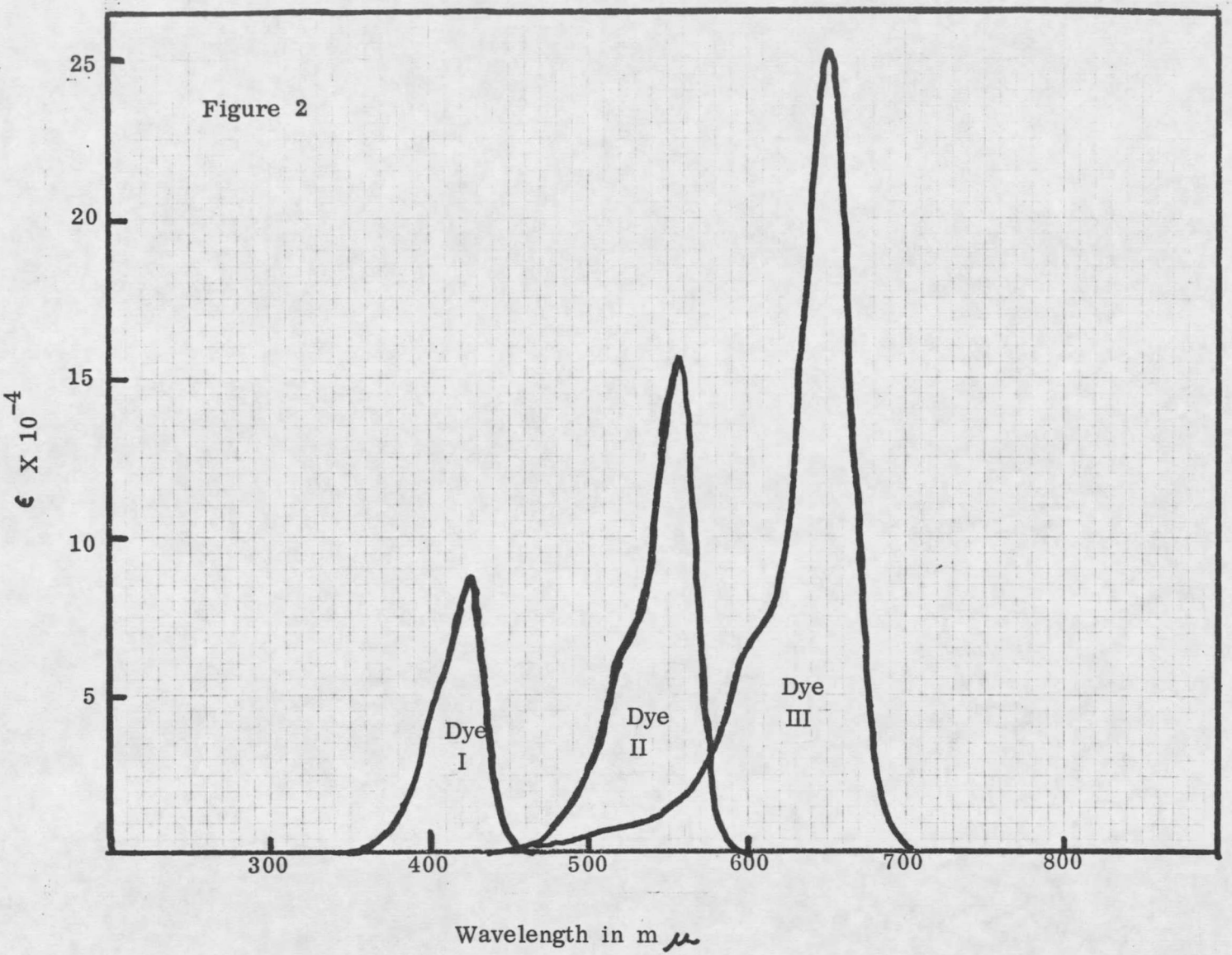


Figure 2
25
20
15
10
5
ϵ X 10^{-4}
Dye I
Dye II
Dye III
300
400
500
600
700
800
Wavelength in mμ

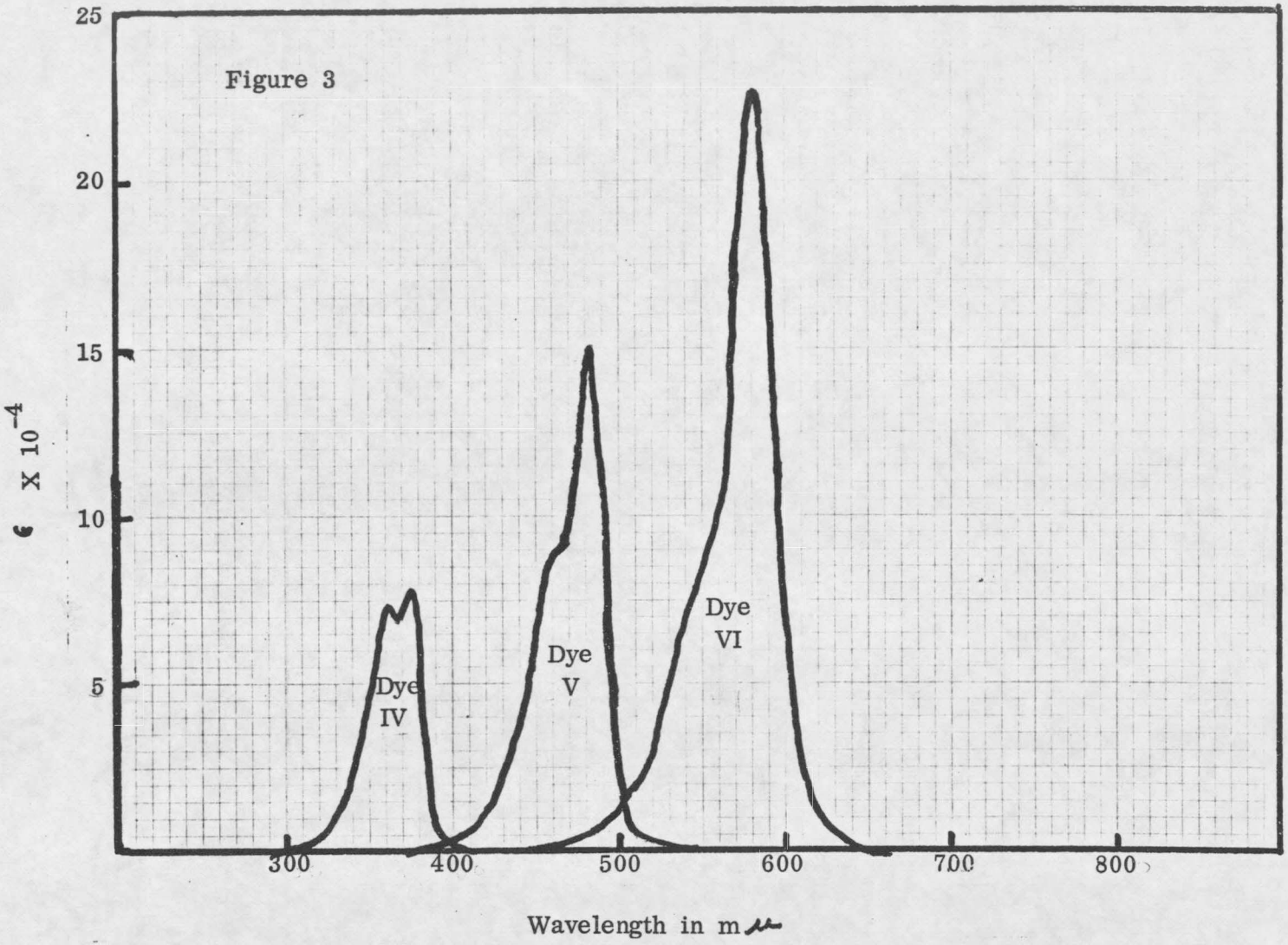


Figure 3
25
20
15
10
5
ε X 10⁻⁴
Dye IV
Dye V
Dye VI
300
400
500
600
700
800
Wavelength in mμ

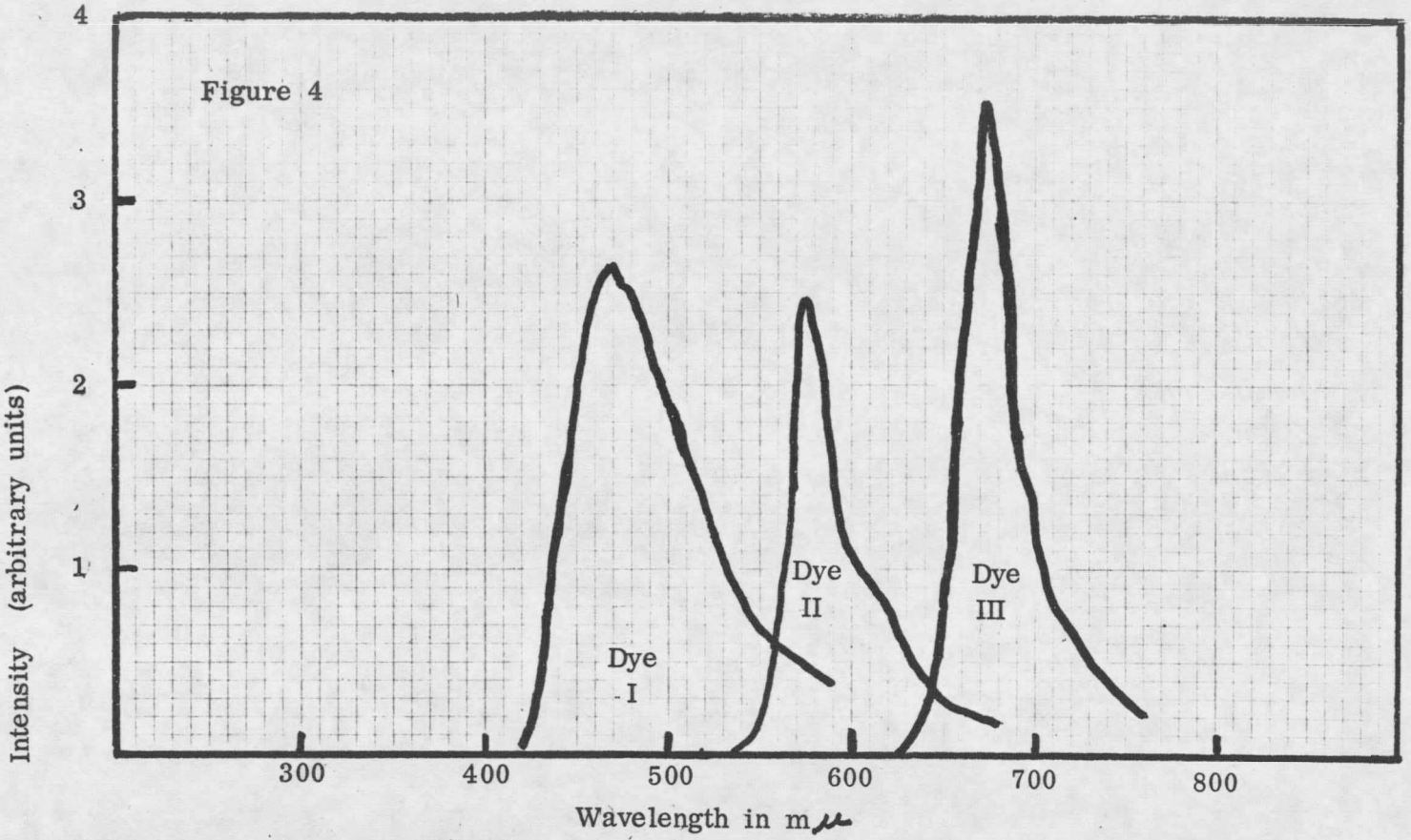


Figure 4
4
3
2
1
Intensity (arbitrary units)
Dye
-I
Dye
II
Dye
III
300
400
500
600
700
800
Wavelength in mμ

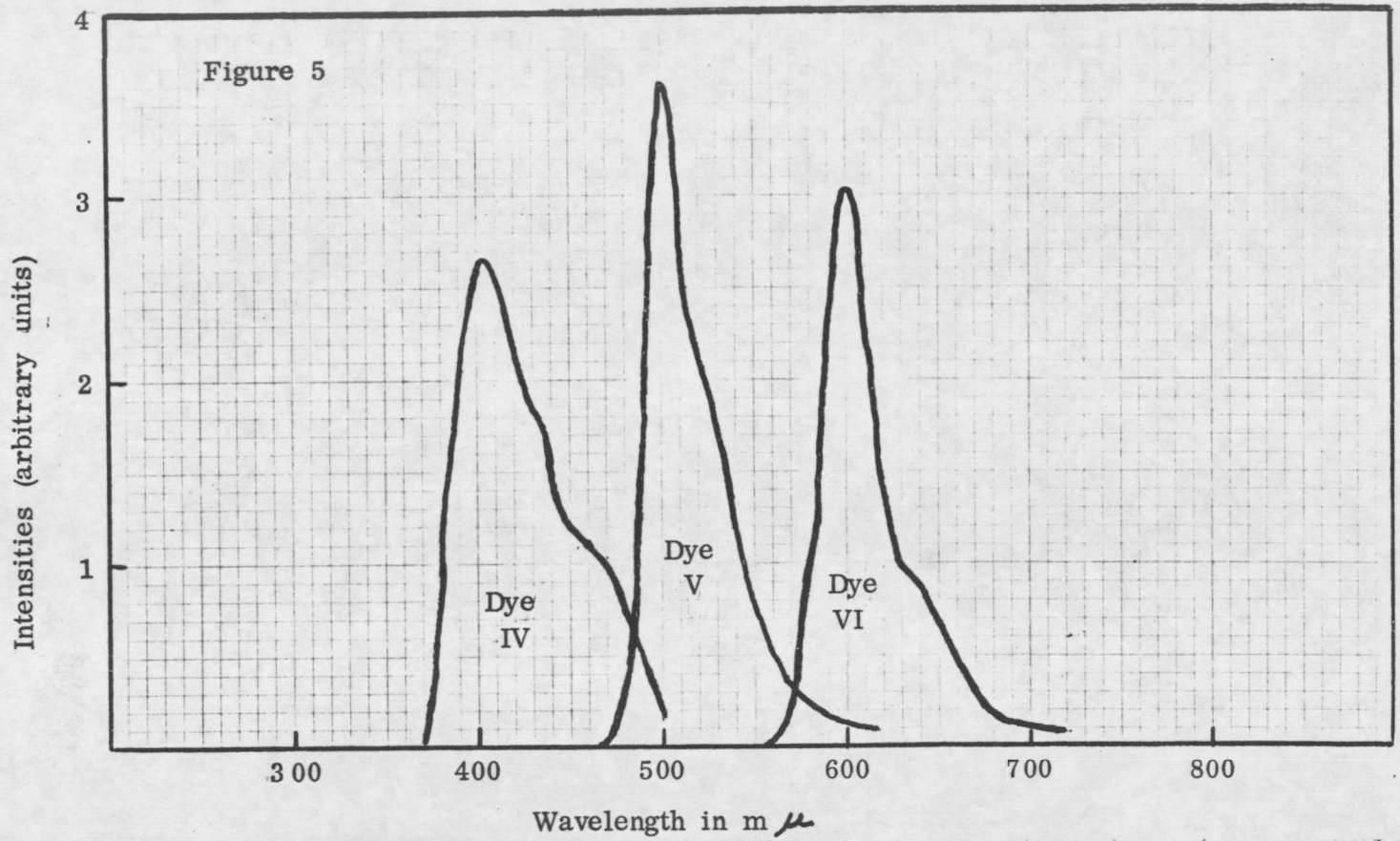


Figure 5
Intensities (arbitrary units)
4
3
2
1
Dye IV
Dye V
Dye VI
300
400
500
600
700
800
Wavelength in mμ

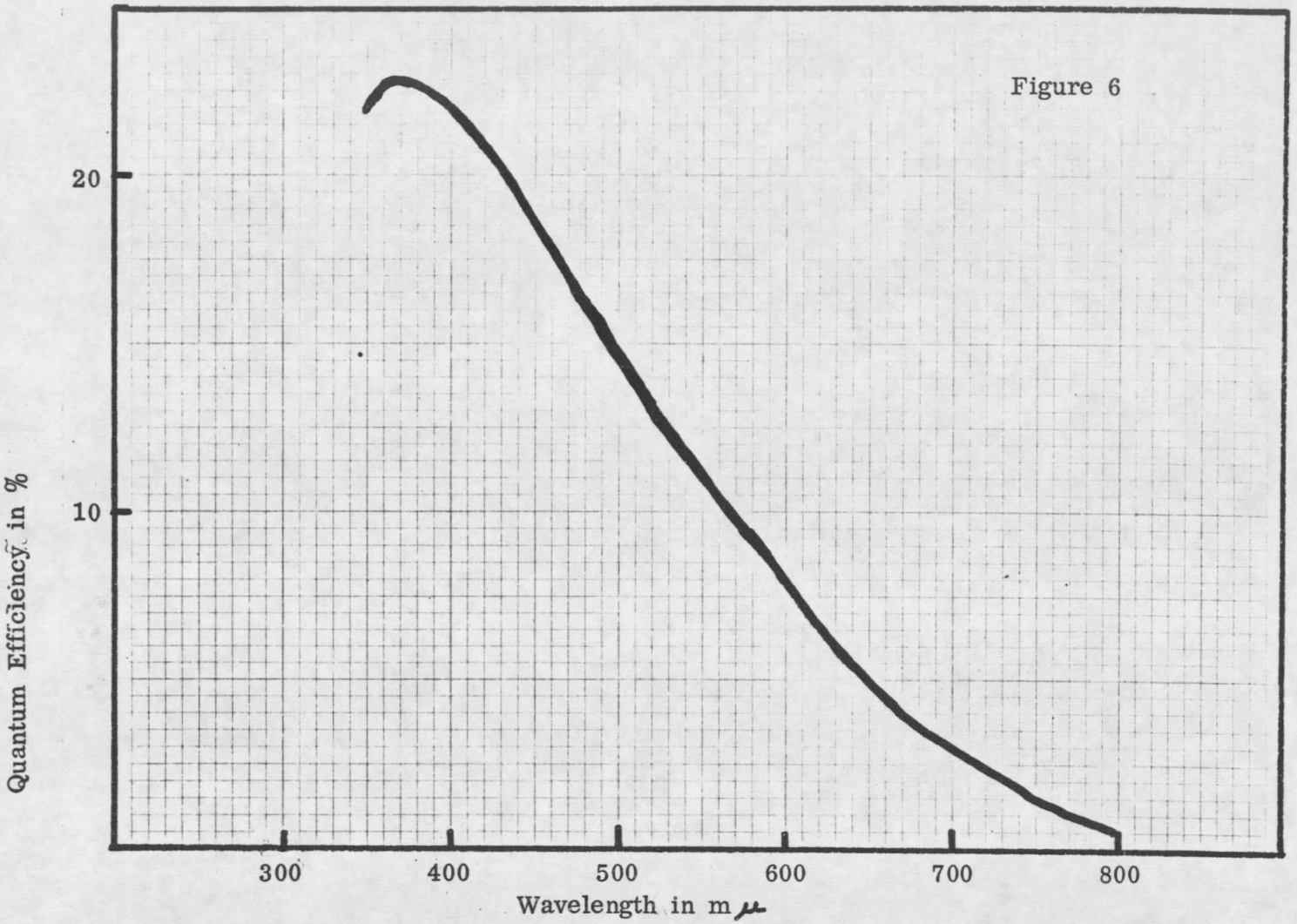

Quantum Efficiency in %
20
10
300
400
500
600
700
800
Wavelength in mμ

Figure 6

Table I

Dye	λmax mμ	ϵ max X 10^{-5}	$\int \epsilon \, d\bar{\nu}$ X 10^{-8}	f
I	424.0	0.868	1.90	0.822
II	556.0	1.56	2.52	1.09
III	651.5	2.53	3.51	1.52
IV	375.5	0.786	2.39	1.03
V	483.0	1.51	2.90	1.25
VI	579.0	2.27	3.42	1.48

where the quantum efficiency in per cent is plotted against the wavelength in mμ. The intensities of the spectra themselves are plotted in arbitrary units.

The lifetime for spontaneous emission, τ_o, is the same as the observed lifetime of the radiative state if there are no other competing processes for the loss of energy. If there are other methods of deactivation, then the observed lifetime is shorter than τ_o by the factor of the quantum yield. Since the lifetime is the reciprocal of the rate constant for the transition, in the case of no competing processes the observed lifetime may be written as

$$\tau_o = \frac{1}{k_e}$$

where k_e is the rate constant for spontaneous emission. When k_d is the rate constant for competing processes, then the observed lifetime τ may be given as

$$\tau = \frac{1}{k_e \quad k_d}$$

or

$$\tau = \frac{1}{k_e} \left(\frac{1}{1 \quad \frac{k_d}{k_e}} \right)$$

$$= \tau_o \left(\frac{1}{1 \quad \frac{k_d}{k_e}} \right)$$

$$= \tau_o \left(\frac{k_e}{k_e \quad k_d} \right) = \tau_o \, \phi$$

where ϕ is the quantum yield. Thus the radiative lifetime may be calculated from the observed lifetime and the quantum yield. However, these two quantities had not been determined for these dyes.

But the radiative lifetime may also be calculated from the absorption and emission spectra. So equation (37) was used to calculate the spontaneous emission

lifetimes of the first electronic excited state for these two series of dyes. Both states of these dyes were assumed to be non-degenerate.

From the Landolt-Börnstein tables[16], a curve such as Figure 7 may be plotted for the index of refraction of methanol versus the wavelength. The values of $n_{uo \rightarrow l}$ and $n_{lo \rightarrow u}$ were obtained from this graph.

The quantity $\langle \bar{\nu}_f^{-3} \rangle_{av}^{-1}$ was calculated by equation (33) where each of the integrals were approximated by the method of the sum of the areas of small rectangles from the emission spectrum and then the integral $\int I(\bar{\nu})\, d\bar{\nu}$ divided by the integral $\int \bar{\nu}^{-3} I(\bar{\nu})\, d\bar{\nu}$. The value of $\langle \bar{\nu}_f^{-3} \rangle_{av}^{-1}$ is probably accurate to about three per cent.

From the absorption spectrum the integral $\int \epsilon(\bar{\nu}_{lo \rightarrow u})\, d \ln \bar{\nu}_{lo \rightarrow u}$ was calculated by the method of the sum of the areas of small rectangles. Since the error in this integral is estimated to be about three per cent, the total error from experimental factors in the calculated radiative lifetime, τ_o, should be approximately six per cent.

The sulfur containing series of dyes, that is Dyes I, II and III, were calculated to have radiative lifetimes, τ_o, around three nanoseconds while the

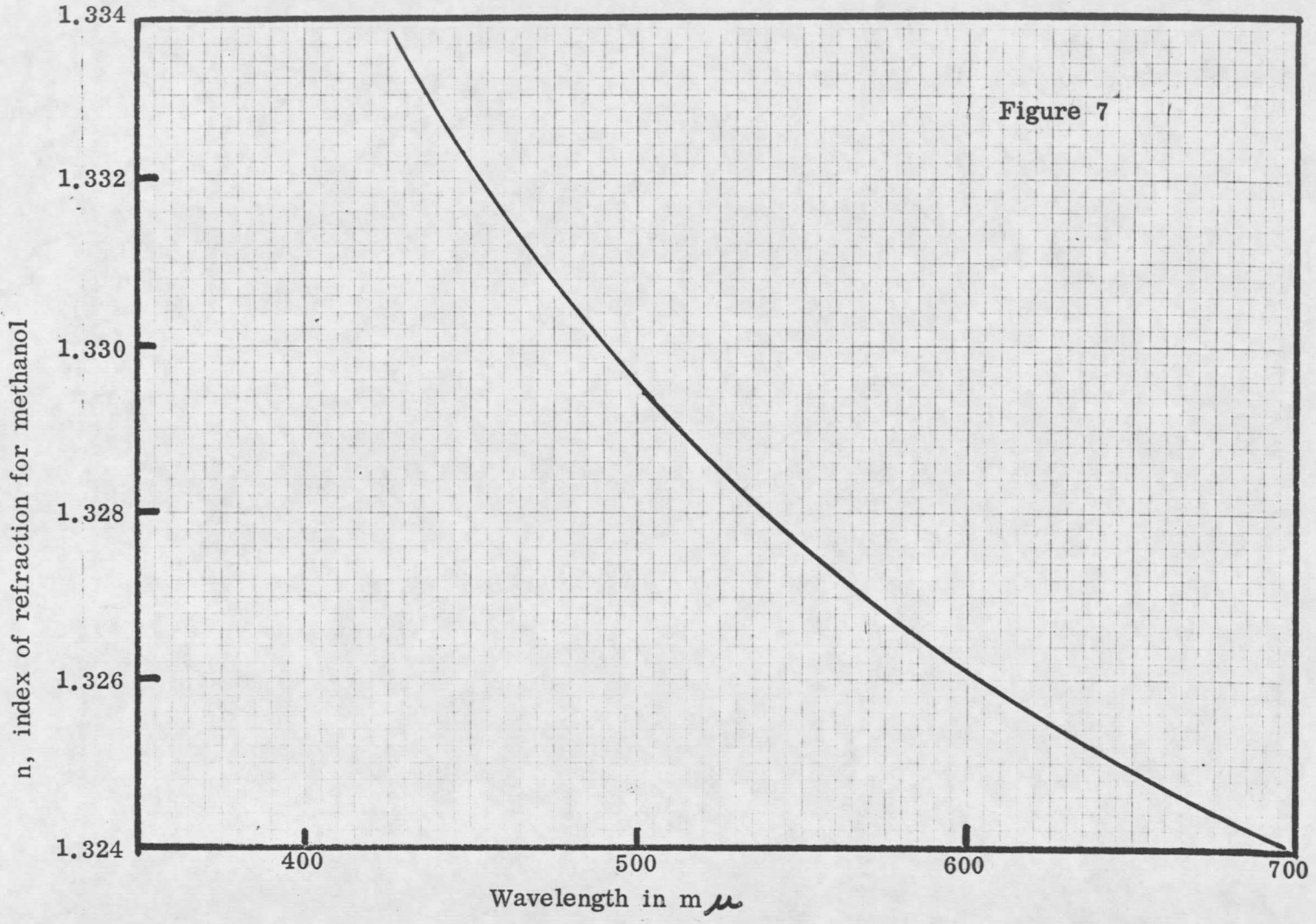

1.334
1.332
1.330
1.328
1.326
1.324
n, index of refraction for methanol
400
500
600
700
Wavelength in mμ

Figure 7

oxygen containing dyes, Dyes IV, V and VI, were calculated to have radiative lifetimes around two nanoseconds. So the radiative lifetime seems to depend more upon the hetero-atom difference between the series of dyes than upon the methylene chain length within each series. The data for the lifetime calculations are summarized in Table II.

As can be seen from Table II, as $\langle \bar{\nu}_f^{-3} \rangle_{av}^{-1}$ decreases the integral $\int \epsilon (\bar{\nu}_{lo \rightarrow u})\, d \ln \bar{\nu}_{lo \rightarrow u}$ increases for each dye series so that the radiative lifetimes are about the same for each series. The difference in lifetimes occurs mainly because the wavelengths of absorption and emission are not the same for the two series of dyes for the same methylene chain length. The oxacyanines are all at shorter wavelengths than the corresponding thiacyanines. Since the mean wavenumber for the fluorescence band is cubed, this increases the difference between the two series, as reflected by the lifetime for spontaneous emission. So the root of the matter is the smaller difference in energy between the ground state and the excited state in the thiacyanine dyes as compared to the oxacyanine dyes.

Considering the sulfurs or oxygens conjugated into the methylene chain as well as the nitrogens for the

TABLE II

Dye	$n_{uo \rightarrow l}$	$n_{lo \rightarrow u}$	$\left\langle \bar{\nu}_f^{-3} \right\rangle_{av}^{-1}$ X 10^{-12}	$\int \epsilon(\bar{\nu}_{lo \rightarrow u})\, d \ln \bar{\nu}_{lo \rightarrow u}$ X 10^{-4}	τ_o X 10^{9}
I	1.3301	1.3348	8.588	0.779	2.94
II	1.3263	1.3281	4.750	1.346	3.09
III	1.3244	1.3256	3.139	2.198	2.87
IV	1.3338	1.3404	13.20	0.863	1.72
V	1.3288	1.3310	7.187	1.351	2.02
VI	1.3258	1.3270	4.335	1.927	2.37

thiacyanines and oxacyanines respectively, then the effect of this conjugation could be described by the terms inductive effect and mesomeric effect. These terms were defined by the classical theory of valence or its quantum mechanical equivalent, valence bond theory.[17]

The inductive effect of a substituent is the effect of the potential field of the substituent on the π electrons of the unsubstituted molecule. The replacement of a CH_2 group by NH, O, or S can be considered as an inductive perturbation. Nitrogen and oxygen being more electronegative than carbon, oxygen even more than nitrogen, will tend to attract electrons from the rest of the molecule. Since sulfur has an electronegativity more nearly that of carbon, this effect will be negligible. Also sulfur could accomodate a partial positive charge more easily than oxygen.[18]

The mesomeric effect of a substituent is defined as the ability of the substituent to extend the space over which the π electrons of the molecule are delocalized. Since the non-bonding electrons of oxygen, nitrogen, and sulfur are $p\pi$ type, these electrons repell the π electrons of the hydrocarbon. This repulsion is due at least in part to the electrostatic field of the $p\pi$ electrons and decreases in the order of

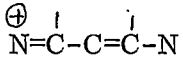
oxygen, nitrogen, sulfur.[19] In addition the low lying d orbitals of sulfur possibly play some role in the extention of the conjugation.

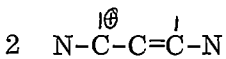
Resonance structures such as those in Figure 8 can be drawn for an open chain cyanine, an oxacyanine and a thiacyanine. Those pictured are for a short methylene chain length but the same type of structures can be drawn for a longer chain with the same resulting conclusions. From the inductive effect and the mesomeric effect, an energy diagram of the structures could be shown in a qualitative way.

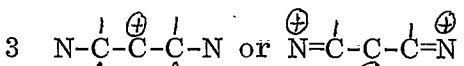
All the structures are considered to contribute to the dye molecule, both in the ground and excited states. In the ground state, however, there will be relatively greater contributions from the low-energy extreme structures where the positive charge is on the nitrogen, while in the excited state the higher-energy structures play a greater part. Thus the energy difference between the ground state and the excited state is less for oxacyanine than for an open chain cyanine, and even less for thiacyanine.

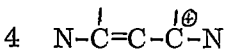
Even though arguments like the preceding give an estimate of the expected difference in the radiative lifetimes of the series of thiacyanines versus the series

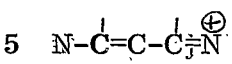
Possible Resonance Structures

1 

2 

3 

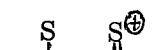
4 

5 

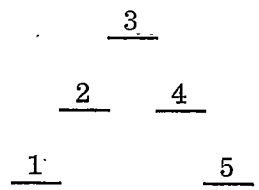
6 

7 

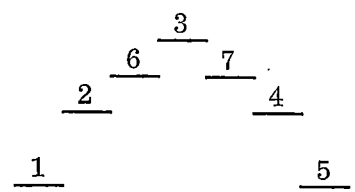
8 

9 

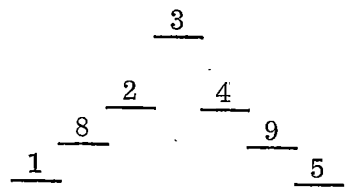
Energy Levels of Structures



Open Chain Cyanine



Oxacyanine



Thiacyanine

Energy Levels of Molecule

E. S. _____

G. S. _____

Open Chain Cyanine

E. S. _____

G. S. _____

Oxacyanine

E. S. _____

G. S. _____

Thiacyanine

Figure 8

of oxacyanines, they do not give a number that is accurate to a six per cent experimental error. To use the lifetime for spontaneous emission in other calculations and to establish the properties of excited states, an accurate figure is needed.

If the fluorescence efficiencies were known for these dyes, then an observable lifetime τ could be calculated with good accuracy even though some of these lifetimes are probably too short to measure with known methods, especially the short chain length dyes. The fluorescence efficiencies were determined by comparison with known standards.

For the comparison to be accurate, the raw fluorescence spectra were corrected for the following wavelength dependent factors; the quantum efficiency of the photomultiplier, the band width of the emission monochromator and the transmission factor of that monochromator. This was done by recording the spectra of the following compounds excited at 366 mμ for which the corrected spectra are known[20,21,22]; quinine bisulfate, 1 μg/ml in 0.1 N H_2SO_4; 3-aminophthalimide, 1 μg/ml in 0.1 N H_2SO_4; 3-nitro-N,N-dimethylaniline, 100 μg/ml in 30% benzene and 70% hexane by volume; and 4-dimethylamino-4'-nitrostilbene, 100 μg/ml in o-dichlorobenzene. The values

chosen were those given by Argauer[22] for dilute solutions at 90° rather than those of Lippert et al.[20] for front surface concentrated solution fluorescence. The fluorescence spectra of these compounds cover the visible spectrum. The least reliable region is that less than 425 mμ since the quinine bisulfate fluorescence is 50% of its maximum at that wavelength and decreasing as the wavelength decreases. So a small error in either the standard curve or in the measured curve will give larger errors in the correction factor at the shorter wavelengths. The spectra of the compounds overlap with at least 70% of their maximum intensity except for the region between 590 and 640 mμ where the two spectra cross at 50% of their maxima. The sodium salt of 2,2'-dihydroxy-1,1'-azonaphthalene-4-sulfonic acid could not be obtained, otherwise, it would have been used, as the fluorescent aluminum chelate, to cover this region.

Then S_λ, the correction for the three wavelength dependent factors, was calculated from equation (40) where $(dQ/d\lambda)_{SL}$ now is the known spectral distribution of one of the standard compounds and R_{SL} is the photomultiplier response of this instrument to the same compound under the same conditions as that for which the standard fluorescence curve is provided. The resulting S_λ's

are plotted in Figure 9. The dotted line gives a maximum uncertainty in the S_λ's in the region less than 425 mμ.

The fluorescence spectra of the dyes I through VI and of the known compounds quinine bisulfate and rhodamine B were determined with the same instrument configuration as that for which the S_λ's were calculated. For the energy correction using peak heights, two of the compounds were excited at the same wavelength and lamp intensity. See Table III for the exciting wavelengths.

Since the fluorescence efficiency of some of the dyes is very low, solutions which absorbed an appreciable amount of the exciting light had to be used. So that any corrections for this would cancel for the two solutions to be compared, all the solutions were made to have an absorbance of 0.4 per cm. at the exciting wavelength as suggested by Parker.[12]

The resulting fluorescence spectra were then used to determine the fluorescence efficiency of the six dyes. This entailed dividing the photomultiplier response at each 5 mμ interval by the corresponding S_λ and then integrating by the method of the sum of the areas of the rectangles formed by the corrected relative intensity versus the wavelength to find the area under the fluorescence curve.

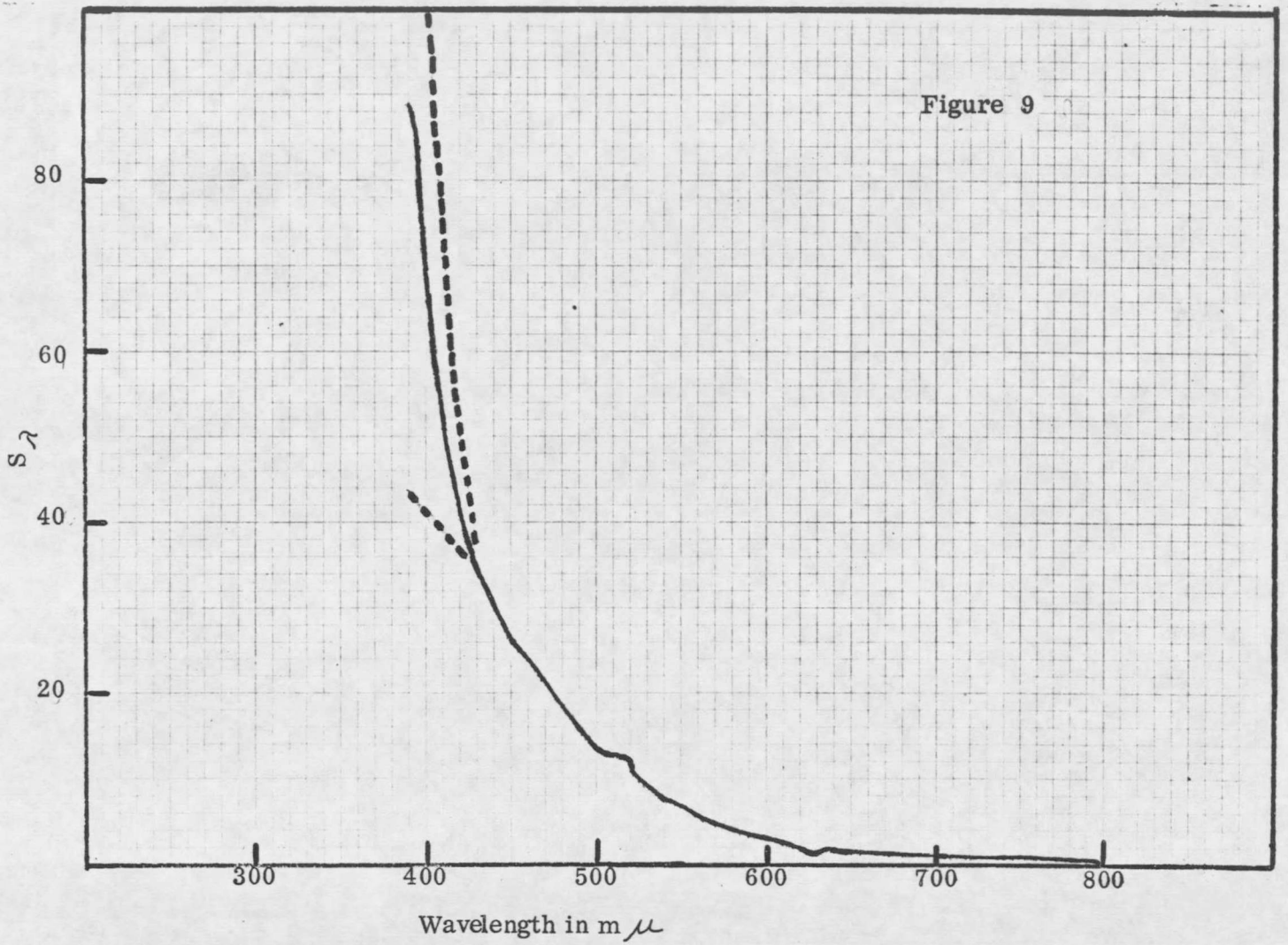


Figure 9
80
60
40
20
S_λ
300
400
500
600
700
800
Wavelength in m μ

TABLE III

Excitation Wavelength mμ	Compound
424	Dye I
556	Dye II
652	Dye III
376	Dye IV
483	Dye V
579	Dye VI
366	Quinine Bisulfate Dye IV
390	Dye IV DyeII
440	Dye I Dye V
500	Dye V Dye II
560	Dye II Dye VI
600	Dye VI Dye III
535	Dye II Rhodamine B

The true absorbances were determined for all the solutions. Then both the areas and the peak heights were corrected to 0.400 by assuming a linear relationship between absorbance and fluorescence quanta. All of the absorbances were within 9 per cent of being 0.400, with most of them very close, so this assumption should be valid.

The correction for the index of refraction was made for the calculation with Dye IV in methanol (n = 1.335 from Figure 7) and quinine bisulfate in 1 N H_2SO_4 (n = 1.338 [23]) and with Dye II in methanol (n = 1.326 from Figure 7) and rhodamine B in ethanol (n = 1.360 [24]). All the dyes were dissolved in methanol so no corrections had to be made for the refractive index.

The fluorescence efficiencies were calculated with quinine bisulfate (ϕ = 0.55 [25]) as the standard and with rhodamine B (ϕ = 0.97 [26]) as the standard. The results of these calculations and the corrected areas under the fluorescence curves are given in Table IV.

As can be seen in Table IV, the ϕ's as calculated from the two standards are disparate. Since rhodamine B has a value of 5.0 from quinine bisulfate, it would appear that the problem must lie in one or more of the four shorter chain-length dyes.

TABLE IV

Compound	Integrated Area	ϕ from Quinine Bisulfate	ϕ from Rhodamine B
Quinine Bisulfate	913	0.55	0.11
Dye I	1.91	0.0030	0.00059
Dye II	491	0.25	0.048
Dye III	4570	1.7	0.33
Dye IV	5.77	0.0037	0.00071
Dye V	282	0.27	0.053
Dye VI	5110	2.2	0.43
Rhodamine B	8290	5.0	0.97

The integrated areas are corrected for everything but the lamp intensity, the band width of the excitation monochromator and the transmission factor of that monochromator. If it is assumed that the last two factors are much smaller than the variation in lamp intensity, then the major correction is the decreasing quanta of light with decreasing wavelength from the tungsten lamp. Now Dye I has an integrated area of 1.91 for excitation

at 424 mμ while Dye IV has an area of 5.77 for excitation at 376 mμ, a ratio of approximately 1:3. If the tungsten lamp is assumed to run at 3000° K and if it is assumed to emit like a blackbody radiator, then at 424 mμ it will emit 2.13×10^{-5} einsteins per μm^{-1} interval while at 376 mμ it will emit 6.73×10^{-6} einsteins per μm^{-1} interval.[27] This is a ratio of approximately 3:1. So it would appear that the ϕ's of Dye I to Dye IV should be at least 1:3 if not greater, but they are not, they are approximately the same.

So the relationship between Dye I and Dye IV, which were calculated one from the other, would seem to be a weak link in the chain of fluorescence efficiency calculations. The wavelength at which both were excited for comparison was one in which neither had a very large absorbance and the energy of the lamp is also very low in this region. Between these facts and the fact that neither has a very large fluorescence efficiency, the instrument sensitivity was pushed to its utmost to give a very small peak height. Thus error could be introduced in this manner. However even all this can not explain an error of a factor of five.

The dyes I and IV are different from the others in that they do not give mirror image fluorescences.

This may mean that there is a molecular rearrangement upon excitation. The fluorescence efficiency calculation has the basic assumption that the fluorescence efficiency is constant across the absorption band. If there is a molecular rearrangement in the excited state, perhaps some excitation energies are more favorable for emission than others. Excitation to some particular vibrational levels might rearrange more readily and then fluoresce rather than decay radiationlessly. Thus the fluorescence efficiency may not be a constant across the absorption band.

Then the best fluorescence efficiencies may be those in which Dye I and Dye IV are calculated most directly from a known standard and not from each other. This gives a ratio of ϕ's of approximately 1:6 for Dye I to Dye IV. The ϕ's from this assumption with their estimated errors, the observable lifetimes calculated from these ϕ's and the calculated intrinsic lifetimes are summarized in Table V. The larger error in Dye IV comes from the uncertainty in S_λ while the error in Dye I comes from the uncertainty in reading the curve. The error for the other four dyes is assessed at the nominal 10 per cent for this procedure since there is not the uncertainty in S_λ and since the

TABLE V

Dye	ϕ	τ_o	τ
I	0.00059 20%	2.94×10^{-9}	1.7×10^{-12}
II	0.048 10%	3.09×10^{-9}	1.5×10^{-10}
III	0.33 10%	2.87×10^{-9}	9.5×10^{-10}
IV	0.0037 50%	1.72×10^{-9}	6.4×10^{-12}
V	0.053 10%	2.02×10^{-9}	1.1×10^{-10}
VI	0.43 10%	2.37×10^{-9}	1.0×10^{-9}

standard rhodamine B has its fluorescence in the same region as these dyes.

The fluorescence efficiencies vary most by chain length in the two series of dyes. As the chain length increases, so does the fluorescence efficiency. The predicted observable lifetimes vary in the same manner. Small differences between the two series for the same chain length dye for the fluorescence efficiency and the intrinsic lifetimes cancel each other out to give approximately the same predicted observable lifetimes except for the shortest members. Dye I is more crowded and thus may be expected to be different from Dye IV.

The rate of all decay processes is the inverse of the observable lifetimes so that for shorter lifetimes the rate is faster. The rate of energy transfer must compete with this fluorescence rate to be effective. If the rate of energy transfer is the same for all the dyes, then it would appear that the longer the chain length the more easily the energy transfer could compete. This correlates very well with the fact that the longer the chain length the better the dye sensitizes photographic films.[28]

Dyes of the 3,3'-diethylthiacyanine series in high concentrations dimerize in water solutions. No evidence of dimer formation in organic solvents at room temperature has been reported.[29] The following studies were done to check for the possibility of dimers.

The original dye solutions prepared for the emission spectra were almost saturated solutions; that is, about 10^{-4} M for Dyes III and VI and about 10^{-3} M for the other four dyes. These solutions plus dilutions of 10^{-5}, 10^{-6} and 10^{-7} M for Dyes III and VI and dilutions of 10^{-4}, 10^{-5} and 10^{-6} M for the other dyes were studied for differences of shape of the emission spectrum. These solutions were also studied for variations of the emission spectrum upon excitation by different

exciting wavelengths. Those wavelengths used were the maximum of the absorption band, the approximate maximum of the absorption shoulder, the dimer wavelength as reported by West and Pearce[29], and a longer wavelength than the absorption maximum.

The major change in the emission spectrum of all six dyes upon a decrease in the concentration of the dye was an increase in the relative height of the major peak versus the shoulder and a shift in the maximum of the major peak toward shorter wavelengths. The weakest two concentrations gave essentially the same spectrum with only more noise in the weaker one. This change with dilution may be attributed at least in part to self-absorption of the shorter wavelength emitted light by the more concentrated solutions.

With the most concentrated solution of dye, excitation at a wavelength longer than the absorption maximum gave a spectrum in which the shoulder to maximum peak heighth ratio was larger than that for excitation at other wavelengths. This anomaly was not observed in any of the more dilute solutions. Since dimers have been reported in water solutions[29], a saturated solution of Dye III was prepared. The absorption spectrum gave approximately equal heights for the monomer and dimer

absorption peaks. The emission spectrum of this solution had the peak maxuma at the same wavelengths as the dilute methanol solutions. This could possibly be explained by the fact that the extinction coefficients for the water solution are much lower than for the methanol solution of similar concentration, so there might be less self-absorption. Different exciting wavelengths did not change the peak height ratios in the water solution.

So whether dimers were the cause of the anomaly in the concentrated methanol solutions is still a moot question. In any case the concentrations of dyes used for the absorption and emission studies were dilute enough to give no evidence of the presence of dimers or self-absorption.

SUMMARY

The absorption and emission spectra in methanol of the dyes of the 3,3'-diethylthiacyanine iodide series and of the 3,3'-diethyloxacyanine iodide series (except for the first member which was the triiodide) were determined. The integrated intensities and oscillator strengths were calculated. In each series these quantities increase as the methylene chain length increases. From the absorption and emission spectra, the radiative lifetimes were calculated. The lifetimes were of the order of three nanoseconds for the thiacyanine series and of the order of two nanoseconds for the oxacyanine series. The fluorescence efficiencies were determined. They increase by a factor of ten as the chain length increases except for the first member of the sulfur series which is lower by a factor of 100 from the next member. The observable lifetimes were calculated from the radiative lifetimes and the fluorescence efficiencies. Since the rate of all decay processes is the inverse of the observable lifetime, a minimum may be placed on the rate of energy transfer from the first excited state for these dyes when energy transfer processes take place.

EXPERIMENTAL

Melting points were determined on a Fisher melting point block and were uncorrected.

Analyses were performed by Galbraith Laboratories, Incorporated, P. O. Box 4187-Lonsdale, 2323 Sycamore Drive, Knoxville, Tennessee 37921.

The ultraviolet and visible absorption spectra were taken on a Beckman Model DK-2 spectrophotometer for 1,1',3,3'-tetraethylbenzimidazolocarbocyanine iodide and on a Cary Model 14 spectrophotometer for all the others.

The emission spectra were taken on a component system. This consisted of a tungsten lamp, two Bausch and Lomb Cat. No. 33-86-45 monochromators with the grating blazed at 3000 Å, an EMI Model 9558 B photomultiplier, and a Hewlett Packard Model Moseley 7030A X-Y recorder.

Methanol, Baker Analyzed absolute, without further purification was the solvent for the cyanine dye solutions for absorption and fluorescence. It had no detectable fluorescence.

Ethanol, 95%, was used without further purification. It had no detectable fluorescence.

o-Dichlorobenzene, Baker Analyzed reagent, contained no fluorescing species, b.p. 180.3-180.5° at 640 mm.

Benzene, Baker Analyzed, was dried over sodium and distilled, b.p. 72.8-73° at 640 mm.

Hexane, Baker Analyzed, was purified with chlorosulfonic acid.[30] About 400 ml. of the hexane was added to 80 ml of Eastman practical chlorosulfonic acid in a 3-necked one liter round bottom flask. After the addition, two of the necks were stoppered, the third had a vent tube to allow the escape of the hydrogen chloride formed during the reaction. The reaction mixture was stirred by a glass-covered magnetic stirring bar. After four days the mixture was placed in a separatory funnel and the acid layer, a dark brown, drained off. The hydrocarbon layer, a light yellow, was washed with 150 ml. of 10% NaOH is small portions. Then it was washed three times with 100 ml. of distilled water and left to dry over Na_2SO_4. The hexane was then dried over sodium and distilled, b.p. 61.8-62° at 640 mm.

Sulfuric acid, 0.1 N and 1 N, were prepared from DuPont Reagent Grade concentrated sulfuric acid and were standardized against standard NaOH.

Quinine Bisulfate, Eastman, gave an O.D. of 0.368/cm. for a concentration of 0.005889 g./100 ml. compared

to 0.0062/cm. for a concentration of 1 μg./ml. for Parker and Rees.[31]

Rhodamine B, J.T. Baker Chemical Co., had an extinction coefficient of 1.06×10^5 at 544 mμ compared to 1.04×10^5 for Parker and Rees.[31]

3-Aminophthalimide, Eastman, was filtered hot and then recrystallized three times from 95% ethanol. Since it sublimes at 640 mm. the melting points were determined using a sealed capillary tube and a Thiele tube filled with concentrated H_2SO_4, m.p. 259.5° (Lit.[20] 260°), $\epsilon_{366} = 3.24 \times 10^3$.

3-Nitro-N,N-dimethylaniline, Pfaltz and Bauer, Inc., was recrystallized once from 95% ethanol, m.p. 59-60° (Lit.[20] 59-60°), $\epsilon_{366} = 1.30 \times 10^3$.

4-Dimethylamino-4'-nitrostilbene, Eastman, gave a very sharp melting point, m.p. 265° (Lit.[20] 256°), $\epsilon_{366} = 5.67 \times 10^3$.

Preparation of 3-Ethyl-2-methylbenzothiazolium Iodide

Ethyl iodide (20.0 g., 0.128 moles) and 2-methylbenzothiazole (10.0 g., 0.0672 moles were refluxed together for four hours. A yellowish-white precipitate

formed. After the reaction mixture was cooled, the precipitate was filtered and was washed three times with acetone (about 10 ml. each time). The yield of white salt was 12.2 g. (0.0400 moles).

Preparation of 3-Ethyl-2-methylbenzoxazolium Iodide

Ethyl iodide (18.1 g., 0.116 moles) and 2-methylbenzoxazole (8.2 g., 0.0614 moles) were refluxed together for 47 hours. The resulting yellow-brown solid, when cooled, was filtered and was washed three times with about 10 ml. portions of acetone. The yield of pale yellow salt was 7.7 g. (0.0267 moles).

Preparation of 1,3-Diethyl-2-methylbenzimidazolium Iodide

Ethyl iodide (10.00 g., 0.0640 moles) and 1-ethyl-2-methylbenzimidazole (7.00 g., 0.0437 moles) were refluxed together for nine hours. The reaction mixture was allowed to cool and then was poured into ether. The ether solution was filtered. The solid salt was washed with ether (about 5 ml.) and with acetone (about 5 ml.). The yield of grey-yellow solid was 12.8 g. (0.0405 moles); m.p. 199-202°.

Anal. Calcd. for $C_{12}H_{17}IN_2$: C, 45.6; H, 5.37; N, 8.85.

Found: C, 45.70; H, 5.39;
N, 8.92.

Preparation of 3,3'-Diethylthiacarbocyanine Iodide

Triethyl orthoformate (1.00 g., 0.00675 moles) and 3-ethyl-2-methylbenzothiazolium iodide (1.00 g., 0.00328 moles) were heated together in 10 ml. of pyridine with magnetic stirring. When the mixture reached reflux temperature and all the solid had dissolved, six drops of triethylamine were added. At this time the solution turned very purple. The solution was refluxed for ten minutes and then allowed to cool. The cooled mixture was poured into about 100 ml. of ether, thoroughly mixed and then filtered. The crude yield was 1.2 g. Recrystallization from methanol three times gave 0.30 g. (0.000609 moles) of shiny grey-purple needles; m.p. 267° with decomposition.

Anal. Calcd. for $C_{21}H_{21}IN_2S_2$: C, 51.2; H, 4.27;
I, 25.8; S, 13.04.
Found: C, 50.96; H, 4.32;
I, 26.07; S, 13.26.

Preparation of 3,3'-Diethylthiadicarbocyanine Iodide

Pyridine (10 ml.), 3-ethyl-2-methylbenzothiazolium iodide (1.00 g., 0.00328 moles) and 1,1,3,3-tetraethoxy-

propane (1.52 g., 0.00681 moles) were heated together until all of the solid had dissolved. Two drops of triethylamine were added. Immediately the color changed to a blue. This mixture was refluxed for ten more minutes after which it was allowed to cool.

Then it was poured into about 150 ml. of ether and was stirred well. Next the crude dye was filtered and recrystallized from methanol three times to give 0.20 g. (0.000386 moles) of shiny yellow-green matted crystals; m.p. 254-6° with decomposition.

Anal. Calcd. for $C_{23}H_{23}IN_2S_2$: C, 53.3; H, 4.44; I, 24.5; S, 12.62.
Found: C, 52.37; H, 4.44; I, 25.29; S, 12.47.

Preparation of 3,3'-Diethyloxacyanine Triiodide

The method used was principally that of Fisher and Hamer[32]. The quaternary salt, 3-ethyl-2-methyl-benzoxazolium iodide (3.50 g., 0.0121 moles) was added to 25 ml. of propionic anhydride which had been heated to 135°. All of the solid dissolved when freshly distilled isopentyl nitrite (1.5 ml., 0.011 moles; b.p. 88-89° at 640 mm.) was added slowly. Then the mixture was allowed to cool.

The solid which first formed was an orange color. The solution was decanted and a purple precipitate formed. Both of these precipitates were filtered, ground with water and with ether and filtered again. Each yielded 0.2 g. of solid.

The ultraviolet and visible spectrum was taken of each. The orange solid appeared to have some of the quaternary salt present and the purple had a larger leading edge to the dye absorption band, otherwise they seemed the same.

The purple solid was extracted three more times with about 20 ml. of ether each time. This purple compound had a m.p. of 300-1° with decomposition.

Anal. Calcd. for $C_{19}H_{19}I_3N_2O_2$: I, 55.4.
Found: I, 55.21.

Attempts were made to reduce the triiodide to the iodide salt. The triiodide was suspended in 10 ml. of methanol cooled in an ice bath and tank sulfur dioxide gas was bubbled through for six hours and for twenty hours. The dye turned lighter in color but upon standing would turn dark again.

Then the dye was recrystallized from methanol, with little difficulty the first time. After the dye dissolved in the second recrystallization a foamy

yellow substance formed which had some purple specks in it. This yellow substance was not soluble in water, methanol or dimethylformamide. It was slightly soluble in ether, much more so than the purple material.

Preparation of 3,3'-Diethyloxacarbocyanine Iodide

Triethyl orthoformate (3.20 g., 0.0216 moles) and 3-ethyl-2-methylbenzoxazolium iodide (3.0480 g., 0.01055 moles) were added to 50 ml. of pyridine in a 20 cm. by 2.5 cm. test tube. This mixture was heated over a small flame. When the solution boiled a total of 21 drops of triethylamine was added. The solid never seemed to dissolve; however, when the solution was cooled and filtered, shiny purple-pink crystals were found. These were recrystallized from ethanol twice to yield 0.55 g. (0.0012 moles); m.p. 281° with decomposition.

Anal. Calcd. for $C_{21}H_{21}IN_2O_2$: C, 54.8; H, 4.56; I, 27.6.

Found: C, 54.69; H, 4.76; I, 27.46.

Preparation of 3,3'-Diethyloxadicarbocyanine Iodide

About 0.1 g. (0.0004 moles) of 3-ethyl-2-methyl-

benzoxazolium iodide, about the same volume of 1,1,3,3-tetraethoxypropane (approximately 0.2 g., 0.0009 moles) and about two ml. of dimethylformamide were heated together in a 15 cm. by 1.5 cm. test tube over a small flame. When the solution started boiling, five drops of pyridine were added. The mixture was allowed to cool and then poured into about 150 ml. of ether and mixed well. This mixture was allowed to stand for several hours with occasional mixing. Then the ether was decanted, fresh ether added and the same process continued until the dye was no longer sticky. Then the dye was recrystallized three times from methanol. The above procedure was repeated four times to yield a total of 0.10 g. (0.00021 moles) of silver-turquoise crystals; m.p. 254-6° with decomposition.

Anal. Calcd. for $C_{23}H_{23}IN_2O_2$: C, 56.8; H, 4.73; I, 26.1.

Found: C, 52.13; H, 4.42; I, 32.10.

A Sample of 3,3'-Diethylthiacyanine Iodide

This yellow dye previously prepared in this laboratory gave satisfactory analysis with m.p. above 315°.

Anal. Calcd. for $C_{19}H_{19}IN_2S_2$: C, 48.9; H, 4.08; I, 27.2; S, 13.75

Found: C, 48.85; H, 4.21; I, 27.03; S, 13.84.

Attempted Preparation of 1,1',3,3'-Tetraethylbenzimidazolocarbocyanine Iodide

An equal volume of 1,3-diethyl-2-methylbenzimidazolium iodide and triethyl orthoformate were reacted together in a series of solvent and base pairs. These included pyridine and triethylamine, dimethylformamide and triethylamine, nitrobenzene alone, nitrobenzene and triethylamine, nitrobenzene and 2,2',2''-nitrilotriethanol, dimethylformamide and sodium hydroxide, and finally nitrobenzene and 3 M sodium ethoxide. The product of this last reaction after five recrystallizations from methanol had an extinction coefficient of 51 at the maximum of 486 mμ so it apparently was not very pure.

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