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I. ENERGY TRANSFER FROM OROTIC ACID DERIVITIVES TO EUROPIUM (III) IONS.
II. THE STRUCTURES OF THE COMPLEXES FORMED BY OROTIC ACID AND EUROPIUM (III) AND NI (II) IONS.
III. PHOTOCHEMISTRY OF 6-ACETYL URACIL IN AQUEOUS SOLUTION.

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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

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A THESIS

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

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ARVIND SARPOTDAR

Norman, Oklahoma

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ABSTRACT

The present work was undertaken to recheck the validity of using europium (III) ions as efficient acceptors of singlet and triplet excited energy from a donor in aqueous solution.

Various orotic acid derivatives have been synthesized and their complex formation with Ni (II) and Eu (III) metal ions in aqueous solutions has been studied. The stability constants for the 1:1 complexes of these derivatives with Ni (II) and Eu (III) ions in a pH range 1 to 7 have been evaluated using Benesi-Hilderbrand type technique. At pH 5.5 europium-orotic acid 1:1 complex has a stability constant = 1.473×10^2 moles^{-1}, liter.

Nature of the Eu^{3+}-orotic acid complex has been elucidated. It has been observed that orotic acid and 3-methylorotic acid form strong 1:1 complexes with either Eu^{3+} or Ni^{2+}, whereas no uv detectable complexes are found to be formed by 1-methyl and 1,3-dimethylorotic acid and isopropyl and methylorotate. Complex stability is found to increase with pH. Orotic acid is thus found to behave as a bidentate in complexing the metal ions through its carboxylate anion and through the anion produced by the ionization of N-1 proton.

The kinetics and mechanism of photodimerization quenching of the above compounds by Eu^{3+} ions in aqueous solutions as well as the excited energy transfer from them to europium ions at various pH values have been studied. Quenching of this photo-dimerization by Eu (III) and also the sensitization of

the Eu^{3+} fluorescence by orotic acid derivatives are seen to be pH dependent. It is apparent from our data that the presence or absence of energy transfer from these compounds to Eu (III) is inherent in their ability to complex Eu (III).

A new kinetic model has been proposed to account for the observed facts. It has been proposed in this model that the observed energy transfer is occurring in a inter-intramolecular fashion from the uncomplexed excited donor to the donor in a 1:1 complex with Eu (III) via the diffusion controlled collisional process and subsequently from excited donor in the complex to the Eu (III) ion. We have obtained the rate constant ($^3k'_t$) for this diffusion controlled energy transfer = 6.79×10^9 sec^{-1}, $moles^{-1}$, liter.

The present work casts a serious doubt on the further use of Eu (III) ions as excited singlet or triplet counters in aqueous solutions.

Photochemistry of 6-acetyluracil in neutral aqueous solution has been studied. The singlet major photoproduct has been isolated by preparative chromatography on silica gel and characterized through its nmr, IR, mass spectrum, elemental analysis, etc. as a new oxetane type photodimer.

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CONTENTS

TABLES

TABLE

LIST OF FIGURES

Chapter I

Chapter II

Chapter III

CHAPTER I

ENERGY TRANSFER FROM OROTIC ACID DERIVATIVES TO EUROPIUM(III) IONS

INTRODUCTION

Strong fluorescence under ultraviolet excitation by a number of lanthanide ions is a well known phenomenon.[1] The strength of the fluorescence depends upon the environment of the cation. Thus the anhydrous chlorides of most of these ions fluoresce strongly. Europium(III) compounds often fluoresce strongly either in solution or in the solid state. Praseodymium(III) and neodymium(III) compounds fluoresce weakly in solution. The fluorescence spectra, where observed correspond closely to absorption spectra and are thus relatable to the shielded 4f electrons.

Trivalent lanthanide ions have an incomplete 4f shell of electrons which is shielded by complete 5S and 5P shells, so that the energy levels appropriate to the free ions are not greatly purturbed in a crystal lattice. The trivalent europium ion (Eu^{3+}) has six electrons in the 4f shell ($1s^2 2s^2 2p^6 3s^2 3p^6 3d^{10} 4s^2 4p^6 4d^{10} 4f^6$) and by Hund's rule the ground

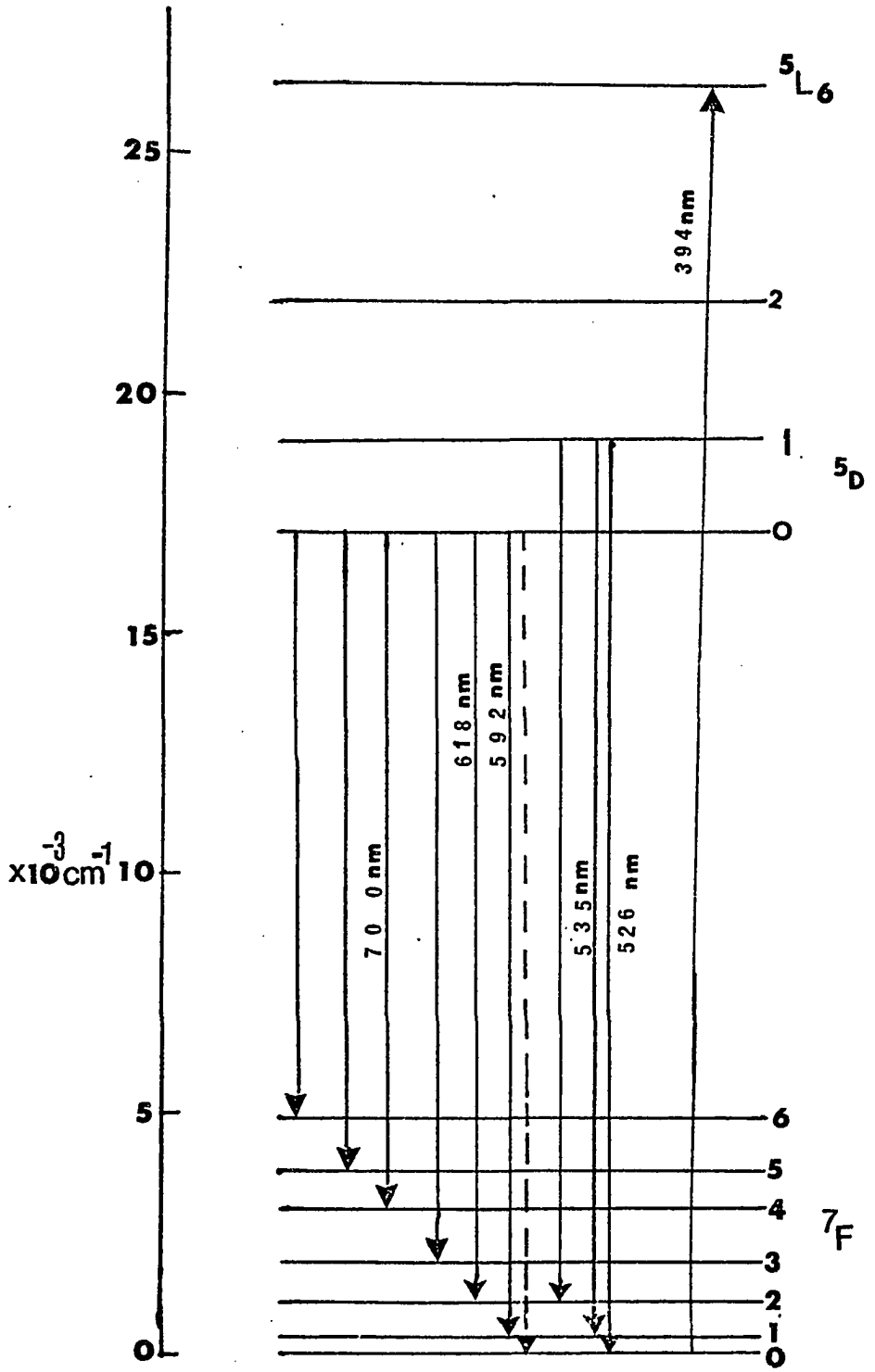


Figure 1. Energy Levels and Fluorescent Transitions in Europium(III)

state is denoted by $^7F_0$ which indicates that none of these electrons is spin-paired. Within 4f shell there can be many other arrangements of electrons; these may be of higher energy, with energy-difference determined by spin-orbit coupling, forming a set of multiplets. These extend over an energy range of several thousand cm^{-1} ($\sim$10 K cal $mole^{-1}$). Luminescence, however, involves the lowest excited multiplet denoted $^5D_{0-4}$. In this state the two electrons are spin paired. Figure 1 shows the energy levels and observed transitions between ground multiplet and the excited one.

Weisman first showed that in many rare-earth chelates energy transfer can occur from excited ligand to central metal ion.[2-4] Crosby and coworkers have investigated in detail the mechanism of intramolecular energy transfer.[5-8] They found that in type A chelates where the lowest triplet level of chelate-ligand lies above the emissive level of the lanthanide ion, sensitized fluorescence of lanthanide ion is observed. This fluorescence is absent in type B chelates where the lowest triplet level of the ligand is below the emissive level of the rare-earth ion. Crosy concluded from these results that the direct transfer of the excitation energy from the excited singlet does not occur and that the principal path of energy transfer to the ion is through the lowest triplet state of the ligand or a nearby excited state (within 500 cm^{-1} of the lowest triplet state).

Fillipscue and coworkers[9] and Charls and Riedal[10] have obtained the quantum efficiency of sensitized fluorescence in the range 0.5-1.0 for many europium and terbium chelates in solutions.

Use of rare-earth chelates as laser materials has been discussed by various workers.[11-13]

Contrary to Crosby's findings, Kleinerman showed that the absence of sensitized fluorescence in type B chelates is due to the quenchings of the ion fluorescence by the low lying ligand triplet rather than the lack of energy transfer to central metal ion.[14] He has studied 600 rare-earth chelates with various ligands and has demonstrated the existence of excited singlet transfer to rare-earth ions. Thus the $S_1 \rightarrow S_o$ fluorescence of ligands such as 8-quinolinol, anthranilic acid, 3-hydroxyflavone, etc. which is observed in the chelates of Lu^{3+}, La^{3+} and Gd^{3+} is quenched by Tb^{3+}, Eu^{3+}, Sm^{3+}, etc. when the above ligands were used to chelate these ions.

Bhaumic and El-Sayed[15-16] were first to show that if the lowest triple level of type A chelates, e.g., $Eu(FHA)_3$ is excited by triplet to triplet intermolecular energy transfer from another donor like benzophenone then the energy can be transferred to the lanthanide ion.[17] Thus they observed a sensitized fluorescence characteristic of Eu^{3+} ions by using benzophenone as a triplet donor to ligands of $Eu(HFA)_3$ chelate.

This spurred the interest of many workers to study the energy transfer from various donors to non-chelated lanthanide ions via an intermolecular process. Heller and Wasserman[18] found that twenty-one out of twenty-five compounds they studied transferred energy to either europium or terbium or to both. Their kinetic rate data for transfer process and the oxygen quenching effect led them to believe that the energy transfer occurred through the triplet state of the donor and as the transfer rate was viscosity dependent the energy transfer was occurring through a diffusion controlled colligional process.

Fillipescue and Mushrush[19-20] have done similar studies with various organic carbonyl compounds and Eu^{3+} and Tb^{3+} ions. Thus in N,N dimethyl formamide as a solvent they observed that the energy transfer was occurring through n,π^* lowest triplet state of carbonyl compound and at 77°K the energy transfer was negligible with only blue phosphorescence of the donor. Raising the temperature to 300°K typical Eu^{3+} fluorescence was seen with donor 2-acetonaphthone in 5:4 ethanol : methanol as the solvent. ($EuCl_3$ = 0.05 M, 2-acetonaphthone = 0.005 M.)

More recently Lamola and Eisinger[21-22] have used Eu^{3+} ion as an acceptor to study energy-transfer from excited states of various biologically interesting compounds such as orotic acid, GMP, UMP, TMP,[23] etc. in aqueous solutions. They have proposed a kinetic model to fit their

data. This model accounts for an excited triplet transfer to Eu^{3+} ions at low Eu^{3+} concentration and a plausible singlet transfer at a much higher Eu^{3+} concentration.

Observations of many workers on intermolecular energy transfer to lanthanide ions in fluid solutions so far have led to confusion and the mechanism of energy transfer is not completely clear. The question still not satisfactorily answered is whether the energy transfer in these systems is a purely diffusion controlled collisional process or the energy is transferred to metal ion through some kind of complex formation or some association between the donor and the lanthanide ions. The observations inconsistent with collisional process of energy transfer are as follows:

1. Heller and Wasserman[18] found that the energy transfer from various aromatic aldehydes and ketones in 4:1 ethanol methanol glass at 77°K with low Eu^{3+} concentration did not occur and only donor phosphorescence could be observable. By increasing the Eu^{3+} concentration even at glassy rigid media (77°K) sensitized Eu^{3+} emission was observable. The authors have considered a possibility of transient complex formation between the rare earths and the donor aldehyde or ketone.

2. Fillipscue and Mushrush[19] noted the failure of nonpolar aromatic hydrocarbons such as naphthalene and triphenylene to sensitize any Eu^{3+} fluorescence. Lamola and Eisinger[21]

have similarly noted the absence of sensitized Eu^{3+} fluorescence with tryptophan as the donor. These compounds otherwise are known to undergo efficient intersystem crossing to triplet excited state under UV irradiation with appropriate light energy. These triplets lie well above the emissive levels of Eu^{3+}.

3. More important is the observation of Wagner and Scott[24] who found that the Eu^{3+} ions are about two orders of magnitude slower in quenching the photoelimination of p-methoxy valerophenone than dienes; which suggests that the effective concentration of Eu^{3+} is about two orders of magnitude smaller than the actual concentration used.

Similar results were obtained by Lamola and Eisinger[21] where they studied acetophenone sensitized Eu^{3+} fluorescence, quenched by HDAC (2,4 hexadienyltrimethyl ammonium chloride). They found that Eu^{3+} is more than two orders of magnitude less effective as an acceptor of triplet excitation from acetophenone than is the diene ($^3k_q/^3k_t = 350$).

Worth mentioning are the observations of some Russian workers. Ermolaev has studied the triplet state quenching of various ketones by trivalent rare-earth ions.[25] The energy transfer constants K_t were 10^5-10^7 which are 3-5 orders smaller than the diffusion controlled rate. The values of K_t/I where I is the overlap integral were found highest for Eu, Sm and Pr and were lowest for Ho, Er, and Nd. They have explained the highest values as a consequence

of the formation of charge-transfer contact complexes between triplet ketone and the rare-earth ion.

Sveshinkova has similarly found that the energy transfer from excited ketones to rare-earth ion occurs through a collision complex and the transfer efficiency depends upon the complex stability.[26] For benzophenone with strong electron donating substituents on the phenyl ring caused the formation of stable complexes with rare-earth ion and the energy transfer rates were about 2 orders of magnitude higher than the rates with electron-withdrawing groups on the aromatic ring.

Grigoryan and Gevorkyan noticed that Eu^{3+} and Tb^{3+} form complexes with p-propoxy, p-isopropoxy and p-isobutoxy acetophenone in ethanol solution and the sensitized fluorescence of Eu^{3+} and Tb^{3+} was not quenched by oxygen.[27] Also in these cases the luminescence spectra were different from the emission spectra of Eu^{3+} and Tb^{3+} alone in ethanol.

We have found that energy transfer from excited orotic acid (OA) is more complicated than reported. Orotic acid (6 carboxy 2,4 dioxypyrimidine) in aqueous solution dimerizes efficiently when photolyzed with UV light of appropriate wavelength. This photodimerization has been shown to occur exclusively through excited triplet state.[28] This photo-transformation is markedly affected by pH (for pH > 3.3 $\phi_{isc} = 0.062$, for pH = 1.1 $\phi_{isc} = 0.13$). Energy transfer efficiency from orotic acid to Eu^{3+} ions was also a function

of pH such that the energy transfer was enhanced with increasing pH; with a maximum at pH $\simeq$ 4.5-5. Also noteworthy was the fact that at pH = 5 to 6.5 degassing (nitrogen bubbling) of the solution of orotic acid and europium (OA = 1×10^{-4} M) decreased the optical density of the solution at λ = 280 nm (λ_{max} for OA) and an increase in the optical density of λ = 300 nm was noticed. Lamola[21] in his work used optically thick solutions where this effect might have gone unnoticed. Furthermore, in the solution of OA (10^{-4} M) with various concentrations of Eu^{3+} ions the emission intensity of Eu^{3+} ions when excited at 394 nm where orotic acid has no absorption, was not proportional to their concentrations. This dependence became more and more conspicuous with increasing pH.

Blocking of the carboxyl group of OA by making isopropyl or methyl esters did not affect its dimerization quantum yield. This photodimerization was also taking place exclusively through the excited triplet state of the ester as revealed by oxygen quenching studies.[29,30] These esters (compared to orotic acid) were less efficient sensitizers of Eu^{3+} fluorescence. Energy transfer work with various orotic acid derivatives suggested that there is certain structural requirements of the functional groups on the donor for efficient energy transfer to occur and it was apparent that some kind of association between orotic acid and Eu^{3+} was responsible for energy migration as opposed to

purely collisional energy transfer invoked by other workers.

We found that orotic acid and 3-methyl orotic acid form 1:1 complex with Eu^{3+} whereas no UV detectable complex formation was noticed with methyl and isopropyl orotates, 1-methyl and 1,3-dimethylorotic acid.[31] We have no more doubt that the energy transfer is occurring through complex formation. Here we present our data on energy transfer studies with various orotic acid derivatives using Eu^{3+} as an acceptor in aqueous solutions; the photodimerization quenching by Eu^{3+} and propose a kinetic model to account for our results. We feel that this model can also help to explain and rationalize some of the earlier observations by other workers in this and related systems. Our results on complex formation studies will be presented in Chapter II.

In our kinetic model we have included only the excited triplet energy transfer occurring as we do not believe the singlet transfer is taking place in solutions of unchelated Eu^{3+} ions. Arguments in support of our model will be provided in the discussion part.

PHOTO-REACTION AND ENERGY TRANSFER KINETICS

The kinetic model is most readily described by the following set of twelve equations, which define monomolecular and bimolecular processes which follow the initial excitation of the donor (D). The donor states are characterized by left superscripts 1 and 3 to represent singlet and triplet states respectively. The lowest excited singlet and triplet states of the donor are assumed to lie at a higher energy state than the emissive levels of Eu^{3+}. [^5D_o level of Eu^{3+} lies at 17.3 kK = 49.48 K cal/mole.]

	Reaction	Rate
1)	$^1D + h\nu \rightarrow {}^1D^*$	I_{abs}
2)	$^1D^* \rightarrow {}^1D + \Delta$ (heat)	$^1k_{nr}\ [^1D^*]$
3)	$^1D^* \rightarrow {}^1D + h\nu'$	$^1k_r\ [^1D^*]$
4)	$^1D^* \rightarrow {}^3D^*$	$^1k_{isc}\ [^1D^*]$
5)	$^3D^* \rightarrow {}^1D$	$^3k_{nr}\ [^3D^*]$
6)	$^3D^* + {}^1D \rightarrow D_2$ (dimer)	$^3k_{dm}\ [^3D^*]\ [^1D]$

	Reaction	Rate
7)	$^3D^* + [^1D\text{-}\ Eu^{3+}] \rightarrow {}^1D + [^3D^* - Eu^{3+}]$	$^3k_t'\,[^3D^*]\,[^1D_{cx}]$
8)	$[^3D^*\ Eu^{3+}] \rightarrow [^1D\text{-}\ Eu^{3+}]$	$^3k_q\,[^3D^*\text{-}\ Eu^{3+}]$
9)	$[^3D^*\text{-}\ Eu^{3+}] \rightarrow {}^1D + Eu^{3+}$	$^3k_{dis}[^3D^*\text{-}\ Eu^{3+}]$
10)	$[^3D^*\text{-}\ Eu^{3+}] \rightarrow [^1D\text{-}\ Eu^{3+*}]$	$^3k_t\,[^3D^*\ Eu^{3+}]$
11)	$[^1D\text{-}\ Eu^{3+*}] \rightarrow [^1D\text{-}\ Eu^{3+}]$	$k_{nr}^{Eu}\,[^1D\text{-}\ Eu^{3+*}]$
12)	$[^1D\text{-}\ Eu^{3+*}] \rightarrow [^1D\text{-}\ Eu^{3+}] + h\nu$	$k_r^{Eu}\,[^1D\text{-}\ Eu^{3+*}]$

Here $[^1D\text{-}\ Eu^{3+}]$ indicates the 1:1 europium (III), donor complex concentration. The definition of most rate constants is self evident. The left superscript of k refers to the multiplicity of the molecules whose energy is being radiated (r), dissipated non-radiatively (nr), quenched (q), undergoes intersystem crossing (isc) or is transferred (t) with the symbol characterizing the particular mechanism appearing as the subscript of k. The subscript cx denotes the donor complex of Eu^{3+}. k_{dm} is the rate at which the uncomplexed donor (orotic acid) undergoes photodimerization when the triplet donor collides with a ground state donor molecule. Not included in this scheme is a process in which an excited donor molecule is quenched by a

ground state donor molecule without producing a dimer. This process can be assumed to have the same probability as that of the dimerization.

Earlier[31] we have found that the value for the stability constant for the process OA + $Eu^{3+} \rightleftharpoons$ [EuOA] is 44.9 at pH = 5.05. This means at pH 5 less than 1% of orotic acid or Eu^{3+} is complexed in a (10^{-4} M) equimolar Eu^{3+} and orotic acid solution. Thus energy absorbed directly by the complex is quite negligible compared to absorption by uncomplexed orotic acid. Nevertheless there will be an energy transfer occurring between uncomplexed excited triplet orotic acid and the complexed orotic acid in a thermoneutral process ($^3k_t'$). Once the orotic acid in the complex is excited to triplet state its triplet energy can be dissipated by three different possible processes indicated by equations 8, 9 and 10. Here ^3k_q represents the bimolecular rate constant by which the Eu^{3+} in the complex can quench the triplet orotic acid in the complex, ^3k_t the energy transfer rate for energy transfer from excited ligand to metal ion and $^3k_{dis}$ is the rate for photodissociation of the complex. When Eu^{3+} has been excited, either by direct absorption of light or by energy transfer, it may lose its energy either radiatively (k_r^{Eu}) or nonradiatively (k_{nr}^{Eu}) with the probability for radiative deexcitation given by

$$\beta = \frac{k_r^{Eu}}{k_r^{Eu} + k_{nr}^{Eu}} \qquad (13)$$

For photodimerization in the presence of Eu^{3+} ions we have

$$I_{abs} = [{}^1k_r + {}^1k_{nr} + {}^1k_{isc}][{}^1D^*] \tag{14}$$

$${}^1k_{isc}\,[{}^1D^*] = [{}^3k_{nr} + {}^3k_{dm}[{}^1D] + {}^3k_t'\,[{}^1D_{cx}]]\,[{}^3D^*] \tag{15}$$

$$\phi^D = \frac{{}^3k_{dm}\,[{}^3D^*]\,[{}^1D]}{I_{abs}} \tag{16}$$

$$\phi^D = \frac{{}^3k_{dm}\,[{}^3\emptyset^*]\,[{}^1D]\cdot\phi_{isc}}{[{}^3\emptyset^*]\,[{}^3k_{nr} + {}^3k_{dm}({}^1D) + {}^3k_t'\,({}^1D_{cx})]} \tag{17}$$

$$\text{where } \phi_{isc} = \frac{{}^1k_{isc}}{{}^1k_r + {}^1k_{nr} + {}^1k_{isc}} \tag{18}$$

Now for the equilibrium

$$OA + Eu^{3+} \rightleftharpoons [Eu^{3+}OA] \quad K = \frac{[EuOA]}{[OA]\,[Eu^{3+}]} \tag{19}$$

The concentration of the complex EuOA can be approximated by $K[OA][Eu^{3+}]$. Putting this value in equation (17) and rearranging gives

$$\frac{1}{\phi^D} = \frac{{}^3k_{nr} + {}^3k_{dm}\,[{}^1D] + K\cdot{}^3k_t'\,[{}^1D]\,[Eu^{3+}]}{\phi_{isc}\cdot{}^3k_{dm}\,[{}^1D]} \tag{20}$$

$$\frac{1}{\phi^D} = \frac{1}{\phi_{isc}}\left[1 + \frac{{}^3k_{nr}}{{}^3k_{dm}\,[{}^1D]} + \frac{K\cdot{}^3k_t'}{{}^3k_{dm}}[Eu^{3+}]\right] \tag{21}$$

This equation predicts that for a fixed orotic

acid concentration ($[^1D]$) plots of reciprocal of dimerization quantum yield verses Eu^{3+} concentration should be linear. The value of K is of course pH dependent.

An expression to relate the quantum yield of sensitized Eu^{3+} emission to Eu^{3+} concentration can be obtained as follows: Energy transferred to complex is given by equation (7).

$$I_t = {}^3k_t'[{}^3D^*]\cdot K[{}^1D][Eu^{3+}]$$

Energy emitted as fluourescence will be given by

$$\phi_r^{Eu^{3+}} = \frac{I_t\cdot {}^3k_t\cdot\beta}{{}^3k_q+{}^3k_{dis}+{}^3k_t}$$

$$\frac{\phi_r^{Eu^{3+}}}{\beta} = \frac{{}^3k'_t[{}^3D^*]\cdot K[{}^1D][Eu^{3+}]\cdot {}^3k_t\cdot\phi_{isc}}{[{}^3D^*][{}^3k_{nr}+{}^3k_{dm}({}^1D)+{}^3k'_t\cdot K({}^1D)(Eu^{3+})][{}^3k_q+{}^3k_{dis}+{}^3k_t]} \quad (22)$$

which gives

$$\frac{\beta}{\phi_r^{Eu^{3+}}} = \frac{[{}^3k_q+{}^3k_{dis}+{}^3k_t]}{{}^3k_t\cdot\phi_{isc}}\cdot\frac{1}{K}\left[\frac{{}^3k_{nr}+{}^3k_{dm}({}^1D)+{}^3k'_t\cdot K({}^1D)(Eu^{3+})}{{}^3k'_t({}^1D)(Eu^{3+})}\right] \quad (23)$$

$$\frac{\beta}{\phi_r^{Eu^{3+}}} = \frac{{}^3k_q+{}^3k_{dis}+{}^3k_t}{{}^3k_t\cdot\phi_{isc}}\left[1+\frac{1}{K}\frac{{}^3k_{nr}+{}^3k_{dm}({}^1D)}{{}^3k'_t({}^1D)(Eu^{3+})}\right] \quad (24)$$

This equation predicts that the reciprocal quantum yield

for sensatized europium emission should be linear with $[Eu^{3+}]^{-1}$ concentration for a fixed orotic acid concentration. The increase in stability constant K will also enhance the europium emission. From equation (21) we obtain

$$\text{intercept/slope} = \frac{{}^3k_{dm}({}^1D) + {}^3k_{nr}}{K \cdot {}^3k_t'[{}^1D]}$$

and from equation (24) $\text{slope/intercept} = \dfrac{{}^3k_{dm}[{}^1D] + {}^3k_{nr}}{K \cdot {}^3k_t'[{}^1D]}$

Thus, if our kinetic system is valid we should obtain identical values by above operations.

Results

Figure 2 represents the plot of $1/\phi_D$ vs Eu^{3+} concentration at pH 5-5.5. Orotic acid concentration was fixed at 10^{-4} M. We obtained a good straight line as predicted by equation 21. The intercept value we obtain from equation 21 at $[Eu^{3+}] = 0$.

$$\frac{1}{\phi_{isc}} \left[1 + \frac{{}^3k_{nr}}{{}^3k_{dm}[{}^1D]}\right] = 37 \qquad (1)$$

Johns and coworkers have reported their $\frac{{}^3k_{nr}}{{}^3k_{dm}}$ value for orotic acid photodimerization[28] which if we use in equation 1 we get

$$\frac{1}{\phi_{isc}} \left[1 + \frac{2 \times 10^{-5}}{1 \times 10^{-4}}\right] = 37 \quad \phi_{isc} = 0.0324 \qquad (2)$$

This value is about half as much as Johns' value (ϕ_{isc} = 0.062). Nevertheless their intersystem crossing efficiency value is at pH 3.3. At pH 5-5.5 in our work the photodimerization quantum yield will be smaller than that at pH 3.3. As mentioned earlier in the text, the photodimerization of orotic acid is an inverse function of pH. In

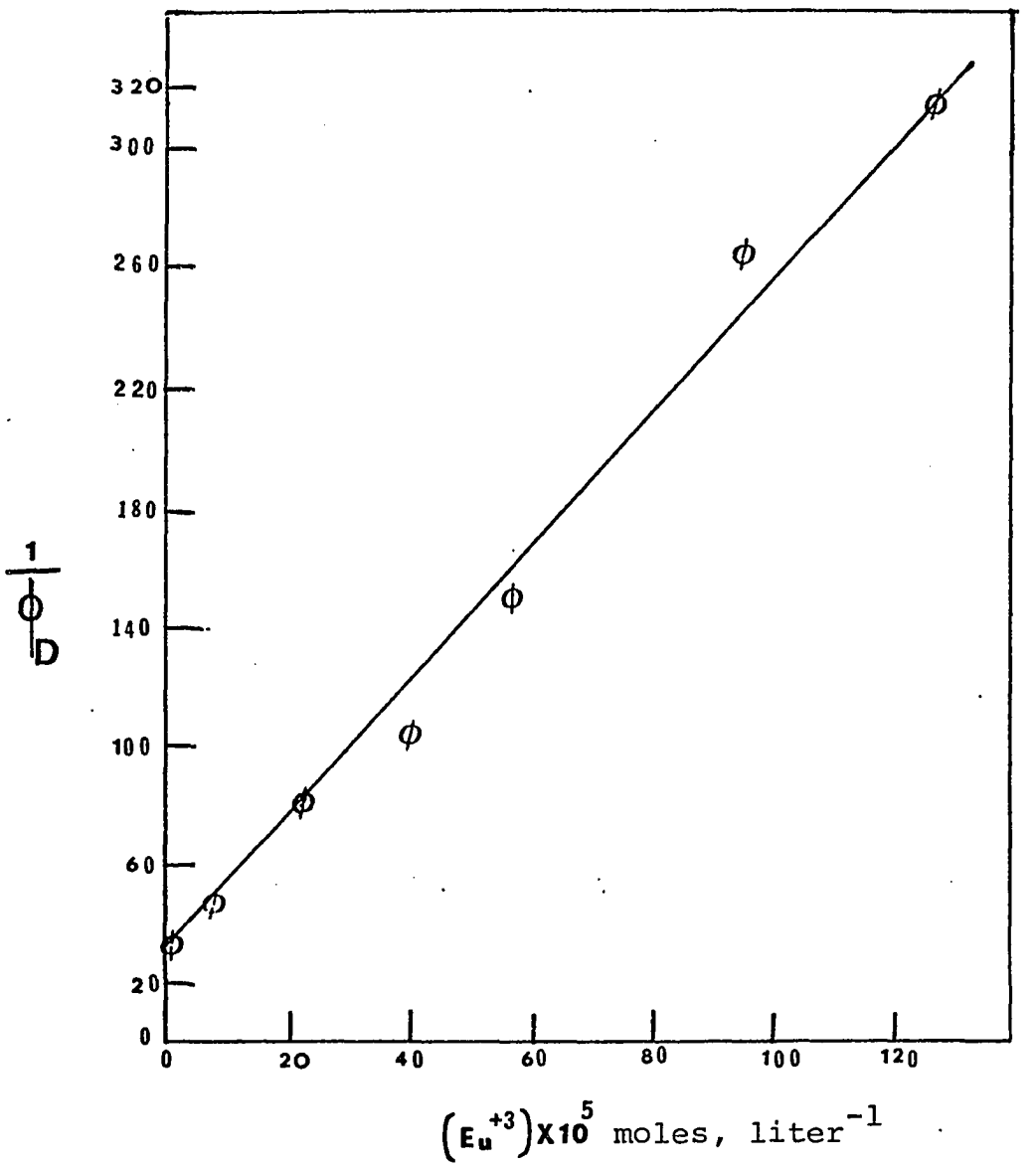


Figure 2. Quenching of Orotic Acid (OA) photodimerization by Eu^{3+} ions at pH 5∿5.5 (Eq. 21). OA conc. is 10^{-4} M in H_2O

essence the $\frac{^3k_{nr}}{^3k_{dm}}$ value at pH 5-5.5 will be smaller than that at pH 3.3. Our ϕ_{isc} value thus is in a reasonably good agreement with Johns' value. Figure 3 is a plot of reciprocal orotic acid sensitized Eu^{3+} emission quantum yield versus $[Eu^{3+}]$ at PD=5. Here again as expected by equation 24 of our kinetic model we obtained an excellent straight line. At the highest concentration of Eu^{3+} used (2×10^{-3} M) we did not see any sharp change in the line slope in contrast to Lamola and Eisinger's observation.[21]

As we have mentioned before a very interesting feature of equations 21 and 24 is that the equation 21 yields intercept/slope =

$$\frac{^3k_{dm}[^1D] + {}^3k_{nr}}{K \cdot {}^3k'_t[^1D]}$$

and the same value is obtained from equation 24 by taking slope/intercept. From Figure 2 we obtain intercept/slope = 1.568×10^{-4} and from Figure 3 gives slope/intercepts = 1.266×10^{-4} in a fairly good agreement with each other. We thus have

$$\frac{^3k_{dm}[^1D] + {}^3k_{nr}}{K \cdot {}^3k'_t[^1D]} \approx 1.5 \times 10^{-4} \qquad (3)$$

We have earlier evaluated K the stability constant value for the equilibrium $OA + Eu^{3+} \rightleftharpoons (OA\text{-}Eu^{+})$

$$K = \frac{[OAEu]}{[OA]\,[Eu^{3+}]} \qquad (4)$$

where OA and Eu^{3+} represent the concentration of free orotic acid (uncomplexed) and free Eu^{3+} ions, respectively, and OAEu is the complex concentration. At pH = 5.5 we have obtained $K = 1.473 \times 10^{2}$. This k value indicates that pH = 5.5 just about 5 to 7% orotic acid is complexed. We can approximate $^{1}D \approx 10^{-4}$ M.

Rearranging equation 3 gives

$$\frac{{}^{3}k_{nr}\left[1 + \frac{{}^{3}k_{dm}}{{}^{3}k_{nr}}\,[{}^{1}D]\right]}{K \cdot [{}^{1}D] \times 1.5 \times 10^{-4}} = {}^{3}k_{t}' \qquad (5)$$

By flash photolysis technique Johns[32] et al. have obtained ${}^{3}k_{nr} = 1.25 \times 10^{-4}$ sec^{-1}. Again using ${}^{3}k_{dm}/{}^{3}k_{nr}$ value from reference (28) we arrive at ${}^{3}k_{t}'$ value equal to 6.79×10^{9} sec^{-1}. This value represents the rate of diffusion controlled energy transfer from free excited triplet orotic acid to the ground state orotic acid bound with europium. Our kinetic model therefore is in perfect agreement with our experimental outcome.

Figure 4 shows a plot of reciprocal Eu^{3+} emission intensity corrected for inner filter effect vs reciprocal orotic acid concentration at pD = 4.5 and constant Eu^{3+}

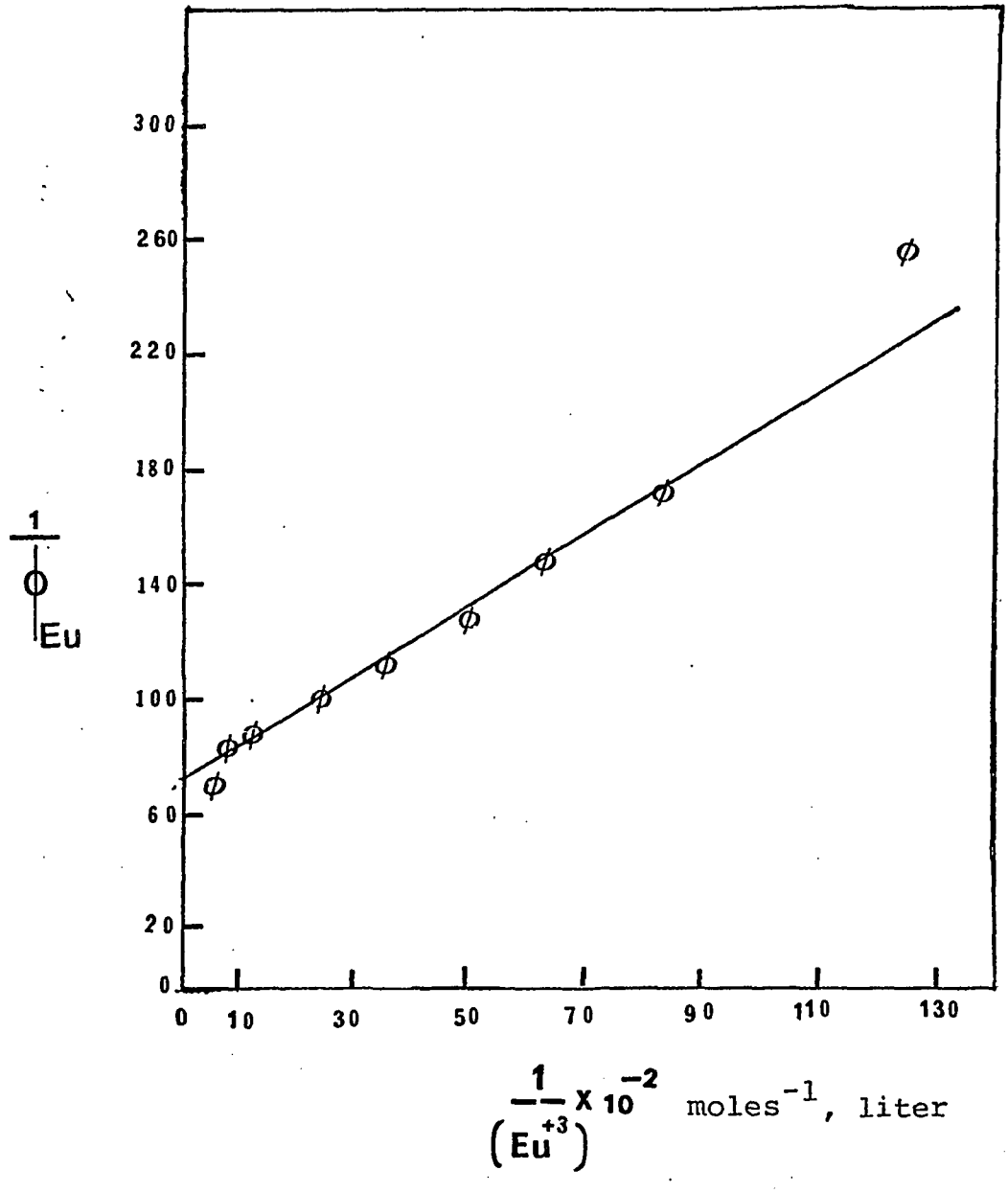


Figure 3. Quantum yield of OA sensitized Europium(III) emission as a function of Eu^{3+} conc. at pD = 5 (Eq. 24). OA = 10^{-4} M in D_2O

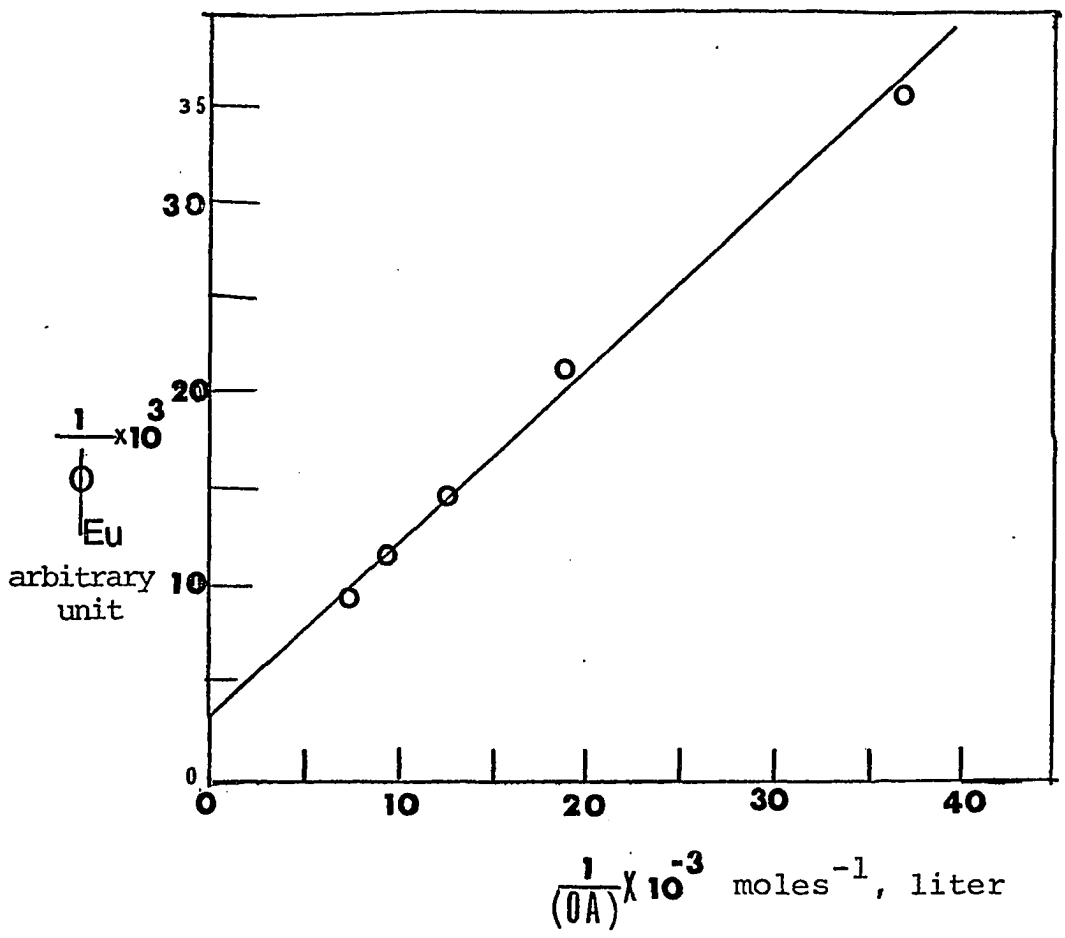


Figure 4. Intensity of Orotic Acid sensitized Eu^{3+} fluorescence as a function of OA conc. at pD = 4.5 (Eq. 24). Eu^{3+} conc. was fixed at 10^{-3} M. (Fluorescence intensities were corrected for inner filter effect.)

concentration (10^{-3} M). Again a good straight line obtained reflects on the validity of equation 24 in the kinetic scheme.

In the absence of Eu^{3+} ions and oxygen the equation 21 takes the form

$$\frac{1}{\phi_D} = \frac{1}{\phi_{isc}} \left[1 + \frac{^3k_{nr}}{^3k_{dm}[^1D]}\right]$$

Figure 5 is a plot of $1/\phi_D$ vs reciprocal orotic acid concentration. From the intercept of this line we find ϕ_{isc} value equal to 0.0386 and the slope of the line gives $^3k_{nr}/^3k_{dm} = 8.9 \times 10^{-6}$ at pH 4.6. This value of $^3k_{nr}/^3k_{dm}$ is in very good agreement with Johns'[28] value (1.8×10^{-5} at pH = 3.3). Our ϕ_{isc} value obtained here matches very well with that obtained earlier from Figure 2 at pH 5.5.

Our results for Eu^{3+} quenching of methylorotate photodimerization are shown in Figure 6 where we have plotted reciprocal of dimerization quantum yield vs Eu^{3+} concentration (pH 4.5). We note here that at pH 4.5 quantum yield of dimerization for ester is about 85% higher than that for orotic acid at the same pH. Moreover there is no significant quenching of methylorotate triplet with added Eu^{3+} ions. At Eu^{3+} concentration (10^{-3} M) orotic acid photodimerization is quenched to the extent of 88% where as for methyl ester the quenching is about 19.6%.

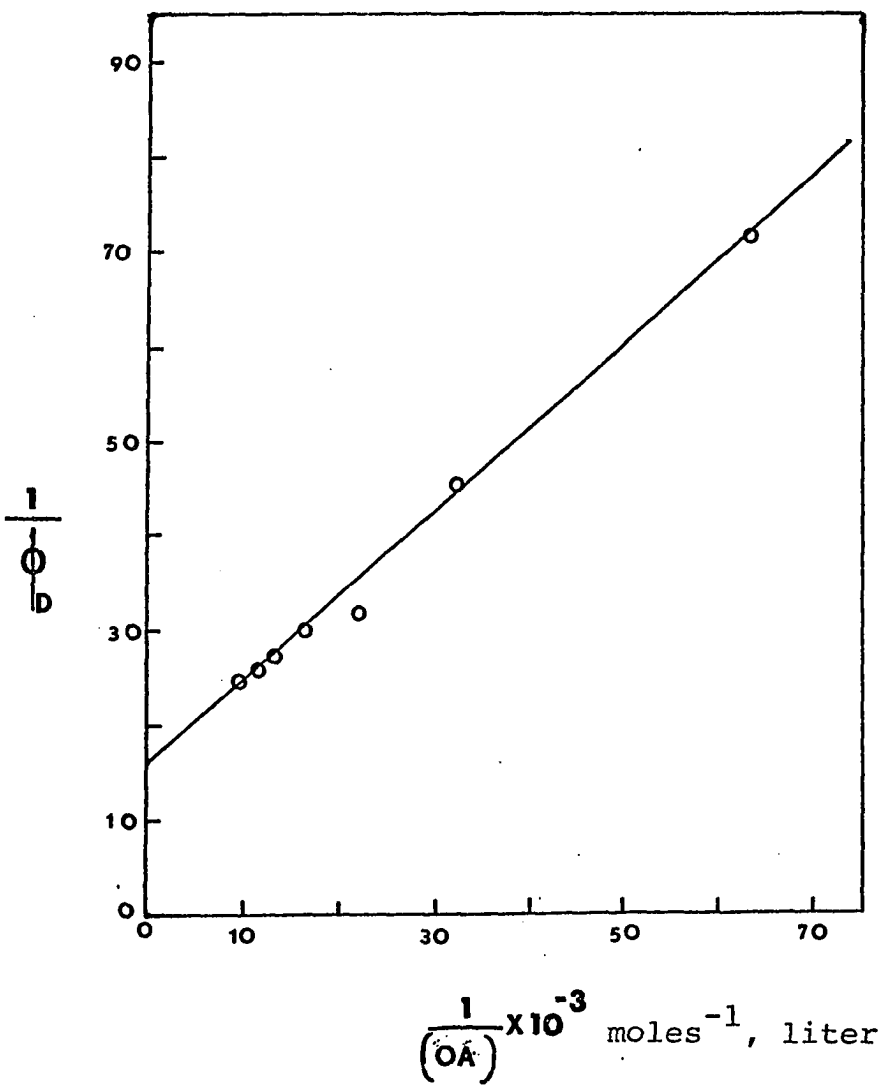


Figure 5. Quantum yield of Orotic Acid photodimerization as a function of OA conc. at pH = 4.5 (Eq. 24). Eu^{3+} = 0.0000

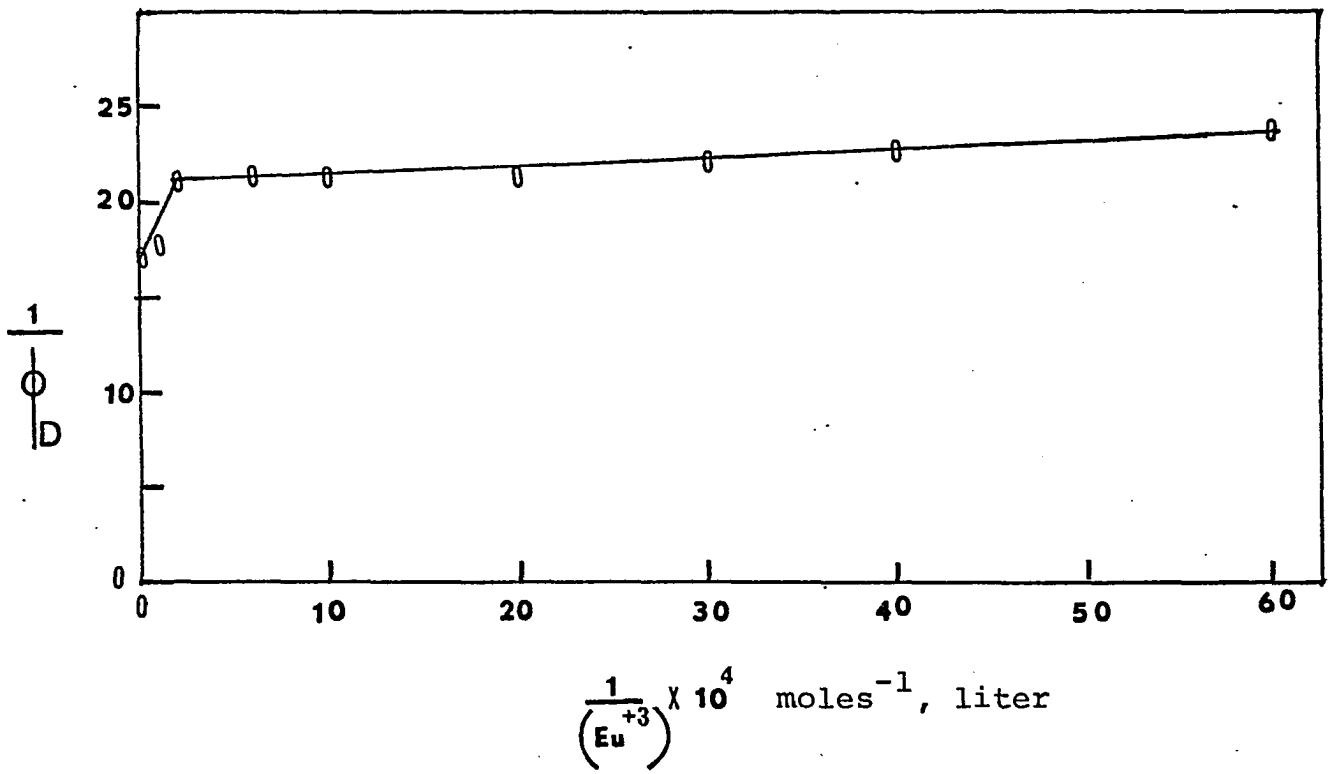


Figure 6. Quenching of Methyl-Orotate photodimerization by Eu^{3+} ions at pH = 4.5 (Methylorotate = 10^{-4} M)

This amount of quenching appears to be fairly constant even though Eu^{3+} ion concentration is increased. In Figure -7 we have plotted inverse of sensitized Eu^{3} emission quantum yield vs $[Eu^{3+}]^{-1}$ for the donor methylorotate at pD $\approx$ 4.6. We can see here that at Eu^{3+} concentration 10^{-3} M emission quantum yield for this ester is about 85% smaller than for corresponding value for Eu^{3+} orotic acid system. Apparently methylorotate even though it undergoes efficient photodimerization through the excited triplet, is a less efficient sensitizer of Eu^{3+} emission. The excited triplet level for methylorotate is not expected to differ from that for orotic acid.[33] We found that 3-methylorotic acid (^{3}D = 60 kcal/mole) photodimerizes with quite identical efficiency to that of orotic acid. It was also very excellent sensitizer of Eu^{3+} fluorescence. Figure 8 shows the energy transfer result for 3-methylorotic acid Eu^{3+} system at pH = 4.7. The slope/intercept value from this plot yielded a value 2.17×10^{-4} which is very similar to that obtained in case or orotic acid. These results parallel with our complex formation studies, where we noticed that 3-methylorotic acid forms 1:1 complexes with various metal ions including Eu^{3+} ion and the complex stability constant had almost the same magnitude as that for orotic acid - Eu^{3+} complex.

1-Methyl and 1,3 dimethylorotic acid are known to undergo photodimerization in frozen media.[29] At room tem-

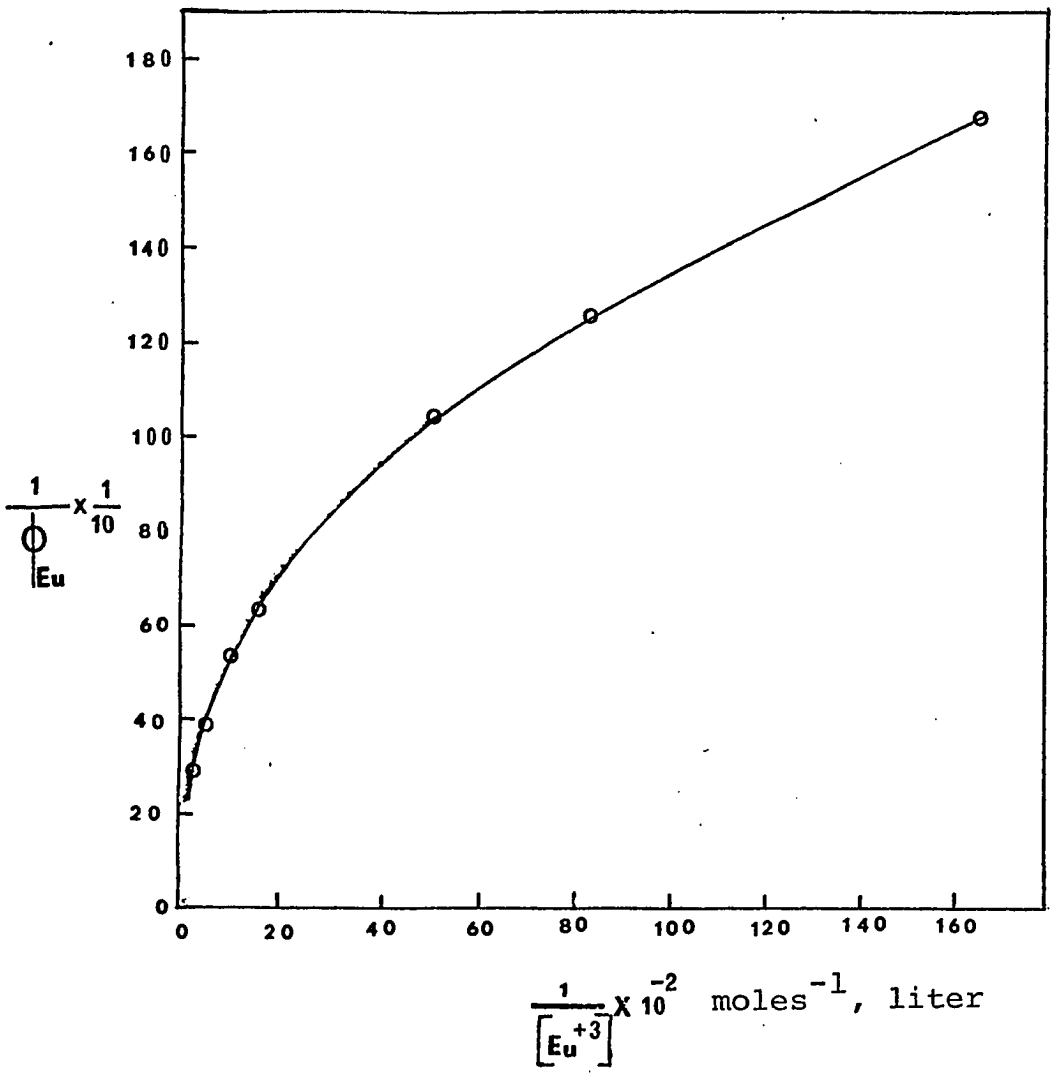


Figure 7. Quantum yield of Methylorotate sensitized Eu^{3+} emission as a function of Eu^{3+} conc. at pD = 4.5. (Methylorotate = 10^{-4} M)

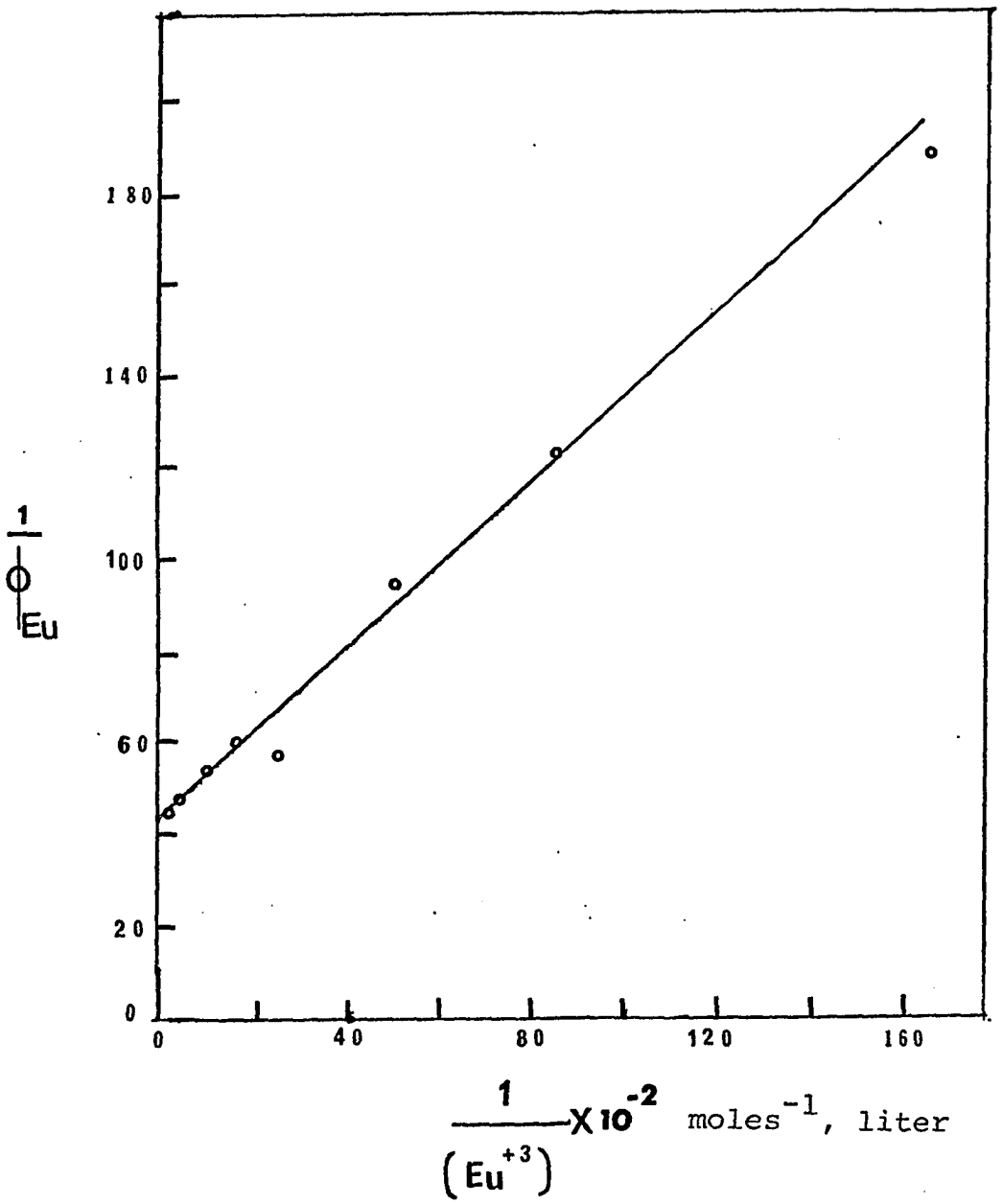


Figure 8. Quantum yield of Europium(III) emission as a function of Eu^{3+} conc. at pD = 4.7 sensitizer, 3-methylorotic Acid (10^{-4} M)

perature we found that for both these compounds quantum yields for photodimerization were much smaller than those for orotic acid, 3-methylorotic acid and methylorotate. These two compounds likewise were poor sensitizers of Eu^{3+} fluorescence, e.g., at pD = 6 even though a very weak europium emission could be observable with 2×10^{-3} Eu^{3+} (10^{-4} M donor) the quantum yields were about two orders of magnitude smaller than those for orotic acid-Eu^{3+} system. Photodimerization yields were immeasurably smaller. Thus for 1,3-methylorotic acid after 2.5 hours photolysis at 270 nm wavelength, under nitrogen atmosphere the original absorbance changed only by 0.072 units (0.690-.618) whereas under the same experimental conditions orotic acid takes less than 5 minutes to give the absorbance difference = .2. Further, for these two orotic acid derivatives the observed photochemical change was not photoreversible on irradiation at 240 nm (orotic acid, methylorotate and 3-Me OA photodimers are almost quantitatively split into monomers by short wavelength uv irradiation[30]). Absorbance change for these compounds can be attributed to their photohydration with low quantum yield. It is quite likely that the triplet state of these compounds may be too short lived to undergo any dimerization. In Figure 9 we have presented our energy transfer results for 1,3 dimethylorotic acid.

We also have noticed that isoorotic acid (5-carboxy-

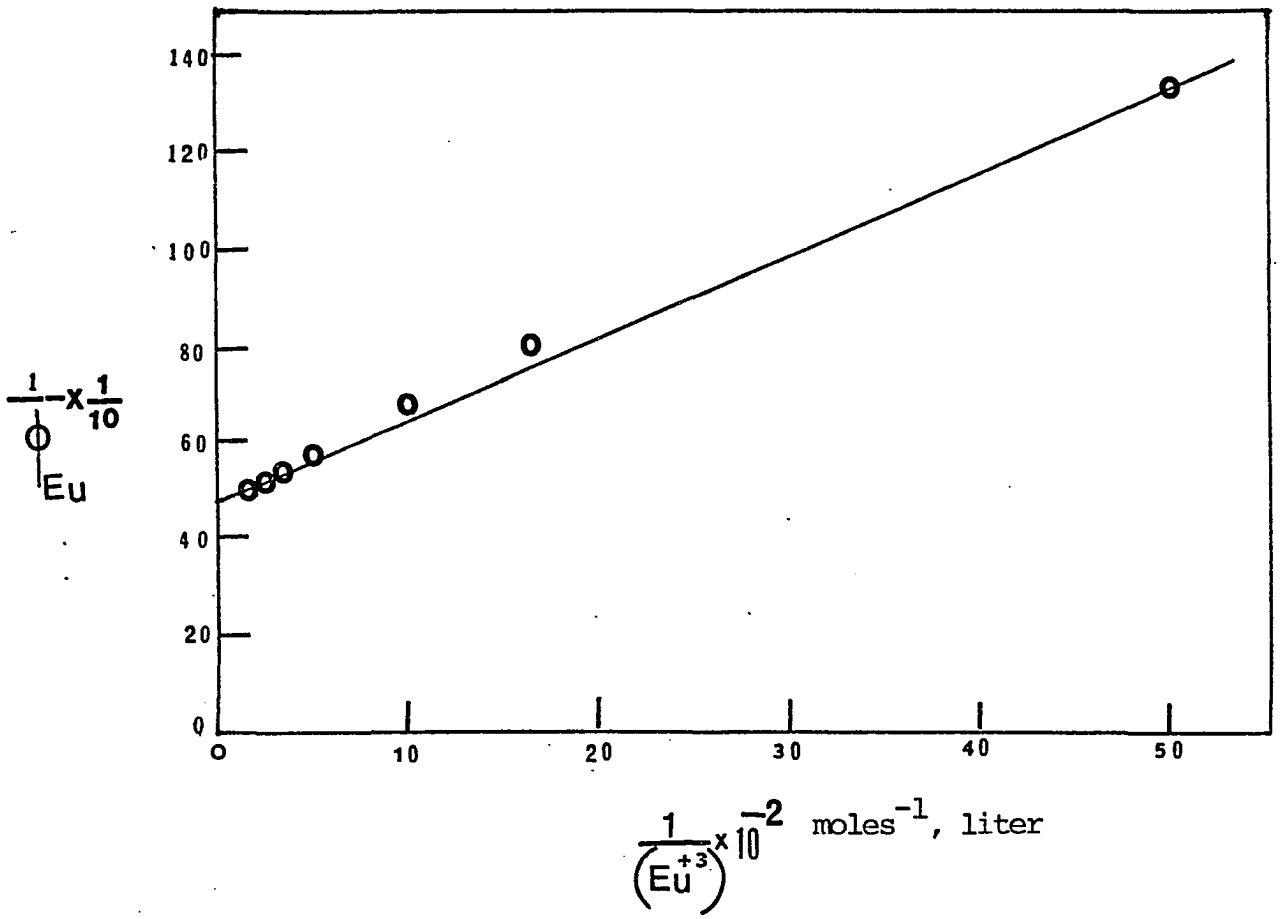


Figure 9. 1,3-dimethylorotic acid sensitized Eu^{3+} fluorescence at pD = 4.7. Emission quantum yield as a function of Eu^{3+} conc. (1,3 diMeOA = 10^{-4} M)

2,4-dioxypyrimidine), 5 methylorotic acid, cytosine-5-carboxylic acid are all resistant to uv irradiation and no photodimerization was observed at room temperature (298°K) photolysis.

Photodimerization efficiency for orotic acid is known to decrease with increasing pH.[28] This fact has been explained as a result of increasing electrostatic charge repulsion between carboxylate anions of triplet excited orotic acid and ground state orotic acid during collisional encounter. For orotic acid we measured the dimerization quantum yield at various pH values and obtained a sigmoidal curve. When quantum yield of sensitized emission was measured as a function of pH (pD) with a constant Eu^{3+} concentration (10^{-3} M) we found that at low pD values the emission was very weak and exhibited a sigmoidal increase with increasing pH, leveling off at pH values above 4 (Figure -10). Midpoint of both these curves is at pH (pD) = 2.8.

Charls and Riedel[10] have obtained similar results when they studied the terbium fluorescence emission in the aqueous solution of terbium ethylene-diamine tetra-acetate anion ($TbEDTA^-$) and the trivalent anion (SSA^{3-}) derived from 5-sulphosalicylic acid (H_3SSA). They found that Tb $EDTA^-$ forms 1:1 complex with SSA^{3-}. Complex stability was enhanced with increasing pH and resultant terbium emission followed a sigmoidal increase with pH.

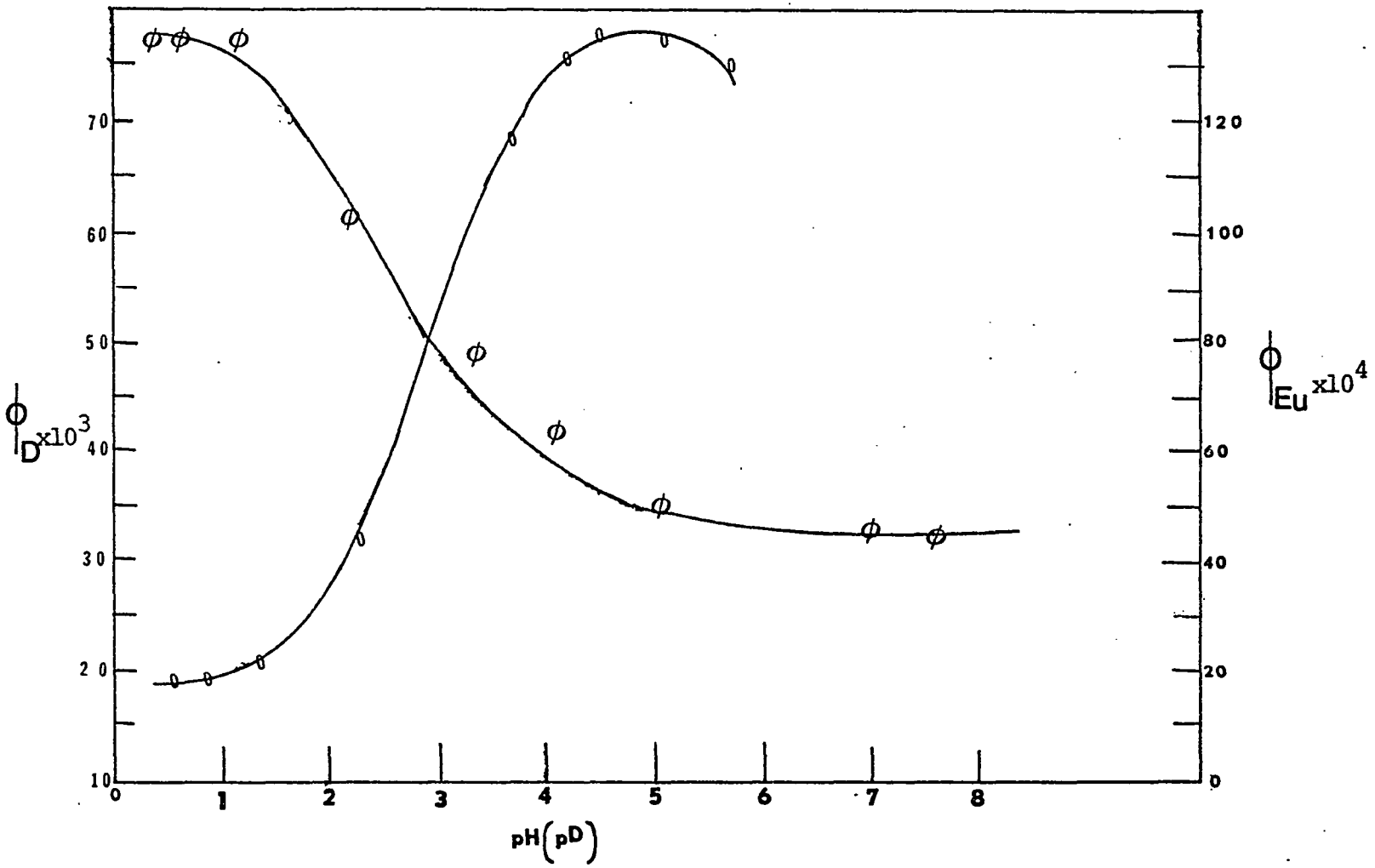


Figure 10. pH dependency of (a) OA photodimerization quantum yield (ϕ); (b) quantum yield of sensitized Eu^{3+} emission (0). OA conc. 10^{-4} M.

From the pH dependent mode of Eu^{3+} fluorescence sensitization exhibited by orotic acid and 3-Me OA we can safely conclude that the energy transfer is in fact occurring through complex formation. It appears from our results that the ground state pK_a of orotic acid is 2.8 rather than 1.8 obtained by Johns and coworkers.[28] The same pK_a value (2.8) is obtained by Bachstez[34] and also by Fox[35] with spectrometric method. For 3-MeOA also we arrived at the pK_a value of 2.8.

Photodimerization quantum yield for methylorotic was insensitive to pH, up to pH 6 as expected. Above this pH rapid hydrolysis of the ester complicated the experiments (Figure 11).

UMP, GMP and TMP[23] all were inefficient sensitizers of Eu^{3+} fluorescence and they were quite resistant to uv light. In all cases we observed mainly photohydration in aqueous solution photolyzed appropriately. Figure 12 shows our ump sensitized europium emission results and Figure 13 those for cytosine 5-carboxylic acid.

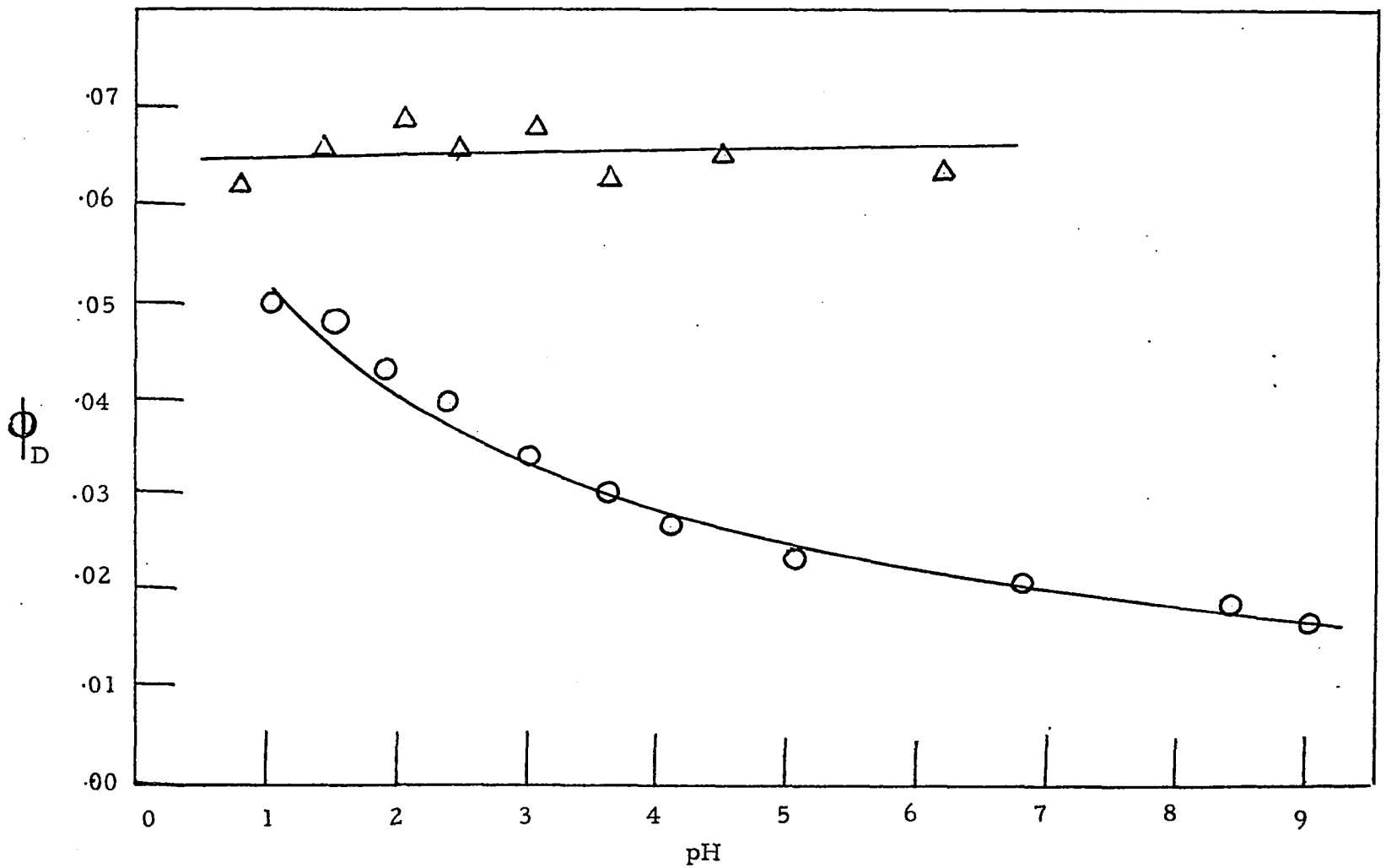


Figure 11. Effect of pH on quantum yield of photodimerization (a) for methylorotate (Δ) and (b) for 3-methylorotic acid (O).

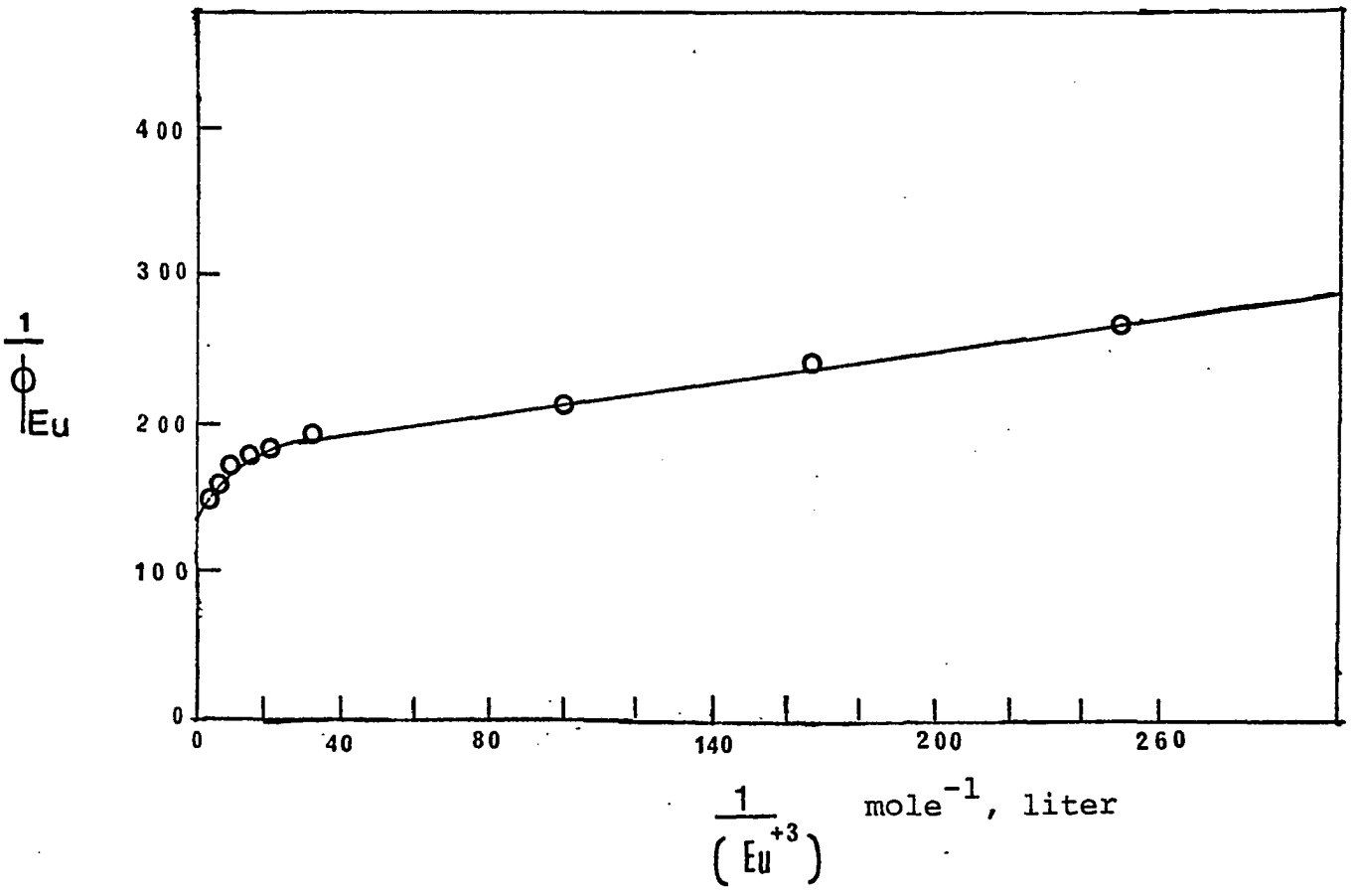


Figure 12. UMP sensitized Eu^{3+} emission quantum yield as a function of Eu^{3+} ions (UMP = 10^{-4} M) pD ≃ 4-5

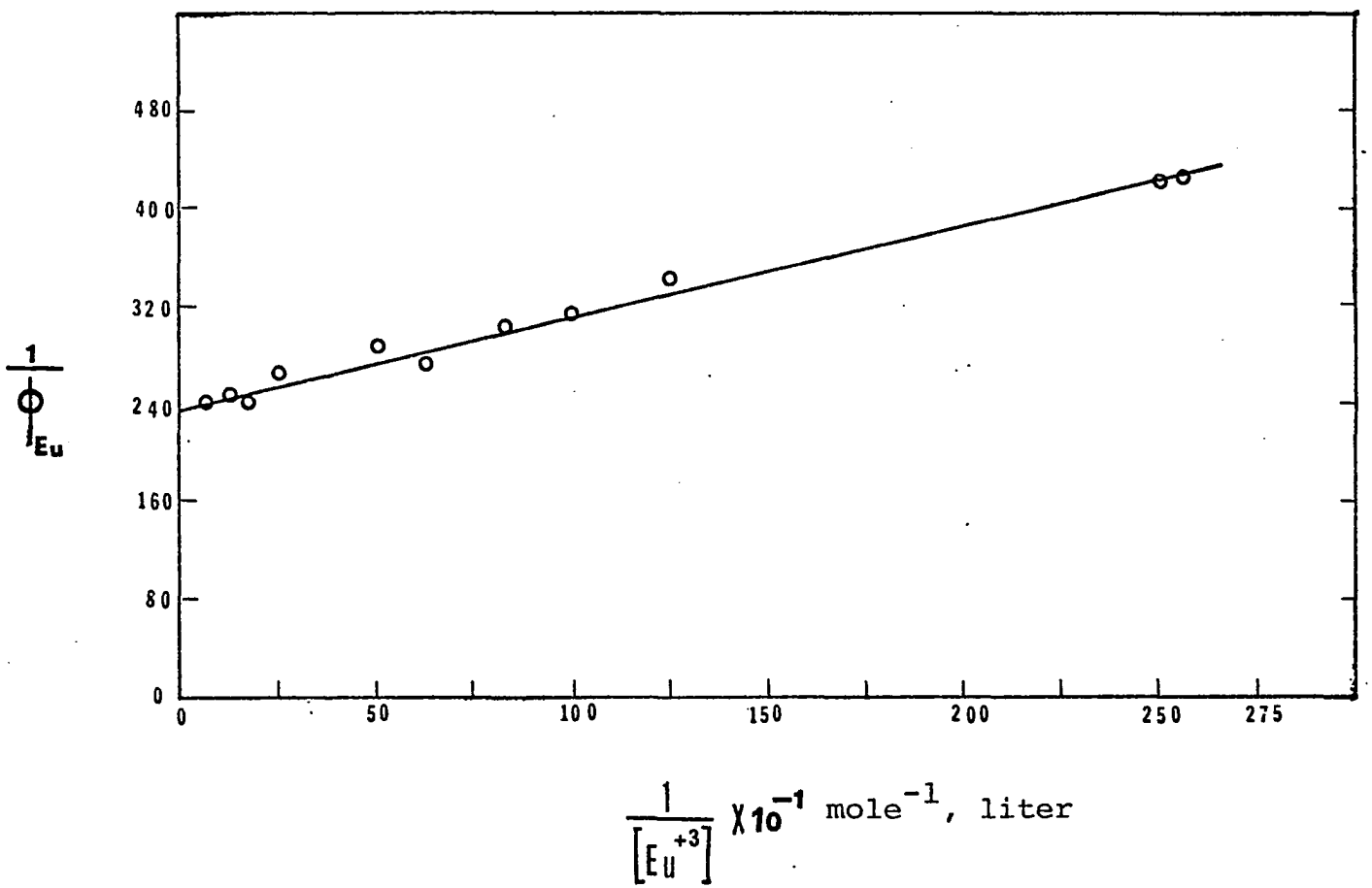


Figure 13. Cytosine-5-carboxylic acid as a sensitizer for Eu^{3+} emission. pD = 5. Donor conc. 10^{-4} M

DISCUSSION

We have shown in our work before that orotic acid and 3- methylorotic acid form 1:1 complex with various metal ions including Eu^{3+} ions.[31] No UV detectable complexes were obtained with methyl or isopropyl orotate, 1-methyl- and 1,3 dimethylototic acid. Our energy transfer results are consistent with the fact that it is this complex formation between Eu^{3+} and donor which is responsible for energy transfer. As explained in our kinetic model the energy transfer is taking place through a diffusion controlled process from a donor in triplet state to the donor complexed with Eu^{3+} ion in a fashion similar to inter-intra molecular energy transfer observed by El-Sayed and Bhaumik in rare earth chelates.[15] This fact is consistent with pH dependent mode of energy transfer, thus with increase in pH there is increase in the donor in complexed form, and the energy transfer process competes with photodimerization. Our $^{3}k_{t}'$ value is consistent with the normal theoretical diffusion control rates in aqueous solutions (5×10^{9} sec^{-1} liters, $mole^{-1}$).

Fillipescue and G. W. Mushush[19] have measured the energy transfer efficiency for various carbonyl compounds

and they observed N,N-dimethyl formamide was the best solvent for energy transfer compared to dimethyl phosphate, dimethyl sulfoxide or acetic anhydride which are not hydroxylic solvents. Thus in the latter three solvents the reduction in Eu^{3+} fluorescence may be due to association of Eu^{3+} with the solvent competing with the association of Eu^{3+} with the triplet donor.

Our model also explains the quenching studies done by different workers.[21,24] Wagner and Scott first noticed that photoreduction of p-methoxyvalerophenone in methanol solution is quenched by Eu^{3+} ions but the $k_q\tau$ value was only 375 ± 25 compared to $k_q\tau$ value for 2,5-dimethyl-2,4-hexadiene ($k_q\tau$ = 10,000 ± 100). Eu^{3+} was thus about 250 times less efficient a quencher than the diene. According to our model the quenching by Eu^{3+} is occurring through complex formation. It is obvious now that the Eu^{3+} concentration in the complex is 250 times smaller than the actual Eu^{3+} concentration used. In the case of orotic acid we have found that at pH 5 about 5-7% Eu^{3+} exists in 1:1 complex form. It appears that only 0.4% Eu^{3+} exists in the complex form with the above ketone and thus is very difficult to detect by conventional pmr or uv spectrophotometric techniques.

At high concentration of Eu^{3+} Lamola[21] has found that in the plots of $[\phi_{Eu}^{-1}]$ vs $1/[Eu^{3+}]$ the linear portion of the curve intercepts the line with greater slope.

We did not find this behavior in the case of orotic acid but ump and TMP did exhibit this sharp curvature. Lamola has invoked that at this point singlet transfer is initiated. Though Kleinerman has demonstrated the excited singlet transfer occurring in many lanthanide chelates where $S_1 \rightarrow SO$ fluorescence is quenched by Eu^{3+} there is no ample evidence so far for a singlet transfer to unchelated rare earth ions in fluid solutions. In light of our model we can explain this behavior. We have found that compared to other metal ions the Eu^{3+} complex is more weakly bound. Thus in energy transfer studies this can be either photo-dissociation or ground state dissociation, which is suppressed with high Eu^{3+} concentration enhancing the energy transfer to increased complex concentration. Williams and coworkers have found by nmr studies[36] that indole-3-yl acetate forms 1:1 complexes with various lanthanide ions at pH = 6. But due to highly dissociative nature of these complexes the ligand to metal ratio 1:30 had to be used to see any observable pseudocontact shifts.

Our model also agrees quite satisfactorily with viscosity and temperature effects.[18,19] Fillipscue and Mushrush have noticed that by changing the viscosity from 0.93 (N,N dimethyl formamide) to 15.85 (N,N dimethyl oleamide) 2-acetophenone sensitized Eu^{3+} emission dropped from .53 to zero. This obviously is due to reduction in ${}^3k_t'$ in our model with increasing solvent viscosity assuming

the association efficiency of Eu^{3+} with 2-acetonaphthone to be invarient in these solvents. Kleinerman[14] has pointed out that the more efficient sensitization of terbium chloride fluorescence in acetic acid than in decanoic acid[18] is not a manifestation of different viscosities of the two solvents but rather shorter triplet lifetime of p-dimethoxy benzophenone donor due to efficient photoreduction in the latter solvent.[37] We differ with Kleinerman's view as in the above mentioned N,N-dimethyl amide solvents used by Fillipescue and Mushrush the photoreduction is unprecedented.

At 77°K no Eu^{3+} sensitizations were observed at low Eu^{3+} concentration with p-anisaldehyde or p-hydroxybenzophenone by Heller and Wasserman.[18] This is consistent with our mechanism. Nonetheless, they could not explain the fact that at high concentrations of rare earth ions even at 77°K, sensitized rare earth emission was observable. At 77°K and with high Eu^{3+} concentration the complex concentration can be much higher than that at 300°K due to increase in stability constant with decreasing temperature and naturally the sensitized Eu^{3+} emission is the result of absorbed energy transformed from the donor in higher complexed concentration to Eu^{3+} in the complex.

Two prominent Eu^{3+} emission lines we observe in aqueous solutions are at 594 and 618 nm. Gallagher[38] has shown that the 618 nm band is the most sensitive to environmental changes, due to the fact that it is mainly an electric

dipole transition, while 594 nm is essentially a magnetic dipole. Complexation with the anion distorts strongly the symmetry about the ion thus allowing electric dipole transition. As a result of complex formation the intensity ratio of 592:618 emission lines is altered. Thus we observed with EDTA as a complexing agent the 618 nm europium emission line is more enhanced in H_2O compared to 594 line. Similar effect has been exhibited by NO_3^- ions and CNS^- ions which are known to form complexes with Eu^{3+} in aqueous solutions.[39] Some workers have argued against any complex formation between donor and Eu^{3+} ions since the emission spectrum of europium was identical in the absence of donor and that observed in sensitized emission.[18,21] Several explanations can be offered to explain the lack of intensity alteration even though the sensitization is occurring through complex formation.

1. For nitrate ions which form weak complex with Eu^{3+} the complex stability is solvent dependent and concentration dependent. Thus in aqueous solution the 618 nm line was of higher intensity than that of 592 nm line when $Eu(NO_3)_3$ concentration was 0.05 M. Reducing the concentration to 0.02 M the 592 line was seen to be more intense than 618 line. In our case the anion concentration of donor was much smaller than Eu^{3+} concentration (Eu^{3+} = 10^{-3} M, orotic acid 10^{-4} M).
2. CNS^- and EDTA form much stronger complex with Eu^{3+}

[log k = 17.35 for 1:1 EDTA Eu^{3+} complex). Complexes in our case are about 14 to 15 orders of magnitude weaker than EDTA complex (log k for Eu^{3+} orotic acid complex at pH 5.5 = 2.167). Thus the symmetry distortion about the cation will be much weaker.

3. More importantly the triplet life time for most of the donors are of the magnitude of 10^{-6} - 10^{-7} sec., whereas for Eu^{3+} ion in D_2O the lifetime for $5D_o$ excited state is 2.27 x 10^{-3} sec . Thus excited Eu^{3+} ions survive 3 to 4 orders of magnitude longer than donor excited triplet state.[40] The second order rate constants for complex formation for trivalent lanthanide ions are much faster: H_2O substitution in the inner coordination spectra of lanthanide ion are as fast as 10^8 sec^{-1} and complex formation rate constant with a bulky molecule like murexide with Eu^{3+} is also as fast as 10^7 - 10^8 sec^{-1}.[41] Thus it is quite likely that after energy transfer the longlived excited Eu^{3+} undergoes a metal exchange at a rate faster than 10^4 sec^{-1} and subsequently undergoes radiative deexcitation to ground state. Essentially then, even though the energy transfer is occurring through the Eu^{3+}-donor complex the europium emission is taking place through dissociated Eu^{3+} ion still in excited state. It is even quite likely that this dissociation is enhanced in the excited state. At present we have not enough information about rate constants for complex formation for lanthanide ions with various carbonyl and carboxylic ligands

but out above explanation is fairly reasonable to preclude any observable change in Eu^{3+} emission spectrum in the weaker complexes and thus the factor β in our kinetic model will be constant for various donors.

Since Eu(III) can form 1:1 complexes with carboxylate anion[36] it is quite possible that above pH 2.8 (pK_a of orotic acid) Eu(III) is associated in a 1:1 complex with -Coo- anion or orotic acid, 3-methylorotic acid, 1-methylorotic acid and 1,3-dimethylorotic acid. Owing to low electronegativity of Eu^{3+} (Chapter II) these complexes may be ionic in character and thus difficult to detect by uv spectroscopy. The concentration of these complexes with monoanionic ligands can be much higher than our estimated values as we have found that orotic acid and 3-methylorotic acid act as bidentate ligands. In this situation a part of the sensitized Eu(III) emission can be due to direct absorption of energy by orotic acid in the complex and subsequent intramolecular energy transfer to bound Eu(III), following intersystem crossing to triplet state. With either increase in pH or increase in Eu(III) concentration then the sensitized Eu(III) emission is expected to enhance as the probability of direct energy absorption by complex orotic acid and the probability of intermolecular energy transfer from free excited orotic acid to that in ground state complex would simultaneously increase. Nevertheless we have to bear in mind that $H_2O + R - COOH \rightleftharpoons R-\overset{O}{\overset{\|}{C}}-O^- + H_3O^+$ ($\tau \approx 10^{-13}$ sec). For 10^{-13} sec any particular association of Eu^{3+} with the carboxylate monoanion will not live long enough to transfer

energy. Poor fluorescence sensitization by 1-methyl and 1,3 dimethylorotic acid is obviously due to their poor intersystem crossing efficiency; which is reflected in their much smaller photodimerization quantum yields observed at room temperature photolysis in unfrozen solutions.

Failure of tryptophan to sensitize Eu^{3+} fluorescence[21] can be due to a possible electron transfer from tryptophan to Eu(III) with the reduction of the latter to bivalent state[53] or as the tryptophan is known to complex with Eu(III) through $-C\bar{O}\bar{O}$ anion[54] the distance between the donor part of tryptophan and Eu^{3+} is too long for an appreciable overlap between the donor and acceptor to occur for an effective energy transfer.

It is certainly difficult to prove that a part of energy transfer is not taking place through diffusion controlled collisional process but the absence of sensitized fluorescence with efficient triplet donors like naphthalene, fluorene, perylene, etc. indicates a lack of complex formation between these nonfunctional compounds and Eu^{3+} cation and efficient transfer from these compounds after adding on a carbonyl or carboxyl function then essentially leads to a weak complex formation. For ketones these may be either ground state complexes or the collision complexes between triplet ketone and the metal ions as indicated by Russian workers.[25,26]

Ketones are also known to undergo a photochemical reaction with Eu(III).[55]

In conclusion from our present knowledge we do not feel that lanthanide ions can be used as efficient triplet or singlet counters.

EXPERIMENTAL

Materials: $EuCl_3$ was purchased from Research Organic/Inorganic Chemical Corporation (ROC/RIC, Sun Valley, Calif. 91352) and was dried thoroughly over P_2O_5 in a vacuum dessicator. Europium oxide was prepared from $EuCl_3$ by the method of Dutt.[42] The amount of H_2O in $EuCl_3$ dried as above was found to be 3 moles of H_2O per mole of $EuCl_3$ by comparing the intensity of 595 nm emission line of Eu^{3+} in solution of $EuCl_3$ in H_2O and of Eu_2O_3 dissolved in aqueous HCl. Further drying was avoided as it normally leads to the formation of europium oxichloride.[43]

Deuterium oxide (D_2O) with 99.8% D was obtained from Stohler Isotope Chemicals (Rutherford, N. J.) or from Bio-Rad Laboratories (Richmond, Calif.).

Orotic and isoorotic acids (Sigma) were recrystallized three times through distilled water.

Deuterium chloride (DCl) was 38% solution in D_2O; 99% D (sIc).

Calcon (Pontachrome Blue Black) (Aldrich), N,N dimethyl 3-nitroaniline (Aldrich), quinine bisulfate (Eastman Kodak), rhodamine B (Aldrich) were reagent grade and were used without further purification.

Thymidine 5'-monophophoric acid and cytosine-5-carboxylic acid were obtained from Sigma and were stored in a dessicator inside a freezer until use.

UV spectra were taken with Hitachi Perkin Elmer double beam spectrophotometer (Coleman 124) in connection with Sargent recorder (Model SRG) and with Cary-18 spectrophotometer.

IR spectra were obtained with Beckman IR 18A with scan speed 22 minutes.

NMR spectra in deuterated solvents were measured on a Varian T-60 NMR spectrometer.

High purity nitrogen was supplied by Big 3 (Oklahoma City). Thin layer chromatography was done on Eastman chromagram sheets (13181 silica gel with fluorescent indicator No. 6060) with following eluting solvent systems.

A. n-butanol/acetic acid/H_2O 5:2:3

B. 1,4 dioxane/H_2O 90:10

C. isopropanol/H_2O 70:30

D. n-butanol saturated with H_2O

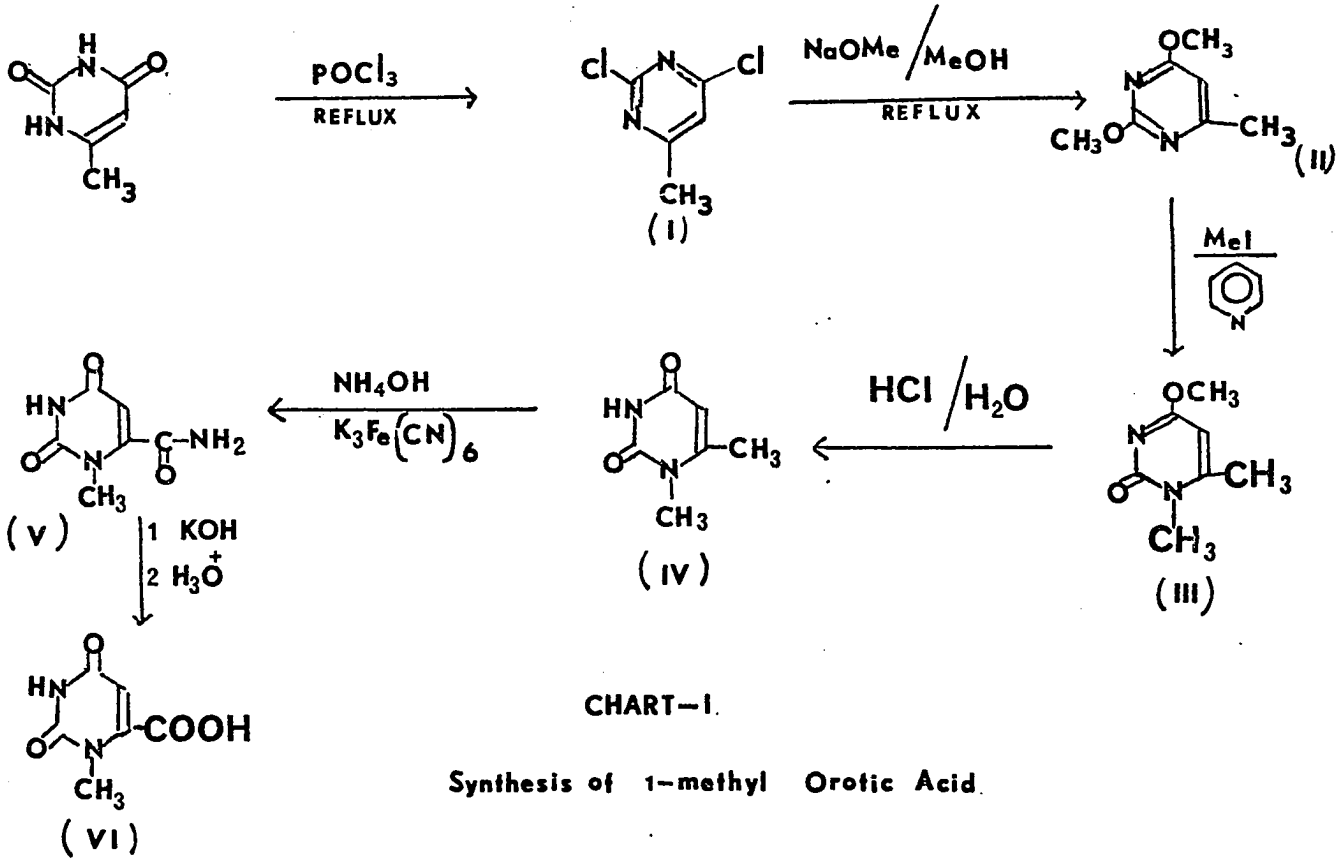
E. n-propanol/H_2O 70:30

F. n-butanol/acetic acid/ethanol 5:2:3

The tlc spots were viewed under short wavelength uv light.

Syntheses of Orotic Acid Derivatives

1-Methylorotic Acid.[44] This was prepared by following sequential steps (Chart I).



CHART—I.

Synthesis of 1-methyl Orotic Acid.

2,4 dichloro 6-methyl uracil (I): 10 g of anhydrous 6-methyl uracyl (Sigma) was refluxed in a hood with 40 ml of $POCl_3$ for 1 hour. The unreacted $POCl_3$ was removed under reduced pressure at 40°. The red yellow liquid was poured in ice cold H_2O to obtain yellow crystals of 2,4 dichloro 6-methyl pyrimidine. The crystals were washed several times with cold water till the filtrate was colorless. Yield; 10 g. (78%) m.p. 46-47°/ The compound was recrystallized from dry chloroform and stored in a dessicator.

2,6 dimethoxy 6-methyl uracil (II): 8 g. of I was dissolved in 50 ml of dry methanol and added slowly to a solution of 3 g of sodium metal in 40 ml of dry methanol with constant stirring. A vigorous reaction was started immediately. A precipitate of sodium chloride was produced and the heat of the reaction raised the temperature of the pot to boiling point. The mixture was refluxed for 1/2 hour, cooled and sodium chloride was filtered off. The methanol was removed under reduced pressure and the residual oil treated with 20 ml of 30% NaOH. From this the dimethoxy compound was extracted with ether. The ether extract was washed several times with H_2O, dried over Na_2SO_4 (anhydrous) and finally ether was distilled off. The yellow oily residue was distilled at 213°C at atmospheric pressure to give yellow oil which rapidly solidified after cooling. m.p. 69-70° yield = 6.8 g (90% of the theoretical yield).

2,4-Dimethoxy 6-methylpyrimidine (III): To a mixture of

II (5 g), 3.5 ml of methyl iodide and five drops of pyridine were added and the neatly stoppered flask allowed to stand for 24 hours at room temperature. A yellowish white solid was deposited on the side of the flask without much trouble. This solid was washed with cold ethanol and then recrystallized from hot ethanol by adding ether. yield, 4.7 g. (95%) m.p. 112.5-113°C.

1,6-dimethyl uracil (IV): 4 g of (III) were treated with 50 ml of 2 N, HCl for 2 hours and then neutralized with 10% aqueous $NaHCO_3$. The neutral solution was evaporated to dryness and the residue triturated with three 50 ml portions of chloroform. Evaporation of chloroform gave yellowish crystals (m.p. 217-18). These crystals were dissolved in hot water, treated with Norit and cooled to give slightly tan crystalline IV. m.p. = 220°C. yield, 2.89g. (75%).

1-Methyl uracil-6-carboxamide (V): 800 mg of IV was dissolved in 32 ml of H_2O containing 8 ml of NH_4OH (conc.). Potassium ferricyanide (12.4 g) was added to this and the flask was loosely stoppered and heated to 50-70°C in a water bath for 9 hours. After addition of another 4 ml of NH_4OH the flask was cooled and the yellow crystalline mass of potassium ferrocyanide [$K_4Fe(CN)_6$] was filtered off. The filtrate was concentrated on a steambath till free from ammonia. The neutral solution was adjusted to a volume of 20 ml and cooled to yield colorless glistening plateletes

of V with slight contamination of ferrous salt. The product was recrystallized from slightly acidic H_2O three times to yield 350 mg of V. Yield; 36% m.p. 327°C dec. (corr.).

1-Methylorotic Acid (VI): 300 mg of (IV) was heated on a steam bath with 20 ml of .2 N KOH during which time the volume was kept constant by repeated addition of water. When the evolution of ammonia ceased the basic solution was filtered and strongly acidified with concentrated HCl. The acidic solution was chilled to give 200 mg of VI. This was washed free of HCl and recrystallized from hot ethanol. Yield; 66% m.p. 273-75°C (with effervescence and resolidification)

UV λ_{max} = 272 nm (ε = 9200) at pH = 3.3

R_f values = .445, .36, .570, .103, .56 in solv. syst. ABCDE.

NMR (dmso - d_6): δ3.3 (3H,S); δ5.9 (1H,S); δ11.52 (1H, b.s.NH)

3-Methylorotic Acid:[38] This was prepared by the following procedure:

3 g of orotic acid (0.0192 mole) was dissolved in 50 ml of H_2O containing .12 moles of NaOH and the solution treated with 7 ml (.07 mole) of dimethyl sulfate. After warming the solution for 20 minutes on a steam bath the solution became homogeneous. At the end of 1 hour the reaction mixture was cooled, filtered and the filtrate was strongly acidified with concentrated HCl. A solid white mass was precipitated. This was collected, dried and triturated several times with hot ethanol to separate 3-methylorotic acid from unchanged

orotic acid. The combined alcoholic extract was cooled to give (1.5 g) of 3 methyl compound. This was purified from any 1,3 dimethyl compound by prep. chromatography on silica gel using solvent system B for elution. Finally the compound was recrystallized from hot water to give white crystalline 3-methylorotic acid. m.p. 311-312°C. UV λ_{max} 280.5 nm (ε = 6495) at pH = 2.1. Yield = 30%.

R_f values: .53; .101; .53; .15; .585 in solv. syst. ABCDE.

NMR (DMSO-d_6) δ3.13 (3H,S); δ6.15 (1H,S) δ11.33 (1H,S NH)

1,3 Dimethylorotic Acid:[45] Orotic acid (3 g) was dissolved in 35 ml of 12% aqueous KOH and was treated with 4.8 ml (.05 moles) of dimethylsulfate for 30 minutes at 15-20°C. At this point 8 ml of 12% KOH and 1.7 ml (.02 mole) of dimethyl sulfate were added in 10 minutes. The mixture was stirred for 3 hours and strongly acidified with 24 c.c. of concentrated HCl. The acidified mixture was allowed to stand for 8 hours at room temperature. The precipitate was filtered, washed with distilled H_2O and after drying was extracted several times with hot ethanol. Evaporation of combined ethanol extract gave 2.2 g of 1,3 dimethylorotic acid. This was further purified by preparative chromatography on thick layer silica gel plates.

The exactly identical compound was obtained by the method of Fox.[44]

m.p. = 150-1°C

R_f values: .47; .178; .553; .189; .615 in sol. syst. ABCDE.

UV: λ_{max} 271 nm (ε = 9434; pH 6)

NMR: δ3.2 (3H,s), δ3.37 (3H,S), δ6.03 (1H,S)

Isopropyl Orotate:[46] 1 g. of orotic acid was stirred at room temperature with 6 ml of thionyl chloride and 5 drops of pyridine for a period of 7 hours. The mixture was then refluxed for 24 hours using a water condenser fitted with a drying tube. The yellowish reaction mixture was allowed to cool to room temperature. After suction-filtration the residue was transferred to a round-bottom flask containing 100 ml of dry isopropanol. A vigorous reaction was noticed with escape of HCl fumes. The flask was fitted with a water condenser and a drying tube and the heterogeneous mixture was stirred for 4 hours at room temperature and then boiled under reflux for 14 hours. At the end of this period all the solid was dissolved in isopropanol. The solution was cooled, filtered and isopropanol was removed under reduced pressure. The yellowish solid with significant odor of sulfur was dissolved in hot water and treated with norit. The recrystallized solid was then dissolved in 100 ml of methanol and passed over a column packed with 8 g Al_2O_3 (Merck acid washed) + 1 g. of decolorizing carbon (Eastman). This removed most of the yellow color and the sulfur odor. The solid obtained after evaporating the methanol was again recrystallized through water to give pale yellow crystalline needles of isopropyl orotate. m.p. 209°C. Yield 350 mg (28 %)

UV λ_{max} 285 nm (ε = 7200) pH = 6.1

R_f values .75; .67; .73; .73 in A,B,C,F solv. syst.

NMR (DMSO-d_6) δ1.3 (6H,d); δ5.1 (1H quintet); δ6.01 (1H,S) δ11.32 and δ11.09 (b.s. 1H for each NH)

Orotic Acid Methyl Ester:[46] 3 g. of orotic acid was added to 750 ml of dry methanol and the solution saturated with dry HCl gas (Matheson). The warm reaction mixture was refluxed overnight and the hot reaction mixture filtered from some unchanged orotic acid. Upon cooling, fine white crystalline needles were obtained. m.p. (243-245°C) (corr. to oil). The product was recrystallized from hot water.

Yield = 2 g (61%)

UV λ_{max} 283 nm (ε = 7243)

R_f values: .62; .63; .591; .448; .577 in A,B,C,D,E. solv. syst.

NMR (DMSO-d_6) δ3.87 (3H,S); δ6.03 (1H,S) δ11.066 (1H, bs NH) δ11.33 (1H bs NH)

Orotic Acid Photolysis

Our light source was a securely shielded (housing similar to Schoeffel[47]) 1000 W, GE BH6 air cooled lamp (operated vertically) combined with aluminum reflectors and a B&L high intensity monochromator with a variable outlet slit. Examination of the output beam with another monochromator showed at half height a peak width of 9 nm and negligible scattered light in the wavelength range 200-300 nm.

The samples were photolyzed at room temperature in a Teflon stoppered Beckman cuvette (1 x 4 x 1 cm). Two small holes were drilled in the Teflon stopper to serve as inlet and outlet for nitrogen gas. Solutions of orotic acid and its derivatives were freshly prepared prior to photolysis in triply distilled water. Nitrogen was passed over a column of silica gel, then bubbled through either H_2O or D_2O prior to entry into photolysis cell to avoid H_2O or D_2O losses due to evaporation during nitrogen bubbling.

3 ml of solution was photolyzed each time. N_2 was bubbled through this solution for a period of 10 minutes prior to photolysis, and during photolysis N_2 was bubbled into theupper part of the cuvette which was not exposed to impinging ultraviolet light. In addition, samples were stirred with a magnetic stirrer using Teflon coated mini (7 x 2 mm) stirring bar (Cole-Parmer). pH of the solution was measured before and immediately after photolysis and

the pH values shown in our data are the latter pH values. Orotic acid and its derivatives were photolyzed at 281 nm or at appropriate λ_{max} for the given compound and the rate of loss of orotic acid or the derivatives was followed by UV absorption studies.

Actinometry

Crystals of potassium ferrioxalate $K_3Fe(C_2O_4)_3 \cdot 3H_2O$ were prepared according to the procedure of Parker and Hatchard[48] and were stored in the dark in an amber colored bottle covered with aluminum foil to avoid any photodecomposition by any stray light. A mixture of 0.1 M potassium ferrioxalate in a 0.05 M H_2SO_4, 0.1% o-phenanthrolin monohydrate in water and the acetate buffer in the proportions of 10:4:6 (final pH = 3.7) respectively was chosen as a ready made actinometer according to a modification suggested by Kurien.[49] Quantum efficiency of the ferrioxalate so prepared was taken to be 1.245 at 281 nm and room temperature (298°K).

3 ml of the actinometric solution was photolyzed in a completely darkened light proof room for various lengths of time. The procedure for photolysis was identical to one mentioned earlier. The optical density of the photolyzed solution was checked at 510 nm rapidly and immediately after termination of photolysis with appropriate blank solution in the reference cell. After subtracting the optical density of unphotolyzed solution at the same wavelength a

plot was made for optical density vs. time of photolysis to obtain mean optical density change per second. The photon flux at a given wavelength of photolysis was then estimated by the following equation.

$$\text{Mean O.D./sec.} \times \frac{1}{1.245} \times \frac{3}{1000} \times \frac{1}{1 \times 10^4} = \text{No. of Einsteins} \quad (1)$$

absorbed per sec (1×10^4 is the molar extinction coefficient for Fe^{2+} at 510 nm).

Actual dose absorbed by the orotic acid sample was obtained by

$$I_o - I_t = I_{abs} = I_o\,(1-10^{-O.D.}) \quad (2)$$

where I_o and I_t are the intensities of incident and transmitted light respectively and O.D. is the optical density of 10^{-4} M orotic acid solution. Validity of the expression (2) was checked by keeping the actinometry solution next to orotic acid solution being photolyzed in identical cuvettes, where the change in actinometer optical density allowed the evaluation of I_t. Where the optical density fell from 0.7 to .4 a mean value of $10^{-O.D.}$ was obtained by taking the mean of $10^{-.7}$, $10^{-.65}$.....$10^{-.4}$ to be used in expression (2).

The quantum yield of dimerization was evaluated as follows:

$$\phi_D = \frac{\text{difference in O.D. at } \lambda = 278 \text{ nm}}{2 \times \varepsilon \times I_o \ (1 - 10^{-\text{O.D. mean}})} \qquad (3)$$

where I_o = Einstein's impinging per litre of photolyzed solution for a given length of time and ε is the extinction coefficient for O.D. at 278 nm. Quantum yields were obtained for various time intervals of photolysis and the plot of quant. yield vs time was extrapolated to zero time. The slope of the straight line portion of the curve was used to obtain instantaneous quantum yield for photodimerization. The reported values are mean of 3 such sets of experiments.

Photodimerization Quenching with Eu^{3+} ions: Stock solutions of $EuCl_3$ in triple distilled water were prepared and pH was adjusted by HCl and Na_2O_3 aq. solutions. Stock solutions were diluted to 25 ml volume to give final orotic acid concentration 10^{-4} M and different Eu^{3+} concentration. Photolysis was done as before. Solutions were unbuffered. Eu^{3+} concentration was varied from 10^{-4} to 10^{-3} M. For higher Eu^{3+} concentration time of photolysis had to be lengthened. Photolysis of other orotic acid derivatives was done in an identical fashion as above.

Fluorescence Measurements

Fluorescence measurements were done at room temperature with Hitachi Perkin Elmer fluorescence spectrometer M.P.F. -3 in connection with PE M.P.F. -3 recorder for output display. This instrument has the advantage that

the variation of the light intensity of the high pressure xenon lamp can be avoided by operating the instrument in ratio mode where the light from xenon lamp is intercepted by a beam splitter before entering the excitation monochromator. The portion of the beam which is deflected by the splitter strikes the reference photomultiplier where a reference signal is produced. This signal is used in a ratio recording mode allowing any correction to be applied for fluctuations in the lamp intensity.

Solutions of orotic acid (10^{-4}M) in D_2O, with various concentrations of $EuCl_3$ were excited in 1 x 1 x 4 cm fluorescence cells (all sides transparent). Fluorescence was viewed at right angle geometry. The fluorescence cells were tightly stoppered with Teflon stopper through which two small holes were drilled to serve as nitrogen inlet and outlet. pH (pD) of the solution was adjusted by DCl or anhydrous Na_2CO_3 dissolved in D_2O. Dry nitrogen was first bubbled through D_2O and then flushed through the cell solution for a period of 15 to 20 minutes. After flushing N_2 the Teflon stopper holes were quickly and tightly sealed with a piece of parafilm to avoid any air infiltration. This procedure was found fairly satisfactory as for a period of 5 to 10 minutes the fluorescence reading was unchanged.

The samples were excited with light of 281 nm with 5 nm excitation slit and 4 mm emission slit. The two

major peaks of Eu^{3+} fluorescence at λ = 594 nm and 618 nm account for 98% of the emission intensity. Hence the emission monochromator was scanned over the wavelength range 560-650 nm with minimum scanning speed (1 nm/sec). Excitation spectra were similarly recorded by setting emission monochromator at 594 nm and scanning excitation monochromator over 230-350 nm wavelength range. Optical density and pD of the solution was measured prior to N_2 bubbling and immediately after taking fluorescence reading. Wherever the change in these two pD values $\geq \pm$ 0.2 units the readings were discarded. Since the europium emission starts appearing around 590 nm a uv cutoff filter was used (uv-35) to avoid any 2nd order scattering.

In our work optical density of most of the solutions was around 0.7. The inner filter effect was very significant. From the observed fluorescence yield (F) true fluorescence yield was obtained by the equation (4):

$$\frac{F_o}{F} = \frac{2.303\ D\ [d_2 - d_1]}{10^{-Dd_1} - 10^{-Dd_2}} \qquad (4)$$

where D is the optical density of the solution and F_o is the true fluorescence yield. The explanation of the remaining symbols and derivation of the equation can be found in reference[48], page 221-22.

Excitation Correction

A recent review has discussed in detail about obtaining correction factors for excitation and emission sides of a spectrofluorometer.[50] The correction factors for the excitation side which normalize mainly the variation of lamp output and change in monochromator bandwidth with change in excitation wavelength were obtained by two methods:

1. Rhoadmine B in ethylene glycol (8 g/liter) was used as a quantum counter. This solution is optically thick in the wavelength region 220-600 nm. The normal rectangular cuvette was replaced by a flat 4 x 1 x 1/2 cm rectangular cell and the excitation curve was obtained by scanning the excitation monochromator over 220-600 nm range. The emission monochromator was set at 640 nm where Rhodamine B has no absorbance. A cutoff filter combination of uv 43 and uv 35 was used at the entrance slit of emission monochromator to avoid any scattered light. Intensity of fluorescence at each wavelength of excitation curve so obtained provides the required correction factors. The corrected excitation spectra were obtained by scanning the spectra for the compounds and then dividing the fluorescence intensity at each excitation wavelength by the correction factor for the same excitation wavelength and manually replotting the new spectrum.

2. White et al. have successfully employed dilute solutions of Al-PBBR chelate (PBBR = pontachrome blue black commerically

known now as calcon, sodium salt of 2,2'-dihydroxy-1,1'-azonaphthalene-4-sulfonic acid to find correction factors for excitation side of the spectrofluorometer.[51]

The stock solution of PBBR in 95% ethanol was 0.01% and aluminum chloride was 50 μmoles of $AlCl_3 \cdot 6H_2O$ per ml in 95% ethanol. For absorption measurements, 4 ml of the PBBR solution were added to 1.65 ml of $AlCl_3$ solution and diluted to 25 ml with 95% ethanol. This mixture results in about 80 μmoles of Al(III) for 1 μmole of dye and ensures that practically all of the dye is chelated. After 1/2 hour the absorption spectrum was determined with 1 cm absorption cell in Cary-18 spectrophotometer. The absorption range of main interest for this chelate is that which lies between 250 and 600 mμ.

Excitation Spectrum: Two milliliters of the solution used for absorption studies with 1 cm absorption cell were diluted to 25 ml with 95% ethanol. This dilution ensured that the distribution of fluorescence intensity along the exciting beam remained uniform at all wavelengths and reduced inner filter effects to a minimum. Further dilution did not produce any relative change in the excitation curve obtained. The excitation curve was determined at an emission monochromator setting of 595 mμ.

If the quantum efficiency of fluorescence is invariant with excitation wavelength then the fluorescence intensity at each wavelength should be proportional to optical density

of that solution. From the relation:

$$F = [I_o(2.3\ \varepsilon\ c.d.)][\phi] \qquad (5)$$

where F = total fluorescence intensity in quanta/sec.

I_o = Intensity of exciting light in quanta/sec.

c = concentration of solution moles/litre.

d = optical depth of solution in cms.

ε = molar extinction coefficient.

ϕ = quantum efficiency of solution

The relative correction factors were obtained by simply taking F/O.D. Where O.D. = ε.c.d. Correction factors obtained by this method matched fairly well with those obtained with Rhodamine B solution but this method was rather tedious. In most of our work we used correction factors obtained by Rhodamine B solution.

Corrected Emission Spectra

Several methods were tried to find correction factors for emission side of the instrument. Emission spectra were obtained with the use of either R106 or 1P28 photomultiplier tubes. So it was necessary to find the correction factors for each emission wavelength owing to variation in photomultiplier response in combination with emission monochromator with change in wavelength of emission spectrum.

The first method used was that due to Melhuish[52]

where the Rhodamine B solution used in the excitation correction was replaced by a triangular aluminum mirror supplied by instrument manufacturers [The performance characteristics of MPF-3 were checked prior to this which include the calibration of emission and excitation monochromators M_2 and M_1 respectively, procedure for which is given in the instruction manual of the instrument. The wavelength dials were adjusted to show correct wavelength of emission and then the dials of both monochromators were set to match each other within 1 nm.] Emission and excitation slits were set at 1 nm. M_2 was scanned at a speed of 1 nm/sec for each wavelength setting of M and peak height of the triangular shaped curve of the reflected light were measured. This was done in the wavelength region 240-580 nm. For wavelengths greater than 580 nm uv cutoff filter uv-35 was used in the exit beam and uv-39 in the emission beam.

Since the reflectance of aluminum mirror is invariant in the wavelength region of 200-800 nm the calibration factors for emission wavelengths were readily obtained from the knowledge of intensity of light falling on aluminum mirror (obtained earlier by Rhodamine B quantum counter). The relative correction factors were obtained by following relation

$$S_\lambda = \phi_\lambda / R_\lambda$$

where Sλ is the relative correction factor at a given wavelength ϕ_λ is the fluorescence intensity at the same activation wavelength for Rhodamine B optically thick solution and R_λ is the response observed by the given PM tube for the reflected light passing through M_2.

This procedure does not take into account the variation of the emission monochromator band width with wavelength variation but for grating monochromator it is constant within ±4%.

Since europium (Eu^{3+}) has sharp emission lines around 590-630 nm and Rhodamine B quantum counter was not effective at this region the correction factors in the region 550-650 nm were obtained by using corrected spectra in the literature. White[45] et al. have reported the corrected emission spectra of following five compounds which cover the emission wavelength range 450-700 nm.
1) Quinine sulfate 2) 3-Aminophthalimide 3) m-Nitro N,N-dimethylaniline 4) PBBR - Al chelate and 5) 4-dimethylamino-4-mitrostilbene.

Melhuish[52] has reported the corrected emission spectrum of quinine bisulfate. Lippert has reported corrected emission spectra of quinine bisulfate, m-nitro-N,N-dimethylanaline and 4-dimethylamino-4'-nitrostilbene.

It was then easy to run the emission spectra of these compounds with our machine and to compare them at each wavelength with the literature spectra. We used quinone

bisulfate, Al PBBR chelate and N,N-dimethyl-3-nitroaniline for this purpose. Emission spectra of these three compounds together cover reasonably well 450-650 nm emission range.

Following is the brief description of the experimental conditions used to run the emission spectra of the above three compounds.

1. Quinine bisulfate: 4.81×10^{-8} M in 0.1 N H_2SO_4. Excitation slit = 11 nm emission slit = 2 nm. λ excitation = 350 nm. Emission filter used was uv 35. Scan speed 1 mm/sec.

2. Al-PBBR chelate: A dilute solution of Al PBBR chelate was prepared as described earlier. λ excitation = 360 nm optical density at λ 360 nm = 0.085. Excitation slit = 8.2 nm. Emission slit = 6 nm. Solvent = 95% ethanol. Emission filter uv 35. Scanning speed 1 nm/sec.

3. N,N-dimethyl m-nitroaniline: concentration 100 µg/ml. Solvent benzene/hexane 30:70. Remaining conditions as above.

Fluorescence Quantum Yields

To obtain quantum yield of Eu^{3+} emission a stock solution of $EuCl_3 \cdot 3H_2O$ was made in D_2O and optical density was measured at λ 394 nm (O.D. = .271). 1 ml from this stock solution was diluted to 5 ml (O.D. = 0.0542). Keeping excitation slit 12 nm and emission slit = 1.5 nm emission spectrum of this solution was obtained with λ excitation = 394 nm using emission filter uv-35. Correction factors

were applied to the emission curve at each 2 nm point and the corrected emission spectrum was traced manually. Area under the two major peaks at 594 and 618 was computed by planey-meter (A).

Quinine bisulfate solution of unknown concentration in 0.1 N H_2SO_4 was prepared and its optical density was measured at λ = 350 nm. (O.D. = .259). 2 ml from this stock solution was diluted to 50 ml (O.D. = 0.01036) with 0.1 N H_2SO_4. With the same experimental settings as above the emission spectrum of diluted solution was obtained at λ excitation = 350 nm. True emission spectrum from this was obtained by applying appropriate correction factors and then the area under the true emission spectrum was obtained as before (A').

The quantum yield of Eu^{3+} emission was obtained by the following formula.

$$Q_1/Q_2 = \frac{A}{I_{394} \times {}^{O.D.}{}_{394}{}_{(Eu^{3+})}} \times \frac{I_{350} \cdot {}^{OD}{}_{350(\text{quinine sulfate})}}{A'}$$

where Q_2 = quantum efficiency of quinine bisulfate (.546)

Q_1 = " " " Eu^{3+} ion.

and I_{394} and I_{350} is the intensity of input light at these two wavelengths. We obtain thus $\phi_{394}^{Eu^{3+}} = 1.563 \times 10^{-2}$. This significantly low quantum yield may be due to a significant amount of H_2O in the system and also due to quenching by

Cl^- ions (Ref. 39).

Literature Values (394 nm excitation leads to $^5L_6$ level)

	$EuClO_4$	$Eu(NO_3)_3$
H_2O	$.57 \times 10^{-2}$	$.73 \times 10^{-2}$
D_2O	20×10^{-2}	16.3×10^{-2}

Quenching power: $CNS^- > Cl^- > NO_3^- > ClO_4^-$

It was difficult to estimate error involved in these calculations due to the presence of H_2O in $EuCl_3$ salt, error involved in finding correction factors and many experimental difficulties. Since we were rather interested in the relative quantum yields we did not try to evaluate absolute quantum yields.

We also used Rhodamine B as a standard for the quantum yield estimation and obtained similar values as above.

Quantum Yield for Energy Transfer

We used standard solution of $EuCl_3; 3H_2O$ in D_2O as a secondary standard for our quantum yields estimation for energy transfer from various uracil derivatives. Due to inner filter effect we had to first evaluate F_o/F value as mentioned earlier. We only noted down the height of the 594 emission line (H_1) when λ excitation was 281 nm and the height of $EuCl_3$ standard solution with known optical density (H_2). Quantum yield of energy transfer was then evaluated as earlier.

$$\frac{Q^{OA}}{Q^{Eu}} = \frac{H_1/E_{281}}{H_2/E_{393}} \times \frac{O.D.EuCl_{3(393)}}{O.D.\ OA_{(281)}}$$

where E_{281} and E_{393} are the relative input energies (Monochromator band width dependent lamp output at these two wavelengths).

$OD_{EuCl_{3(393)}}$ = optical density of $EuCl_3$ solution at 393 nm.

$OD_{OA(281)}$ = " " " orotic acid solution at 281 nm.

Fig. 14 shows the uncorrected and the corrected emission spectrum of Eu^{3+}.

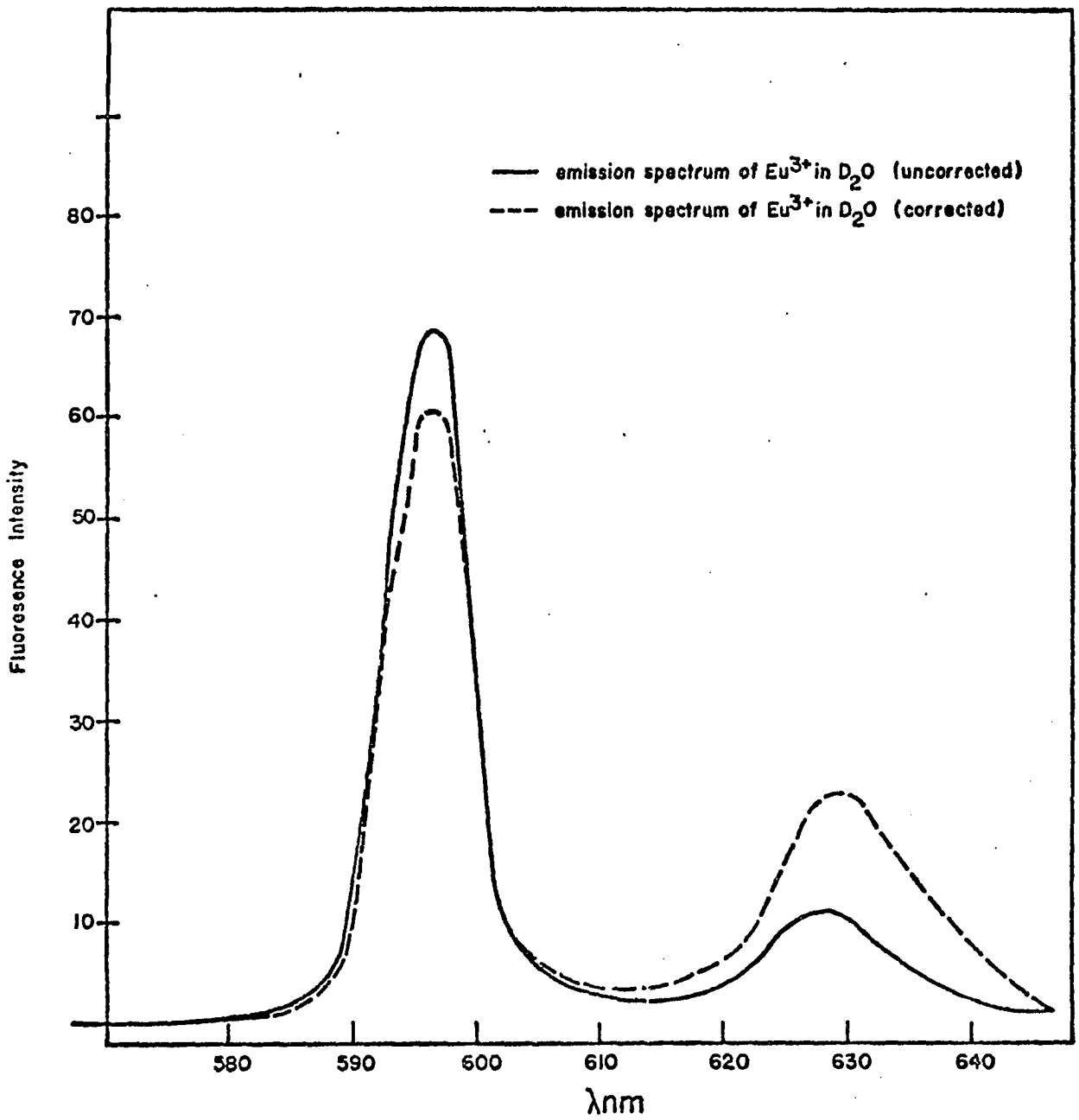


Figure 14. Emission spectrum of Eu^{3+} in D_2O (only two major emission lines are shown here).

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CHAPTER II

THE STRUCTURE OF THE COMPLEXES FORMED BY OROTIC ACID AND EUROPIUM(III) AND Ni(II) IONS. THE STABILITY CONSTANTS AS FUNCTION OF pH

INTRODUCTION

In Chapter I we have described our results on intermolecular energy transfer from various orotic acid derivatives to Eu(III) ions. We noticed that whereas orotic acid and 3- methylorotic acid were efficient donors of excited triplet energy, the transfer efficiency was greatly diminished for 1- methylorotic acid, 1,3- dimethylorotic acid and for the two orotic acid esters, at pH values where the suspected ground state complexes of orotic acid were not reported. Nevertheless we suspected an involvement of such ground state complexes to be important in the energy transfer process, i.e., the energy transfer was not simply a collisional process nor an exciplex process.

We found many examples in the literature where Eu(III) had been found to form 1:1 complexes with simple carboxylate as well as with other varieties of ligands.

Complex stability in each case was a function of pH. A few examples can be cited here for a rapid review.

Holleck[1] has shown by polarographic studies that Eu(III) form 1:1 complexes with citric, tartaric, lactic, malic, mandelic and salicylic acids. Though he did not find any evidence for complex formation between Eu(III) and amino acids Lal[2] has observed the existence of tryptophan-Eu(III) complex by polarographic method. He estimated the instability constant for the complex [Eu-Tryptophan]$^{2+}$ to be 1.63×10^{-7}. Williams[3] et al. have done nmr studies on complex formation between various trivalent lanthanide ions including Eu(III) and indole-3-yl acetate. At pH 6 with their pseudocontact shifts and line broadening results they demonstrated a 1:1 stoichiometry of metal ion and the carboxylate group in the complex. Due to highly dissociative nature of the complexes they were forced to use high ligand to metal ratio (1:30).

The literature is abundant with accounts of 1:1 complexes of Eu(III) and various oxygen containing donors.[4-7] Among the various compounds forming such complexes are glycolic acid, lactic acid, imino diacetic acid, α-hydroxy butyric acid, nitrolitriacetic acid, EDTA, kojic acid, tropolone, picolinic acid, pipiridine 2,6 dicarboxylic acid, etc. The last two compounds have structural analogy to orotic acid.

Kallistratos[8] by paper chromatographic method

found that La, Eu, Gd, Tb, Sc and Y form characteristic fluorescent complexes with derivatives of tryptophan and orotic acid. We found a paper spotted with an aqueous mixture of Eu(III) + orotic acid exhibited a bright red fluorescence under uv light whereas the fluorescence of a corresponding spot of Eu(III) aqueous solution was extremely weak.

Haug[9] found that various bivalent metal ions (Ni^{2+}, Co^{2+}, Zn^{2+}, etc.) form 1:1 complexes with orotic acid and also inhibit the photodimerization of orotic acid.

We have observed that orotic and 3-methyl orotic acid form 1:1 complexes with Eu^{3+}, the complex stability was strongly pH dependent. Haug[9] in his work has not evaluated any stability constants for ($OA-Ni^{2+}$) system below pH 7. Also the nature of the complex was not completely clarified. We therefore have repeated his work using Ni^{2+} ions and various orotic acid derivatives to throw some light on the structure of these complexes. We have slightly modified Haug's procedure and obtained much more accurate values for the stability constants. The work reported here establishes the existence of a relatively high concentration of europium(III) ion-orotic acid complexes at pH values where energy transfer was observed, and establishes the fact that the presence and absence of such energy transfer from various orotic acid derivatives is caused by the chelate nature of these complexes.

EXPERIMENTAL

Details about the synthesis, etc. of the orotic acid derivatives has been summarized in Chapter I. $NiSO_4 \cdot 6H_2O$, reagent grade, was Allied Chemicals and was used without further purification. Uv spectra were recorded with a Varian Cary 118 model and optical density was measured accurately within ±0.0007.

All stock solutions were freshly prepared immediately prior to use in doubly distilled water.

Analysis of the Orotic Acid Metal Ion Complex

Haug reported[9] that orotic acid forms 1:1 complex with various metal ions. We confirmed this in the case of orotic acid and 3- methylorotic acid with Ni^{2+} ions at various pH values, using a spectral method. Using this method it is necessary to infer two parameters from a set of spectral measurements at various concentrations, namely the equilibrium constant (K) and the extinction coefficient (ε) of a spectral band having an absorbance which vary in direct proportion to the concentration of the molecular complex. The Benesi-Hilderbrand technique is probably the method most commonly used to obtain these properties.[10]

Using Haug's notations the equilibrium constant between orotic acid and metal ion at a given pH may be written as

$$O + M \rightleftharpoons OM \tag{1}$$

$$K = \frac{[OM]}{[O]\,[M]} \tag{2}$$

Here O and M are the concentrations of uncomplexed orotic acid and metal ions respectively and OM the concentration of the equimolar complex. Equation 2 can be rewritten in the form

$$K = \frac{[OM]}{[O_o - OM]\,[M_o - OM]} \tag{3}$$

where O_o and M_o are the initial molar concentrations of orotic acid and metal ions respectively. Substituting concentrations by optical densities $c = \frac{D}{\varepsilon \cdot \ell}$ we obtain

$$K = \frac{D_{OM}/\varepsilon'}{\left[\frac{D_O}{\varepsilon} - \frac{D_{OM}}{\varepsilon'}\right]\left[M_o - \frac{D_{OM}}{\varepsilon'}\right]} \tag{4}$$

ε and ε' are the extinction coefficients for orotic acid and the complex species respectively. D is the optical density, ℓ the path length is omitted since we used only 1 cm photometric cells for our measurements. For low metal ion concentrations D_{OM}/ε' can be neglected in comparison

to D_O/ε. Equation 4 after rearrangement takes the form:

$$\frac{D_O}{D_{OM}} \cdot \frac{1}{\varepsilon} = \frac{1}{\varepsilon'} + \frac{1}{K \cdot \varepsilon' \cdot Mo} \qquad (5)$$

Thus if Beer's law is obeyed by the complexed absorbing species then orotic acid concentration can be fixed (D_O/ε) and the metal ion concentration can be varied to obtain experimentally determined values of D_{OM}. Then a plot of 1/M against $D_O/D_{OM} \cdot \ell \cdot \varepsilon$ can be constructed to yield a straight line from where K value can be obtained from the ratio, intercept/slope. Haug[9] has neglected the absorption due to uncomplexed orotic acid at the λ_{max} of the complex for obtaining his values of K. At low pH values we found this procedure rather erroneous. To obtain a better accuracy of the complex concentration we measured the absorbance of orotic acid + metal ion mixture at two different wavelengths; concentration of the complex could be evaluated with the use of the following equations:[11]

$$A' = \varepsilon \cdot C_{OA} + \varepsilon'' \cdot C_{OM} \text{ (at } \lambda') \qquad (6)$$

$$A'' = \varepsilon''' \cdot C_{OA} + \varepsilon' \cdot C_{OM} \text{ (at } \lambda'')$$

Values of ε and ε''' were obtained for pure orotic acid with known concentration and at a given pH value. The values of ε' and ε'' for completely complexed orotic acid with known

concentration were obtained by using higher conc. of metal ions at pH ≈ 9.2. The absorbance by metal ions was negligible.

Results

Spectra of equimolecular solutions of Ni^{2+} ions + OA. And Ni^{2+} + 3 Me OA: Fig. 1 represents the spectra of equimolecular (1×10^{-4} M) solutions of OA and Ni^{2+} ions at different pH values. The reference cell contained in all cases corresponding amount of $NiSO_4$ with the same pH. With our instrument (Cary 18) we found a sharply defined isosbestic point at 292 nm. In Fig. 1 O' represents the completely complexed orotic acid (1×10^{-4}) with 2×10^{-4} M Ni^{2+} ions at pH = 9.2. Adding more Nickel ions at this pH did not alter the shape and intensity of Ni^{2+} OA complex spectrum.

Fig. 2 shows the Ni^{2+}-3MeOA spectra for 3 different pH values. Solutions were unbuffered above pH = 6 and in the region between pH 4-6 acetate buffer was used. It is evident from the similarity of spectra for orotic acid and 3-Me orotic acid that both these compounds have similar complexing ability. In each case there's a sharply defined isosbestic pt. at 292 nm.

We found for orotic acid the completely complexed Ni-OA species has λ_{max} = 311 nm with extinction co-

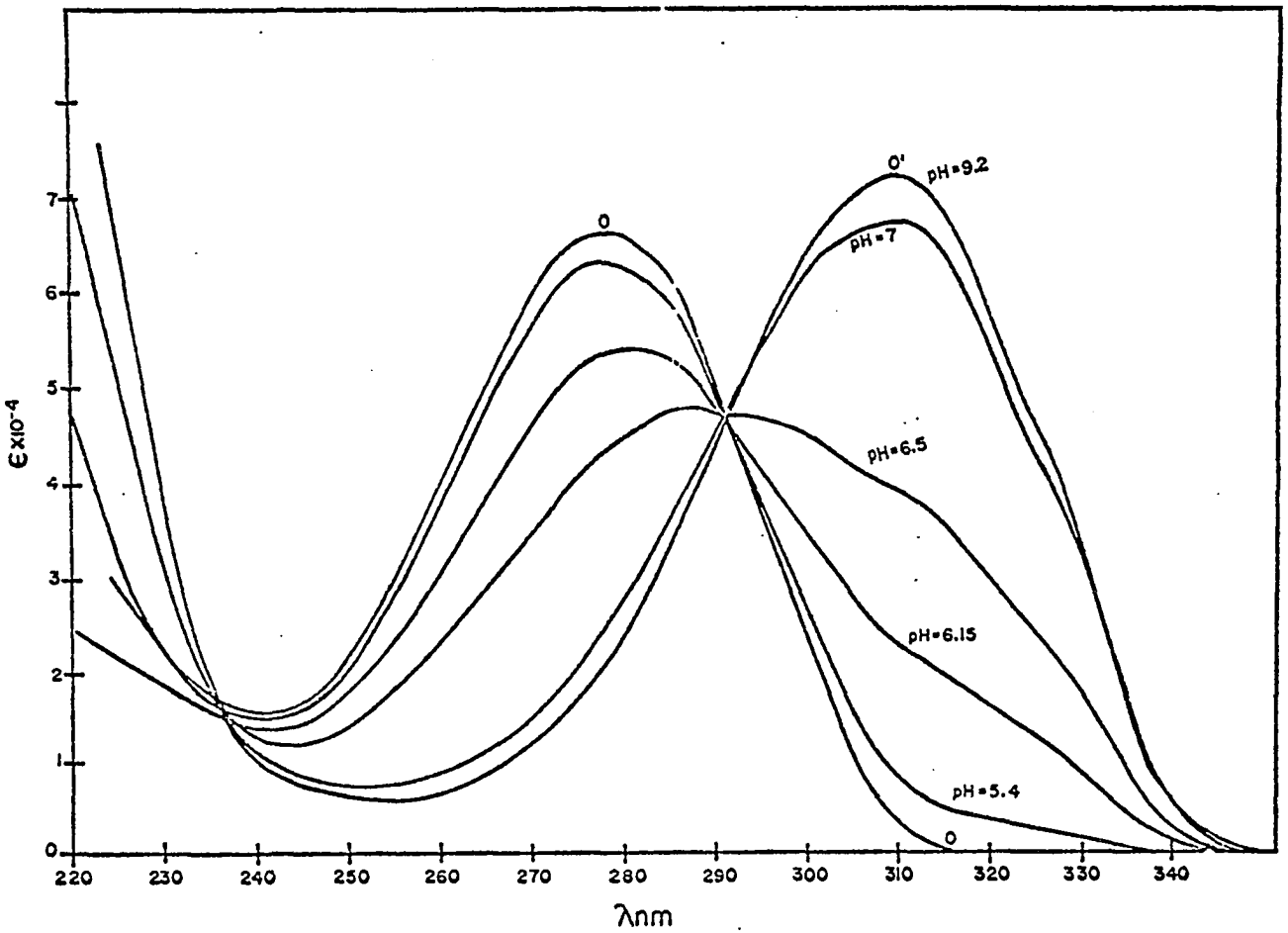


Figure 1. Absorption spectra of equimolar solutions (10^{-4} M) of orotic acid and nickel ions at different pH. The curve O is the absorption spectrum of free acid at pH 5-6 and O' is the absorption spectrum of completely complexed 10^{-4} M orotic acid (2 x 10^{-4} M Ni^{2+}) at pH 9.2.

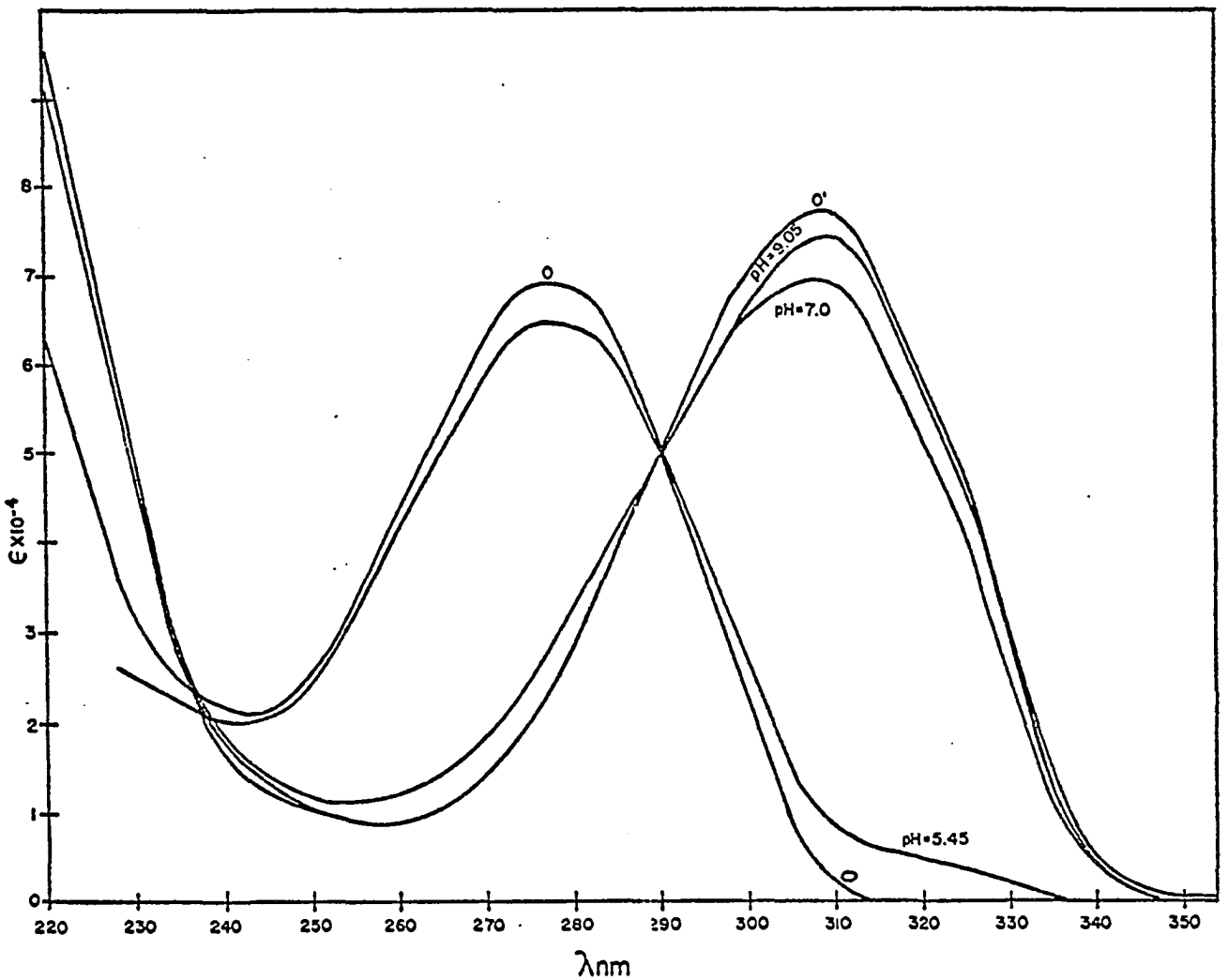


Figure 2. Absorption spectra of equimolar solutions (10^{-4} M) of 3-methylorotic acid-nickel ions at different pH. The curve O is the absorption spectrum of free 3- methylorotic acid at pH 5-6 and O' is the absorption spectrum of completely complexed 10^{-4} M 3-Me-orotic acid (2×10^{-4} M Ni^{2+}) at pH = 9.2.

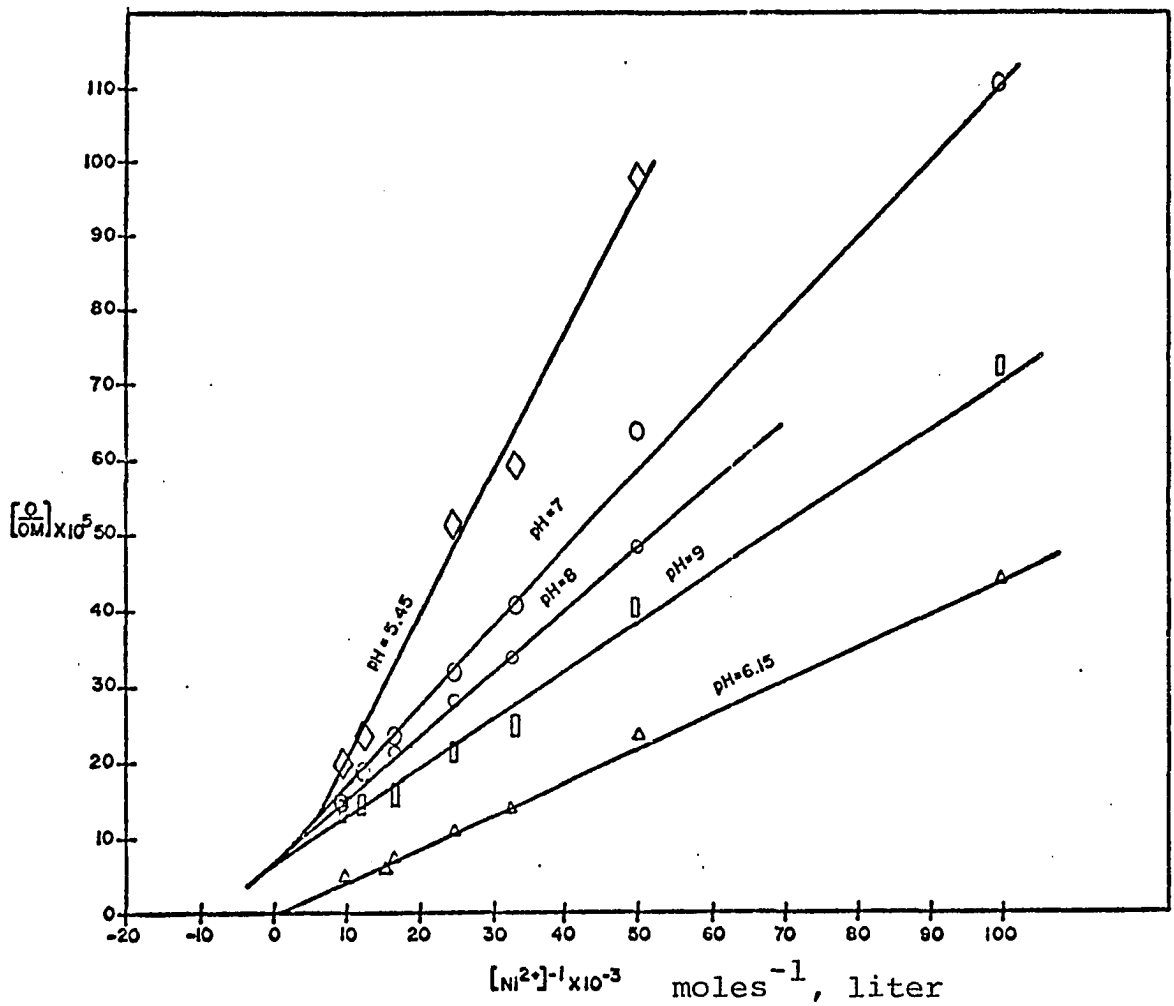


Figure 3. Benesi-Hilderbrand lines (eq. 5) for equimolar complex of orotic acid and nickel ions at various pH values. For pH = 5.45 and pH = 6.15 ordinate values are $[\frac{O}{OM}] \times 10^4$.

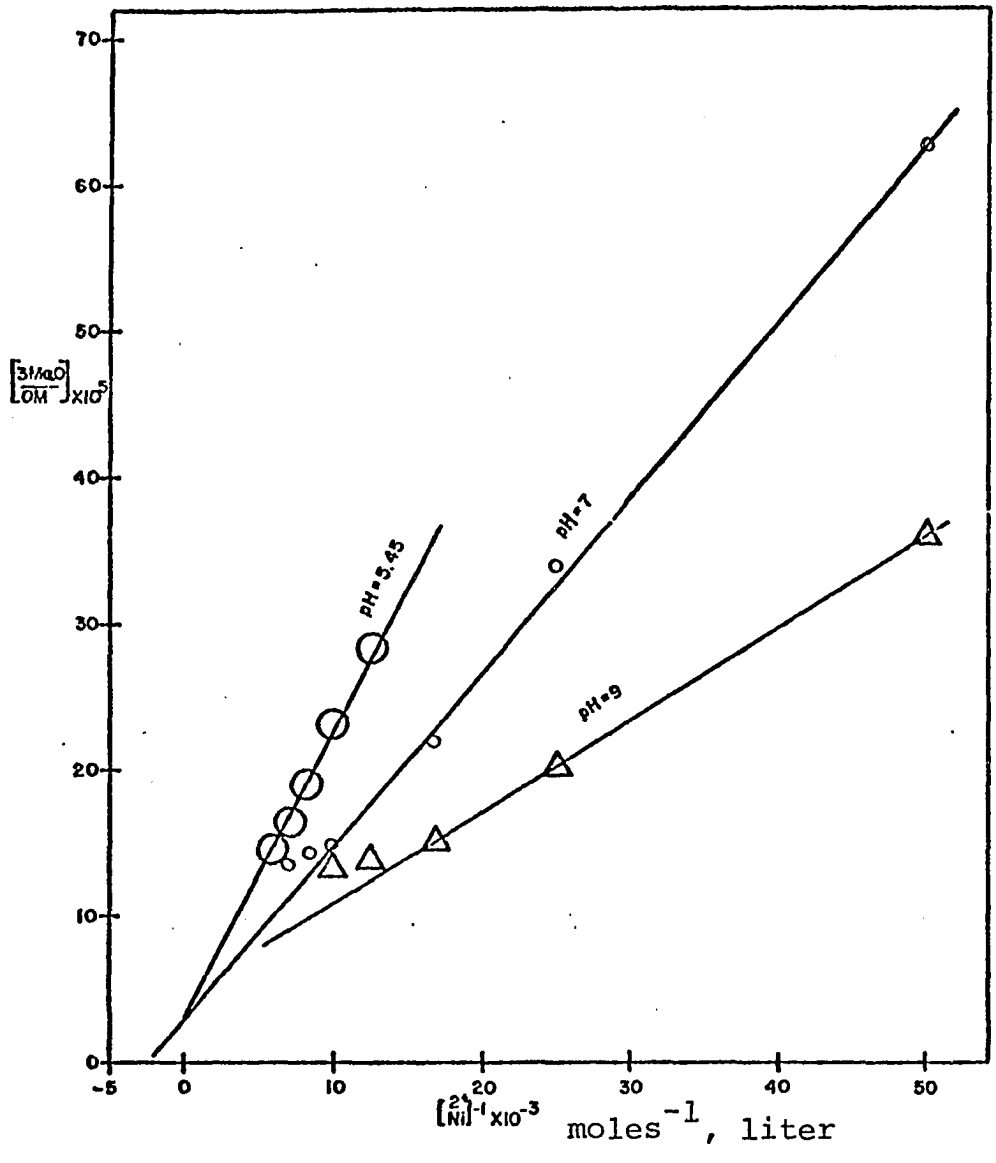


Figure 4. Benesi-Hilderbrand lines (eq. 5) for equimolar complex of 3-Me-orotic acid and nickel ions at three pH values.

efficient = 7177 (ε'). At 280 nm the ε" value was 2608. Extinction coefficients for pure OA at these two wavelengths were obtained separately and using equations and the concentration of complex was calculated for each pH value at various metal ion conc. The metal ion conc. was varied from 1 x 10^{-5} to 1 x 10^{-4} M for fixed (10^{-4} M) orotic acid concentration. Plots were then constructed using equation 5. Fig. 3 shows such plots for OA-Ni system at various pH values. At alkaline pH values for higher nickel ion conc. the Beer Lambert law was not obeyed. To find the slope in this case only points with low metal ion conc. were used. From the slope of each line K value was evaluated. Analogous treatment of 3-Me OA + Ni^{2+} system yielded K values for this system. Fig. 4 shows such plots for 3 Me-OA Ni^{2+} system and Table (1) lists the stability constants. We found it unnecessary to maintain constant ionic strength. Using KCl or NaCl for this purpose did not alter K values in few trial experiments. Our K values are about an order of magnitude smaller than Haug's values. It is also clear from Table (1) values that the presence of a methyl group in 3 position of the uracil ring has apparently no effect on complex stability. Acidifying the solution to pH 2 by HCl solution destroyed the complex spectra regenerating the spectra of uncomplexed OA and 3-Me OA respectively.

We found that Eu^{3+} also exhibits pH dependent mode

Table 1

STABILITY CONSTANTS FOR OA AND 3 MeOA

WITH Ni^{2+} IONS AT VARIOUS pHs

pH	K for orotic acid Ni complex	K for 3 Me-OA Ni complex
4.5	1.345×10^2	
5.45	6.846×10^2	5.52×10^2
6.15	3.646×10^3	
6.5	1.18×10^4	
7	1.315×10^4	1.107×10^4
8	1.67×10^4	
9	2.594×10^4	2.118×10^4 (pH=9.05)

of complex formation with orotic acid and three methyl-orotic acid. Fig. 5 represents the absorption spectra of Eu^{3+} OA system at various pH values. Fig. 6 shows the corresponding spectra for 3-methylorotic acid and Eu^{3+} ions. Here also we have a sharply defined isosbestic point at 290-91 nm. For Eu^{3+} + OA system the λ_{max} for complex was centered at 305 nm. Same value for λ_{max} was obtained for Eu^{3+} + 3 Me OA system.

Several problems were encountered in the Europium-OA system.

1) Europium orotic acid complex is much more labile than the Ni + OA complex. Hence at lower pH values an equimolar concentration of Eu^{3+} was insufficient. At pH 4.6 and pH 5.05 Eu^{3+} conc. was varied from 7.55×10^{-4} to 5.66×10^{-3} M. At pH 5.5 it was varied from 3.77×10^{-4} to 3.02×10^{-3} M and pH = 5.95 - 6 it was varied from 3.8×10^{-5} to 3.02×10^{-4} M. Orotic acid concentration in each case was fixed at 10^{-4} M.

2) Acetate buffer used in the pH range 4-6 for OA-Ni^{2+} system was found to give erroneous results for Eu^{3+} system. In acetate buffer an increase in Eu^{3+} concentration caused the optical density at λ270-280 nm (λ_{max} for OA) to rise rather than decrease. This can probably be attributed to the complex formation between Eu^{3+} and acetate anions. Fortunately at higher concentrations of Eu^{3+} used (vide supra) pH was found to remain constant once adjusted, at-

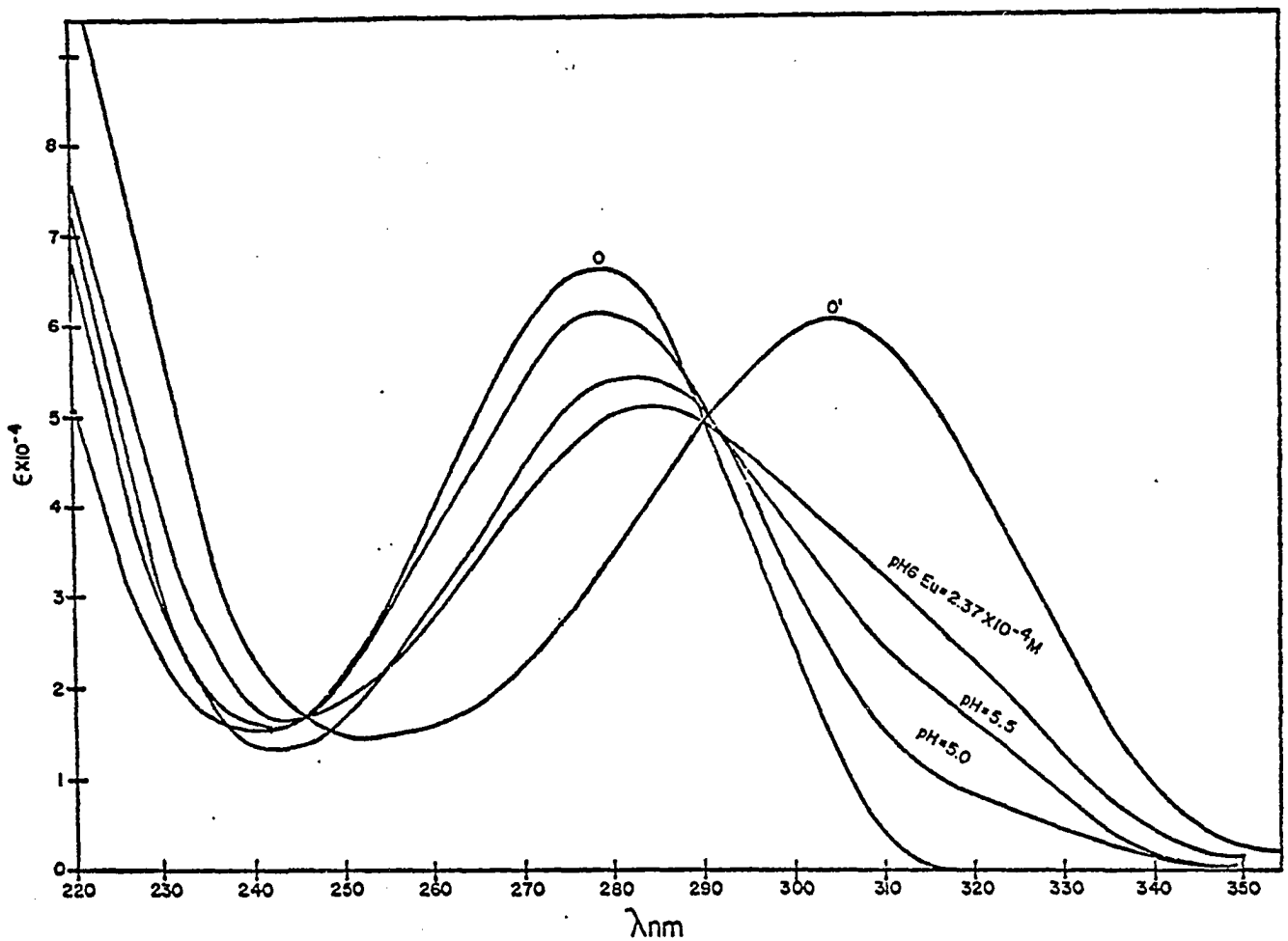


Figure 5. Absorption spectra of orotic acid (10^{-4} M) - Eu^{3+} complex at various pH values. The curve O is the absorption spectrum of free orotic acid and O' is the absorption spectrum of completely complexed orotic acid (10^{-4} M); (Eu^{3+} = 7.2 x 10^{-4} M) at pH = 6.3-6.4.

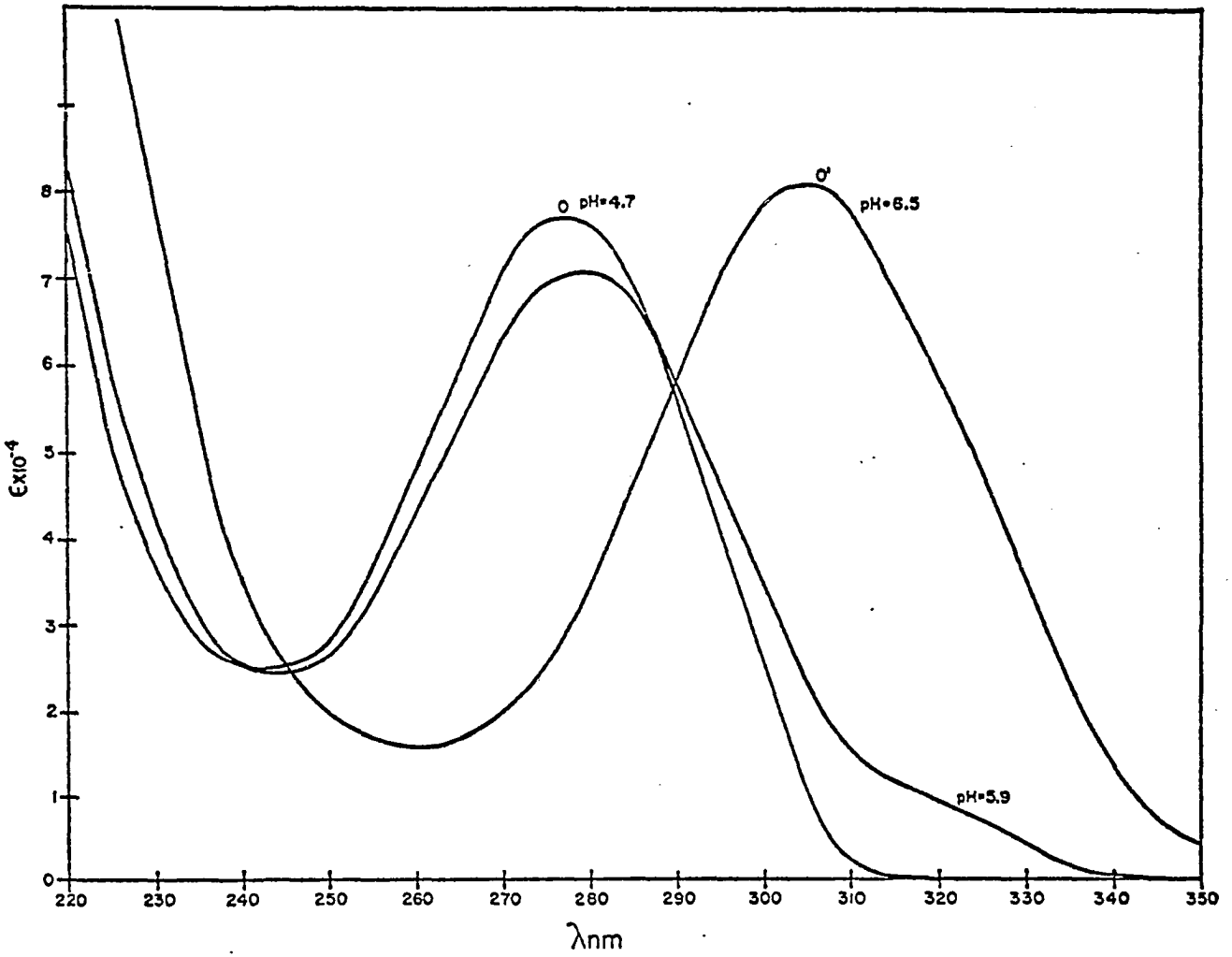


Figure 6. Absorption spectra of 3-Me-orotic acid-Eu^{3+} complex at various pH values. Curve O is the absorption spectrum of free 3-Me-orotic acid and O' the completely complexed 3-Me-orotic acid (10^{-4} M) at pH 6.4 (Eu^{3+} = 7.2 x 10^{-4} M).

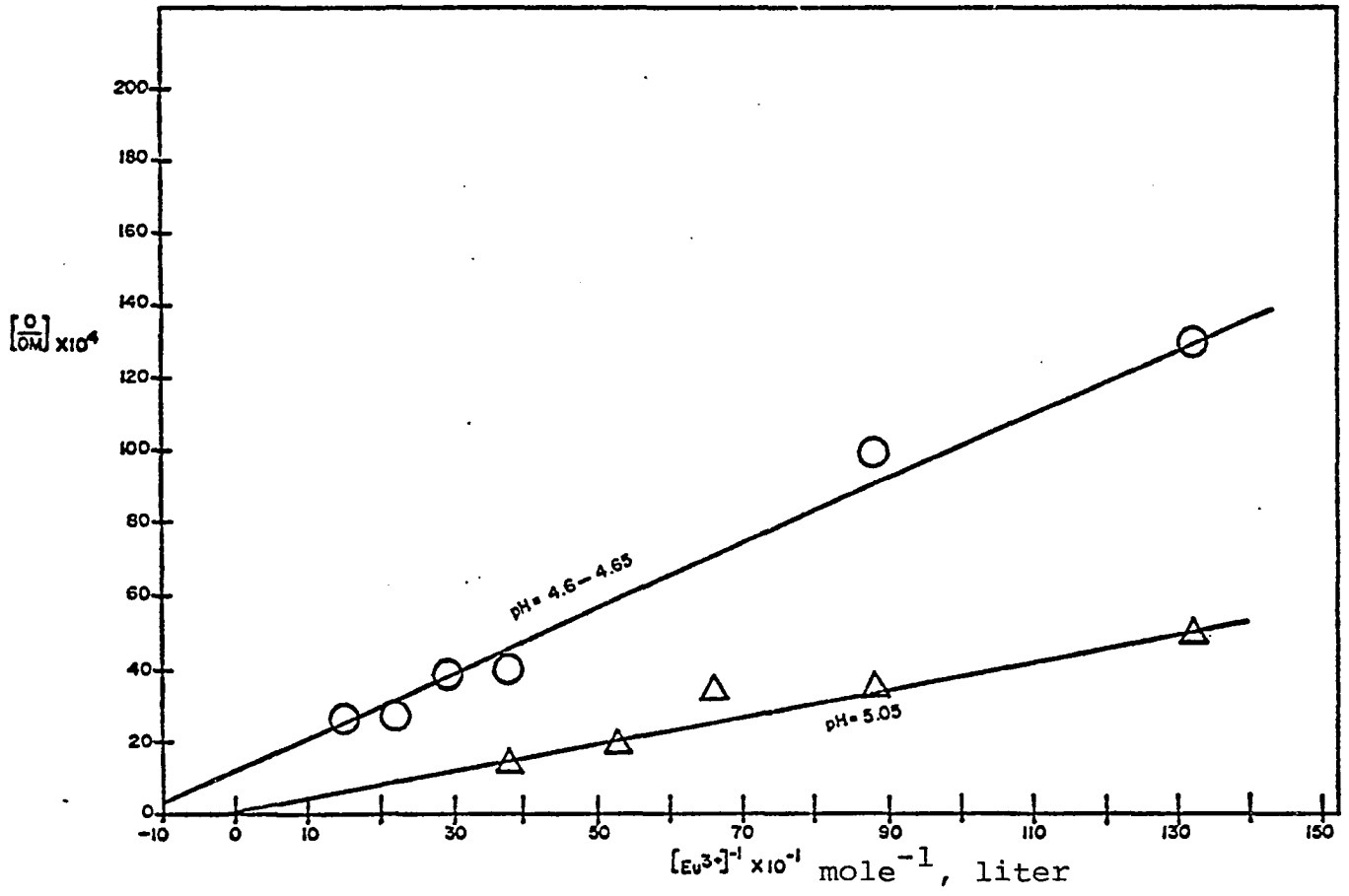


Figure 7. Benesi-Hilderbrand lines for 1:1 complex of orotic acid and Eu^{3+} ions at various pH values.

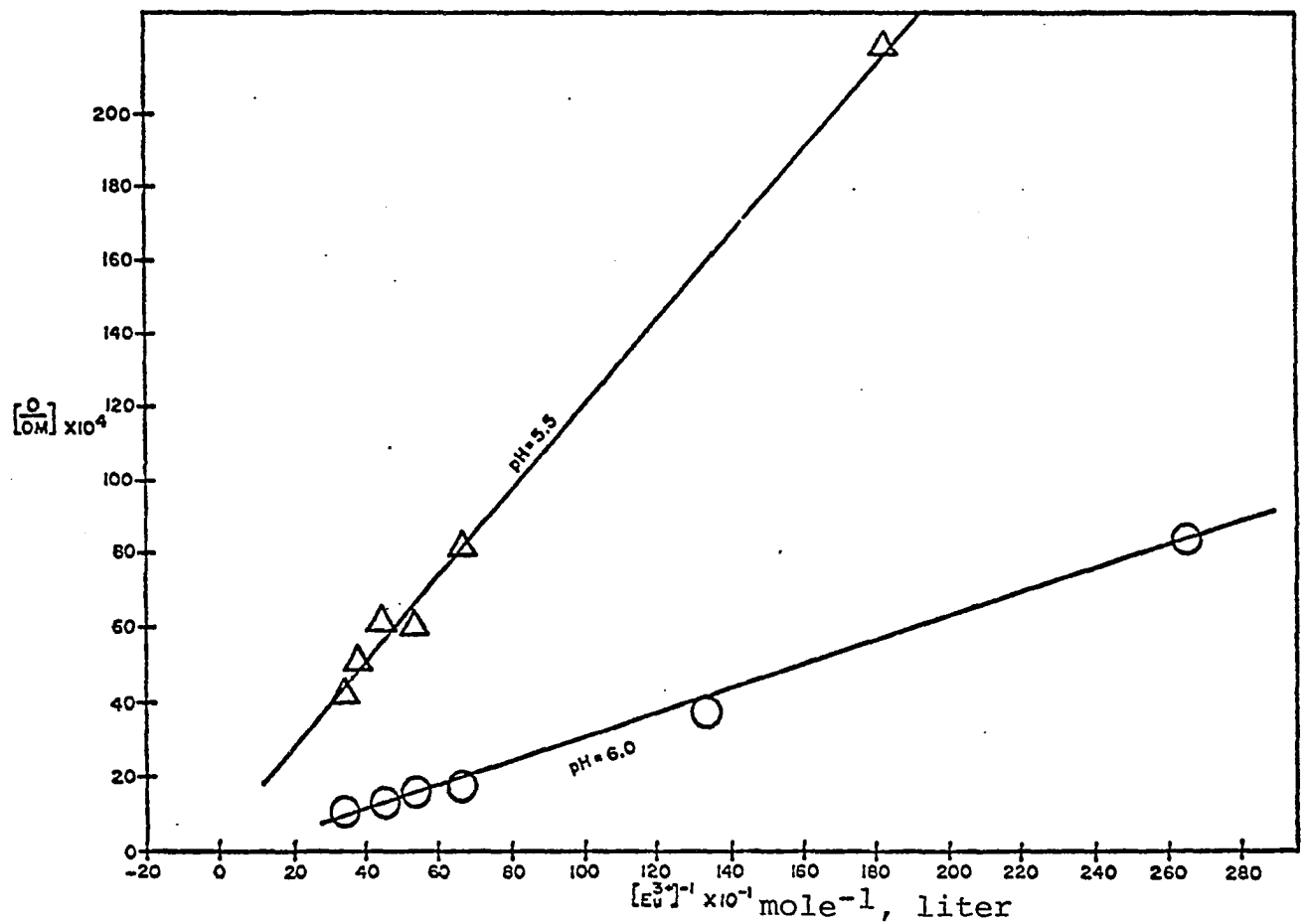


Figure 7'. Benesi-Hilderbrand lines for 1:1 complex of orotic acid and Eu^{3+} ions at pH values 5.5 and 6. At pH 5.5 the ordinate values are [O/OM] x 10^5 and at pH 6 the abscissa values are $[Eu^{3+}]^{-1}$ x 10^{-2} moles^{-1}, liter.

least for a period of 2 hours. Obviously the problem of holding pH constant without buffer was automatically eliminated.

3) Most importantly above pH = 6 $Eu(OH)_3$ starts precipitating (depending upon Eu^{3+} conc.) complicating the spectral measurements. So we had to limit our measurements below pH ≃ 6.5.

The Eu^{3+} - OA complex has a λ_{max} at 305 nm with ε = 6171. Stability constants were obtained as shown for the OA-Ni^{2+} system. Table (2) shows the stability constants thus obtained and Fig. 7 shows the plots fitting equation 5.

Stability constants for 3 Me-OA + Eu^{3+} systems were not evaluated as they are expected to have similar values as above from our experience with Ni^{2+} - 3 Me OA system. Excellent straight line plots in Fig. 7 fitting our data in Equation 5 indicate a 1:1 complex formation between Eu^{3+} and OA.

Examination of Tables (1) and (2) shows that at a given pH Eu^{3+} - OA complex is about 7 to 8 times less stable than Ni^{2+} - OA complex. The following characteristics are those which produce this weaker complex:

1) In the ground state, each cation of Ln^{2+} presents to incoming ligands essentially a noble-gas atom outer electronic arrangement, with both 4f orbitals and the electrons occupying them being effectively screened. Any

Table 2

STABILITY CONSTANTS FOR OROTIC ACID Eu^{3+} SYSTEM AT DIFFERENT pH VALUES

pH	K
4.6-4.65	17
5.05	44.9
5.5	1.473×10^2
5.95-6	5.079×10^2

involvement of metalion orbitals in bonding must be restricted to higher-energy orbitals. The ligand-field stabilization is correspondingly small (ca. 1 kcal/mole).

2) Each Ln^{3+} cation is comparatively large. Thus covalent interactions with ligands are minimized in a second way, and electrostatic interactions are reduced over what they might be for cations of each charge type.

3) Water is particularly a strong ligand. In aqueous media, any ligand added is in competition with large quantities of water for coordination sites on the Ln^{3+} ion. Furthermore, once a coordination site has been occupied by a molecule of water, the displacement by another ligand is commonly very difficult. Thus only strong ligands, in particular those that are chelating, form complexes of sufficient thermodynamic stability to be isolable.

4) Ligand exchange reactions are nearly always very rapid when carried out in solution. This situation both limits the number of isolable lanthanide complexes and minimizes the number of geometrical and/or optical isomers that can be carried unchanged through the reactions essential to their investigation.

The net result is that in comparison with d-transition metal ions the lanthanides ions as a whole both form far fewer complexes and yield complexes with significantly different properties.

Haug has found that by changing the metal ion a shift in the absorption peak of orotic acid complex resulted.[9] Table (3) shows our values of λ_{max} for various orotic acid metal ion complexes.

There doesn't seem to be a direct correlation between λ_{max} for a given metal complex and the electronegativity of the metal ion on the other hand increasing ionic radius is causing a decrease in the red shift of orotic acid λ_{max} indicating a weaker interaction with metal ion.

Free energy for the orotic acid nickel complex can be calculated from the relation $\Delta F = -RT\ln K$. We obtained the values of free energy in Table (4).

Oscillator strength[12] for the charge transfer transition can be calculated with the expression:

$$f = 4.704 \times 10^{-7} \nu max \ \mu^2 EN$$

Where νmax is the frequency in cm^{-1} of the maximum intensity of the band and μEN is the transition dipole. The simplified expression is:

$$f = 1.35 \times 10^{-8} \ \varepsilon max \ (\nu max - \nu_{1/2})$$

Where εmax is the molar extinction coefficient for the complex, $\nu_{1/2}$ is the wave number at the long wavelength

Table 3

POSITION OF THE ABSORPTION PEAK OF OROTIC ACID-METAL ION COMPLEX AND THE PROPERTIES OF METAL IONS USED.[b]

	Ni^{2+}	CO^{2+}	CU^{2+}	Zn^{2+}	EU^{3+}	Hg^{2+}
λ_{max} nm	311	310	309	303	305	303
Ionic Radius A°	.69	.72	.72	.74	.98(.950[a])	1.10
Electronegativity						
Sanderson	1.47	1.47	1.74	1.86	.94	1.92
Pauling	1.8	1.8	1.9	1.6	1.1	1.9

[a]"Comprehensive Inorganic Chemistry" Ed. J. C. Bailar, H. J. Emeleus, Sr. Ronad Nyholms, A. F. Trotman-Dieckenson, Vol. 4. Lanthanides. Transition metal compounds. Page 7, Publ. Pergamon Press, 1973.

[b]"Inorganic Chemistry" G. C. Demitras, C. R. Ross, J. F. Salmon, J. H. Weber, G. S. Weiss, Page 73 and 39, Prentice Hall, Inc., Englewood Cliff, New Jersey, 1972.

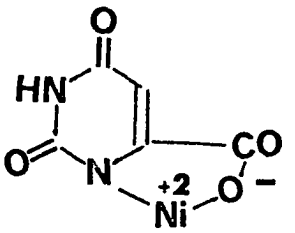
Table 4

Compound	Metal Ion	pH	-ΔF
Orotic Acid	Ni^{2+}	4.5	2.84 Kcal/mole
Orotic Acid	Ni^{2+}	6.15	4.75 Kcal/mole
Orotic Acid	Ni^{2+}	9	5.89 Kcal/mole
Orotic Acid	Eu^{3+}	4.6	1.64 Kcal/mole
Orotic Acid	Eu^{3+}	6	3.61 Kcal/mole
3-Me OA	Ni^{2+}	9	5.71 Kcal/mole

side at the half band width of the absorption band.

For orotic acid-Ni^{2+} system taking λ_{max} for the complex=311 nm at pH = 9 we obtain f = 0.161 and for Eu^{3+} - OA at pH ≃ 6.2 taking λ_{max} = 305 nm we obtain f = .192; $\nu_{1/2}$ value in both cases were obtained at λ = 328 nm. These values of oscillator strength indicate a strongly allowed transition similar to the original transition (f for orotic acid itself = .204).

Nature of the Complex: How the metal ion is bound to the orotic acid was an intriguing question. As mentioned in the introduction our energy transfer work prompted us to explore more in this area. Haug[9] originally suggested two possible modes through which OA-metal ion complexing could be deemed possible. One possibility was the complexing occurring between $Ni(OH)^{+}$ and free carboxylate anion of orotic acid. A second type he tentatively suggested was through a five membered ring skeleton where the N-1 proton on orotic acid is made labile through metal ion interaction as shown below. To gain some more insight into



the true nature of the complex we studied various orotic acid derivatives namely 1-methylorotic acid, 3-methyl OA,

1,3 dimethyl OA, methyl and isopropyl orotates and 6 acetyl uracil.

Results

We have shown earlier that 3 methylorotic acid behaves analogously to orotic acid.

1-methyl and 1,3 dimethylorotic acid: For these compounds in the pH range 1-9 we did not find any evidence of complex formation with Ni^{2+} ion, added even in excess than equimolar amounts. Thus there was no change in the λ_{max} in uv spectra for these compounds or change in optical density with Ni^{2+} ions at a given pH compared to spectrum of pure donor at the same pH.

Isopropyl and methylorotate: Methyl ester of orotic acid was rather difficult to study as it undergoes rapid hydrolysis to orotic acid in alkaline pH. Fig. 8 shows the uv spectra of 10^{-4} M isopropyl orotate at various pH values. Some noteworthy features of these spectra are (1). These spectra have a sharply defined isosbestic point at λ = 305 nm. At pH 6 a new absorption shows its appearance at longer wavelength side of the spectrum and at pH 8 and above we have a broad completely new spectrum with λ_{max} centered at 310 nm. Acidifying this solution with HCl to any pH below 6 regenerated the original spectrum with λ_{max} = 283 nm. Up to pH = 6 we did not observe any complexing occurring with Ni^{2+} ions and isopropyl orotate. Isopropyl-

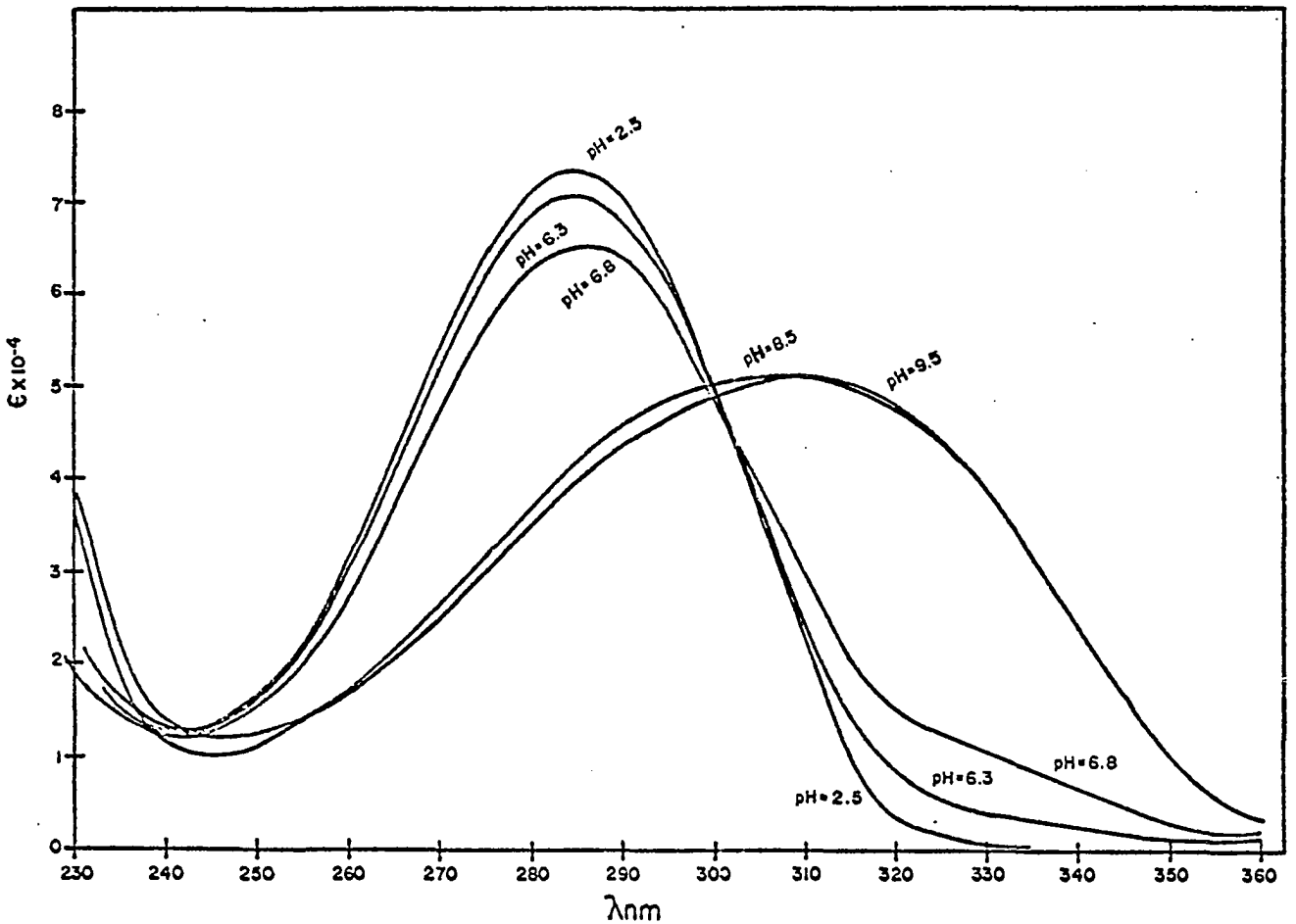
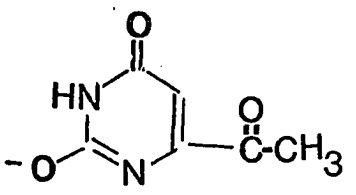
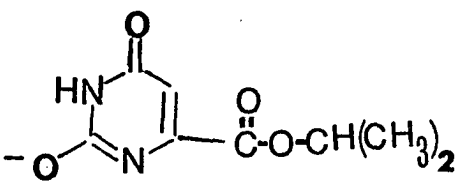


Figure 8. Absorption spectra of isopropyl orotate (10^{-4} M) at various pH values.

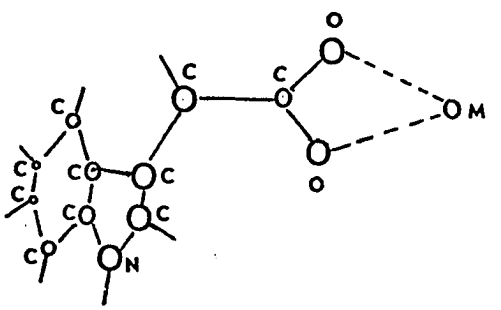
orotate also undergoes hydrolysis to orotic acid in alkaline solution though this hydrolysis occurs relatively slowly compared to methylorotate. We noticed that at pH = 8 this hydrolysis was very rapid in the presence of nickel ions and we obtained OA-Ni^{2+} complex spectra. Haug also did not observe any complexing occurring between Ni^{2+} and butylorotate.

6-acetyl uracil: Fig. 9 shows the uv spectra of 6-acetyl uracil at various pH values. Here also we see a sharp isosbestic point at λ = 311 nm and a new broad absorption maxima at 320 nm at pH = 9.6. Adding Ni^{2+} ions did not alter shape and intensity of 10^{-4} M solution of this compound up to pH 6. At pH 8 and above we were surprised to see a drastic change in spectrum with Ni^{2+} ions (Fig.10). A possibility of Ni^{2+} complexing was felt at the beginning but when we acidified this solution with aq. HCl the 6-acetyl uracil spectrum at pH 2 was not regenerated and we obtained a new spectrum having two overlapping peaks. It appears that 6-AcU in alkaline solution is undergoing a new reaction in the presence of Ni^{2+} ions. We are trying to find out more about this new reaction. Almost identical shifts in the λ_{max} ($\approx$ 25 nm) for isopropyl orotate and 6 acetyl uracil on changing from acidic to alkaline pH values can be safely concluded to have origin in the ionization of N-1 proton of the uracil ring to give a monoanionic species





Williams[3] at al. have observed Eu^{3+} forming 1:1 complex with carboxyl group of indol-3-yl-acetate at pH = 6. From their nmr shift data in aqueous solution they arrived at the following conformation of the complex.



In orotic acid and 3- methylorotic acid formation of this type of complex is not impossible since acetic acid, nicotinic acid and so many monocarboxylic acids are known to form complexes with rare earths ions.[13] But since we did not find any spectrophotometric evidence for complex formation between nickel ions and 1-MeOA, 1,3 dimethyl OA and orotic acid esters it appears that kind of complexes which we are able to detect by uv studies may have the nature where the metal ion complexed with the carboxylate

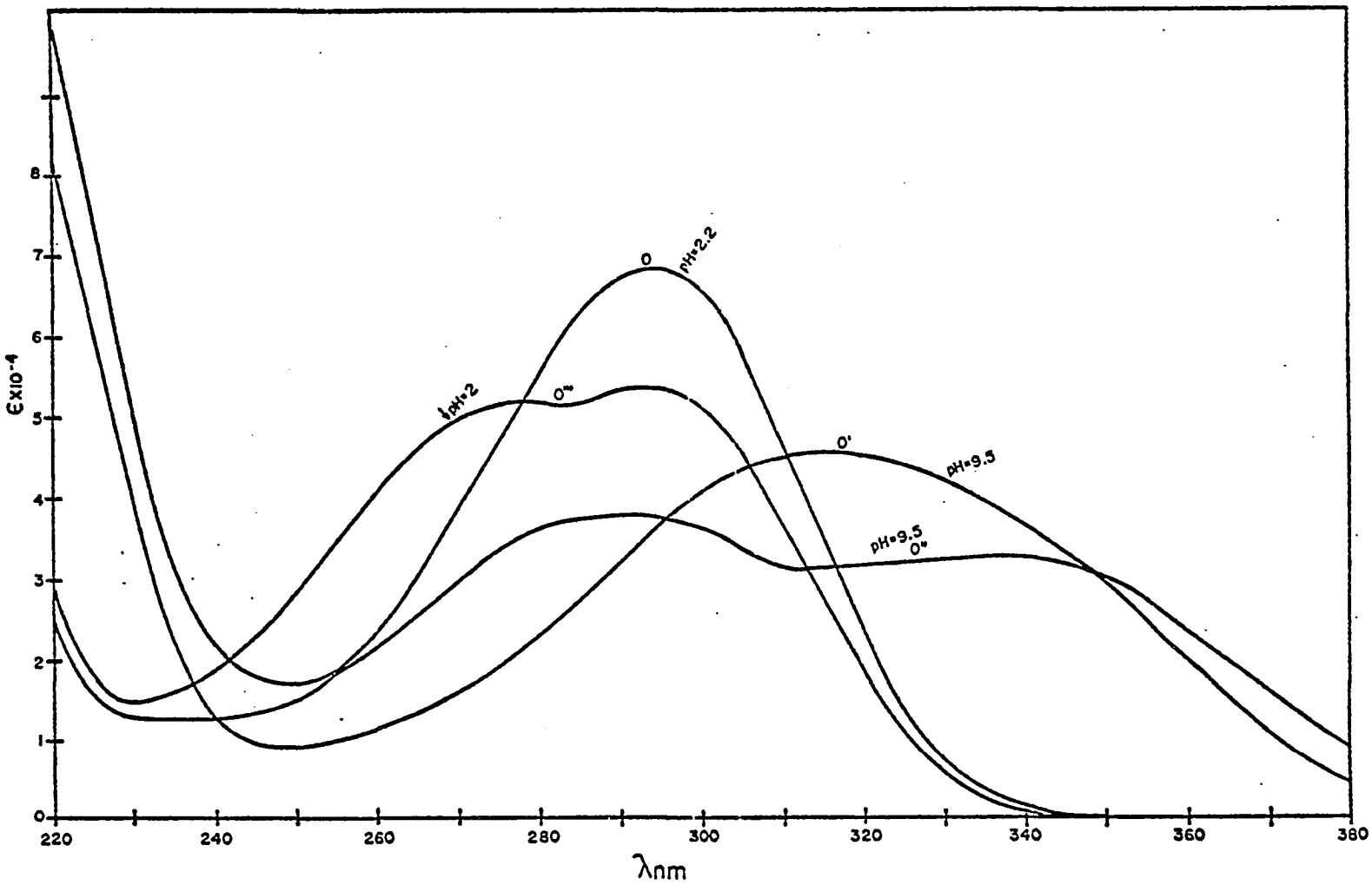
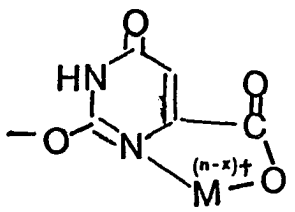
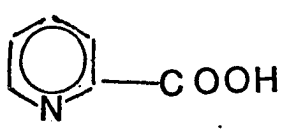


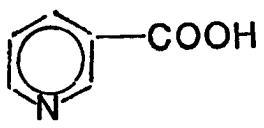
Figure 10. Absorption spectra of equimolar (10^{-4} M) 6-acetyl uracil and nickel ions. The curve O is the absorption spectrum of 6 acetyl uracil without Ni^{2+} ions at pH 2.2 and O' at pH 9.5. Curve O" is the absorption spectrum of equimolar solutions of Ni^{2+} and 6 acetyl uracil at pH = 9.5. O"' is the absorption spectrum of equimolar solutions of Ni^{2+} and 6 acetyl uracil after changing the pH from 9.5 to pH 2.



group is pulling off the N-1 proton making orotic acid bidentate. In case of 2-picolinic acid L. C. Thompson[13] arrived at a similar conclusion. Thus he found that picolinic acid forms 1:1 complexes with various rare earth ions. The formation constants for these complexes were much higher than simple carboxylate complexes. [Thus nicotinic acid complexes have a K value of Log K ≈ 2 compared to stability constants of Log K = 4.07 for Eu^{3+} picolinic acid systems.

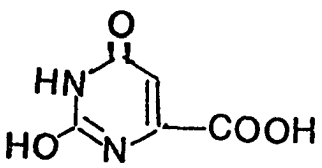


PICOLINIC ACID

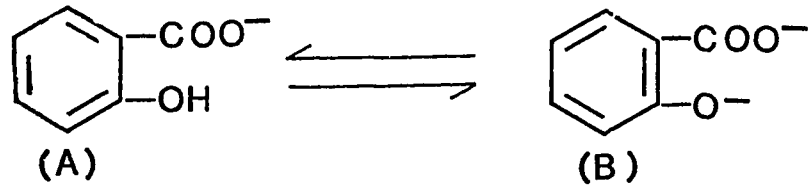


NICOTINIC ACID

In our system we reach an identical situation if we enolize the N-1 proton.

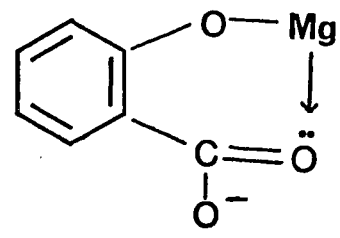


The pK_a values for orotic acid are 1.8, 9.45 and >13[14,15] blocking the carboxyl group can lower the pK value of N-1 proton. In our complex formation studies it is apparent that the metal ion complexed with carboxyl group is lowering the pK of N-1 proton which is no more in an anionic environment. This is not surprising since similar effect has been observed in the case of salicylate anion.[16] In the absence of Mg^{2+} ion at pH = 10 salicylate fluoresces as the mono anion A (λA = 296, λF 420 nm). The fluorescence of dianion B which is more than twice as intense as the monoanion is not seen below pH 12 (λA = 316, λF 410 nm).



(A) (B)

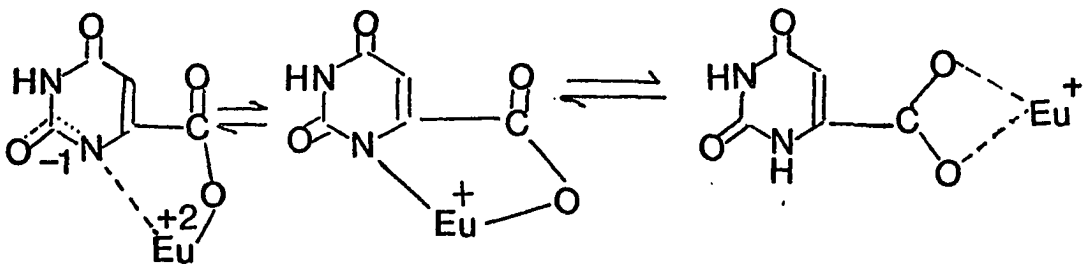
In the presence of Mg^{2+} ions the fluorescence of dianion is observed at pH = 10, implying that the Mg^{2+} has enabled the phenolic -OH group to ionize by forming a complex C.



(C)

Since 3- methylorotic acid yields complex spectra very similar to those of orotic acid itself it is evident that the change in the λ_{max} as a result of complex formation in either case is probably due to ionization of the N-1 proton on the pyrimidine ring. The uv spectra of all the compounds we studied display only one sharp isosbestic point indicating only one well defined equilibrium. Furthermore, the isosbestic point in each series of spectra is separated by exactly 15 to 16 nm from the λ_{max} for the particular compound in its neutral or monoanionic form. It is quite likely since then that as a result of complex formation with metal ions orotic and 3- methylorotic acids and with increase in pH the two esters and the 6-acetyl uracil are undergoing similar structural changes. The most probable and the common structural change for these compounds appears to be the dissociation of N-1 proton.

We therefore conclude that the 1:1 complex of orotic acid-metal ions which exhibits a distinct uv spectrum has the following structure.



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CHAPTER III

PHOTOCHEMISTRY OF 6-ACETYL URACYL IN AQUEOUS SOLUTION

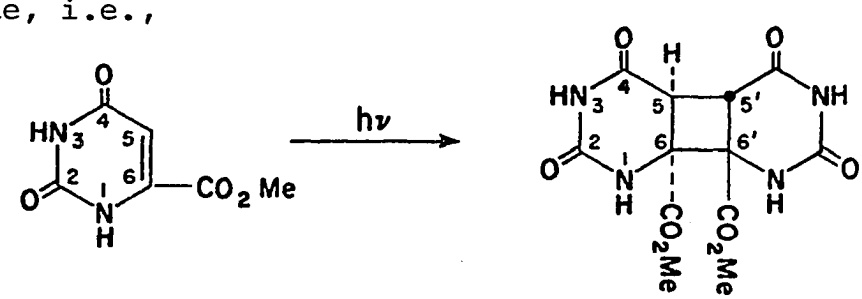
INTRODUCTION

As discussed in Chapter I and II we observed that 10^{-4}M orotic acid, 3- methylorotic acid and orotic acid esters undergo efficient photodimerization through their triplet excited states when photolyzed with uv light of 250-300 nm, whereas for similar concentrations of 1-methyl, 1,3 dimethyl-orotic acid, 6-methyl uracil, and isoorotic acid the photo-dimerization yields were immeasurably small at room temperature photolysis. Orotic and 3- methylorotic acid were also found to form strong uv detectable complexes with various metal ions (Zn^{2+}, Ni^{2+}, Eu^{3+}, etc.) whereas for 1-methylorotic acid, 1,3-dimethylorotic acid and orotic acid esters no complex formation with metal ions was noticed. It was seen in energy transfer studies that orotic acid and 3-methylorotic acid trans-ferred the excited triplet energy to Eu^{3+} quite efficiently whereas 1- methylorotic acid, 1,3-dimethylorotic acid and orotic acid esters were poor sensitizers of europium

emission. In conclusion, it was apparent that those compounds which can undergo efficient intersystem crossing to their triplet state to yield photodimers and at the same time are capable of forming ground state complexes with europium can also transfer energy and excite europium fluorescence. Orotic acid esters thus gave good yield of photodimer via their triplet excited state but their inability to complex Eu^{3+} made them poor sensitizers of Eu^{3+} emission. The quantum yield of photodimers seemed to be related to the existance of an N-1 proton and a carbonyl function at the 6- position. 6-Acetyluracil should be an interesting candidate to test this hypothesis, since it contains an N-1 proton and a carbonyl function at the 6-position of the uracil ring. This compound was not found to complex with metal ions (Chapter II) and would be a poor sensitizer of Eu^{3+} fluorescence. This compound should thus behave analogously to methylorotate and would provide an additional evidence to substantiate the fact that the energy transfer to Eu^{3+} in these systems in aqueous solution is not a simple diffusion controlled collisional process and the observed Eu^{3+} fluorescence sensitization is essentially linked to the ground state complex formation.

Photolysis of aqueous solution of 6-acetyl uracil,

(10^{-4}M) in fact, produced a new dimeric species which did not appear to be the expected 2 + 2 cyclobutane-type dimer forming on 5,6 double bond of pyrimidine ring obtained from orotic acid, 3-methylorotic acid or methyl and isopropyl-orotate, i.e.,



Furthermore, the reaction was probably occurring via a singlet excited state since nitrogen bubbling did not increase the rate of disappearance of 6-acetyl uracil. Photodimers of orotic acid, etc. are quantitatively split into monomer in aqueous solutions by uv photolysis[1] at short wavelength radiation (λ <240 nm). This new photoproduct of 6-acetyl uracil, λ_{max} around 270 nm, was unchanged even after a prolonged photolysis at 240 nm. The possibility of the new photoproduct being formed via photohydration across 5,6 double bond was also eliminated since heating the photolyzed solution at 90-100° for eight hours did not bring about any change in the uv spectrum. This anticipated thermal dehydration otherwise is a typical process occurring in 5,6 dihydro-6-hydroxy pyrimidines.[1] We isolated and characterized the new product and found it to be an oxetane type product.

Experimental

Materials:- Pyruvic acid, triethyl orthoformate and guanidine carbonate were purchased from Aldrich and were used without further purification. Melting points were determined with Fisher Jones apparatus and are uncorrected. Infrared spectra were recorded on a Beckman IR 12, ultraviolet spectra on a Cary-18 spectrophotometer, nmr spectra on a Varian T-60 or Varian XL-100 machine. Mass spectra were taken on Hitachi-Perkin Elmer REM-5E. Elemental analyses were performed by Chemalytics, Inc. (Tempe, Arizona) and all samples were dried at 92°C, 3 torr, over P_2O_5 prior to analysis.

Thin layer chromatography was done on Eastman chromagram sheets (13181 silica gel with fluorescent indicator No. 6060) with the following eluting solvent systems.

A) n-butanol/acetic acid H_2O 5:2:3

B) 1,4 dioxane/H_2O 90:10

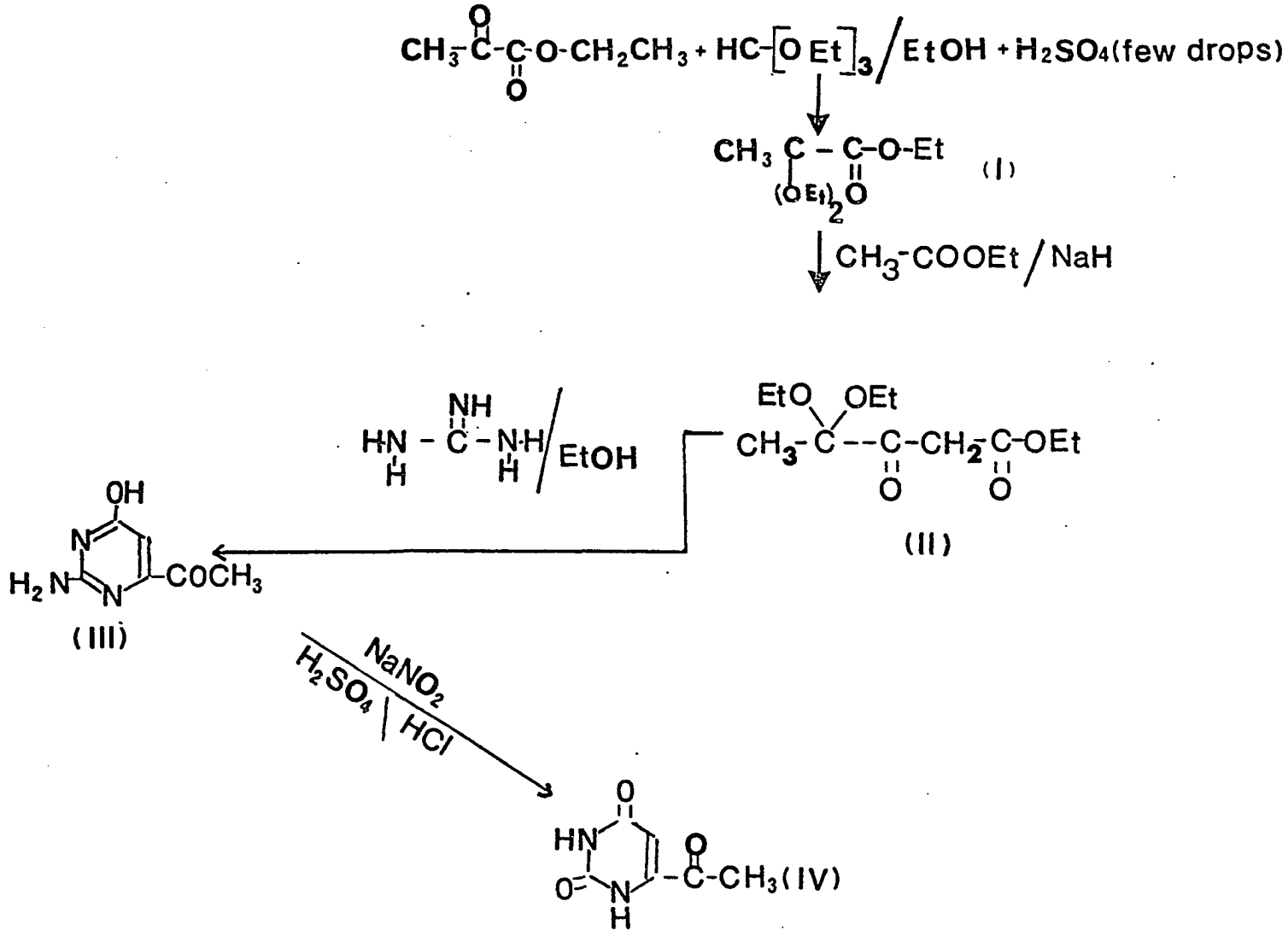
Synthesis of 6-Acetyl Uracil

The synthetic steps involved in the preparation of 6-acetyl uracil are outlined in Chart I.

Ethyl α,α-diethoxypropionate[2] (I):- In a 250 ml round bottom flask were placed 21.66 g (.187 moles) of ethyl pyruvate, 46.24 g (.31 moles) of triethyl orthoformate,

CHART-1

SYNTHESIS OF 6-ACETYL URACIL



38.32 g (.83 moles) of absolute ethanol and 5 drops of concentrated sulfuric acid. The flask was fitted with a water cooled reflux condenser and a calcium-chloride drying tube. The mixture was allowed to stand for 22 hours at room temperature. After refluxing the mixture for 12 hours most of the ethanol was removed by simple distillation at atmospheric pressure. The compound then was separated from acid catalyst by vacuum distillation at 4 mm (90°C) and was collected into another flask immersed in dry-ice acetone bath. The product was then fractionated to give 33.6 g (95%) of ethyl α,α diethoxy propionate, a colorless liquid, b.p. 93-94°C (26 mm).

Ethyl 3,4-dioxovalerate 4-diethylketal (II)[3]:- To a 500 ml two necked round bottom flask equipped with condenser, drying tube and a dropping funnel were added 38 g (.2 moles) of (I). The flask was kept in ice-water bath and stirred for a period of one hour with magnetic stirrer. To this was then added 17.6 g of sodium hydride (as a 54.5% suspension in mineral oil). Ethylacetate, 26 g. (.295 moles) was added to this mixture dropwise with stirring over a forty minute period. The solution was then stirred at room temperature for another three hours. At the end of this period a solid gummy mass was obtained which was poured over 100 g of crushed ice. The resulting solution was adjusted to pH 4 with cold 6 M hydrochloric

acid and extracted 6 times with 50 ml portions of ether. The combined extract was washed two times with 50 ml portions of water, dried over anhydrous magnesium sulfate and the ether was distilled off at atmospheric pressure. From the remaining liquid ethyl acetoacetate was removed at 45-50° (4-6 mm) and then the β-ketal ester was distilled off at 110°C (4 mm) into a flask cooled in dry ice-acetone bath. This liquid was then further fractionated to yield 16 g of (II) (52%) b.p. 85°C (3 mm).

2-Amino-6-acetyl-4 (3H)-pyrimidone (III):- A mixture of 21.4 g of (II), 300 ml of dry ethanol and 20.2 g of guanidine carbonate was placed in a 500 ml round bottom flask and refluxed overnight. The ethanol from this dark musky red colored solution was removed with rotary vacuum evaporator and the solid obtained was dissolved in cold dilute hydrochloric acid. The volume of the solution was adjusted to 200 ml and pH was kept at 1. The solution was then boiled under reflux for 20 minutes and filtered after treating with norit. The filtrate was cooled and neutralized to pH 7 with saturated aqueous sodium bicarbonate solution. A tan solid precipitated which was filtered after cooling. This was again dissolved in aqueous hydrochloric acid solution, treated with norit as above and recovered back at pH 7 by adding sodium bicarbonate solution. Yield 4.5 g (31.7%). R_f values .59, .52 in solvent systems A and B

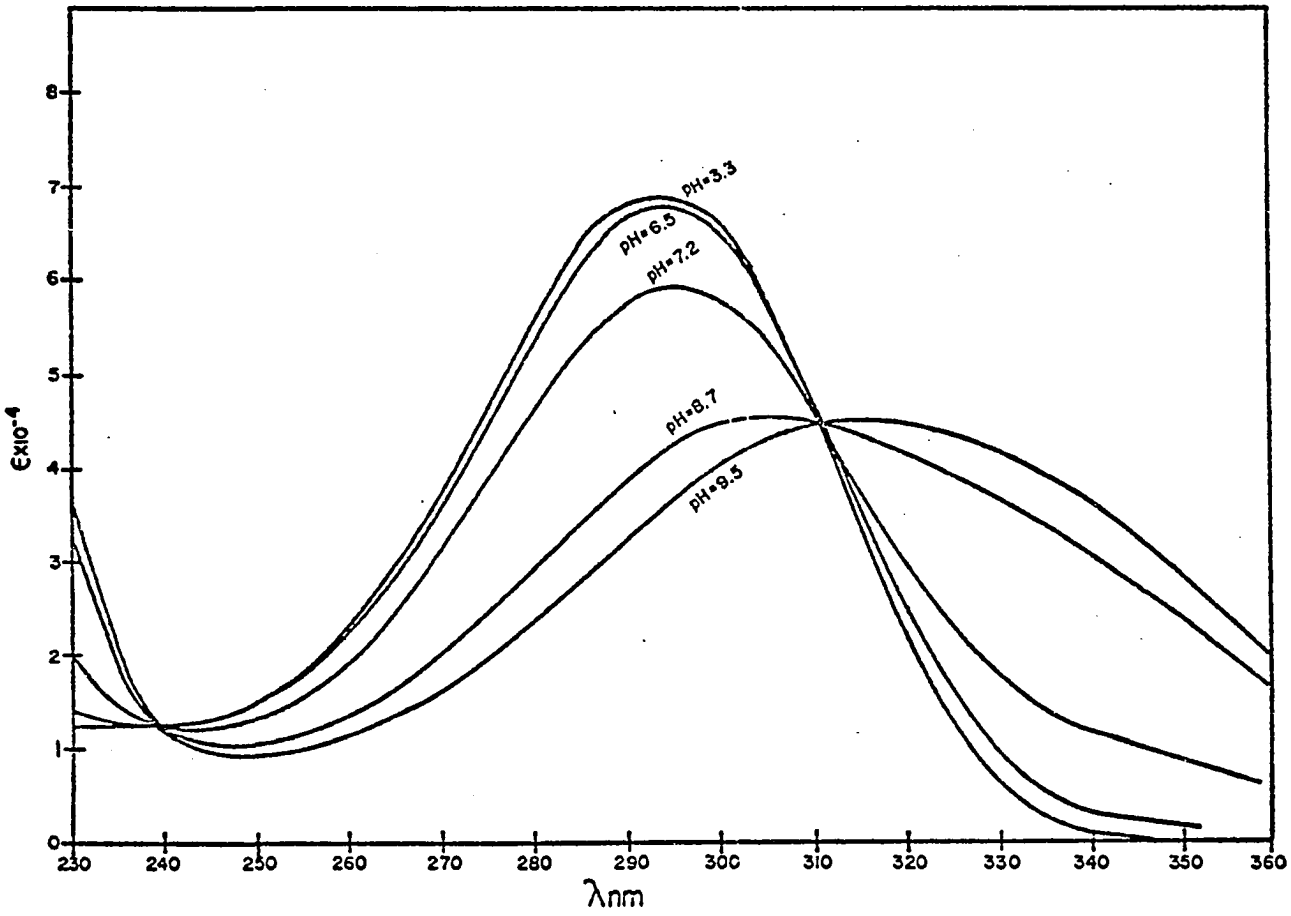


Figure 1. Absorption spectra of 6-acetyl uracil (10^{-4} M) at various pH values.

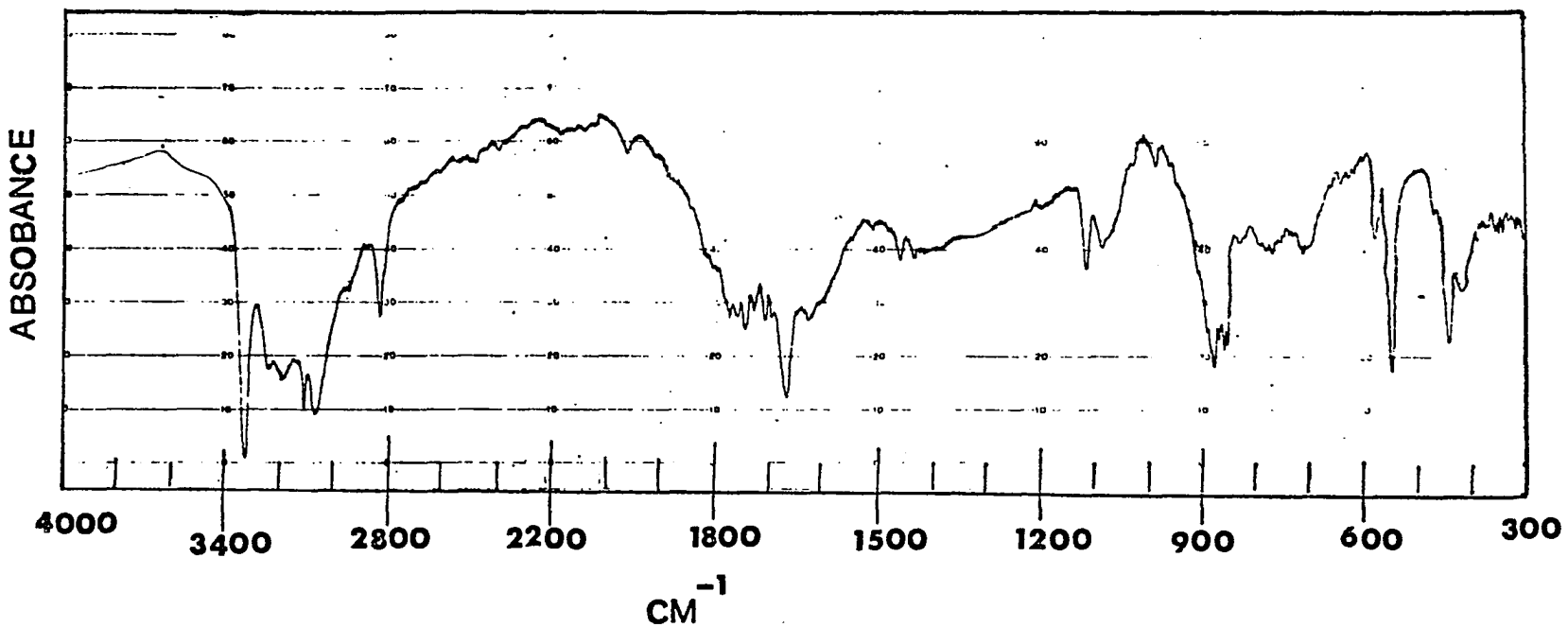


Figure 2. Infrared spectrum of 6-acetyl uracil in potassium bromide pellet.

The compound did not melt below 300°C.

6-acetyl uracil (IV):- To 4.5 g of (III) was added 30 ml of 6 M hydrochloric acid, 15 ml of concentrated sulfuric acid, and 45 ml of H_2O. The mixture was stirred with a magnetic stirrer and to it was added in a dropwise fashion a solution of 18 g of sodium nitrite in 30 ml of distilled water over a period of 30 minutes. The mixture was stirred for another 3 hours at room temperature and chilled to give 2.1 g of crude 6-acetyl uracyl. The compound was recrystallized from boiling water to give colorless needles of (IV). Yield 1.5 g (33%) m.p. 255°C. R_f value .66; .65 in solvent systems A and B. Fig. 1 shows the uv spectra of 6 acetyl uracil at various pH values.

NMR (D_2O) δ 2.7, 3H(s); δ 6.9, 1H(s)

Mass spectrum (m/e 154, 140, 126, 112, 111, 84, 68, 57, 55, 53, 44)

IR is shown in Fig. 2.

Photolysis of 6-Acetyl Uracil

A schematic diagram of the photolysis set up used is shown in Fig. 3. Our light source was a Hanovia Medium Pressure mercury arc lamp (679 A 36). A Kimax filter (uv cut off below 300 nm) was used to avoid the further photolysis of photoproduct which was found to absorb uv light below 300 nm. Lamp and solution were cooled with chilled

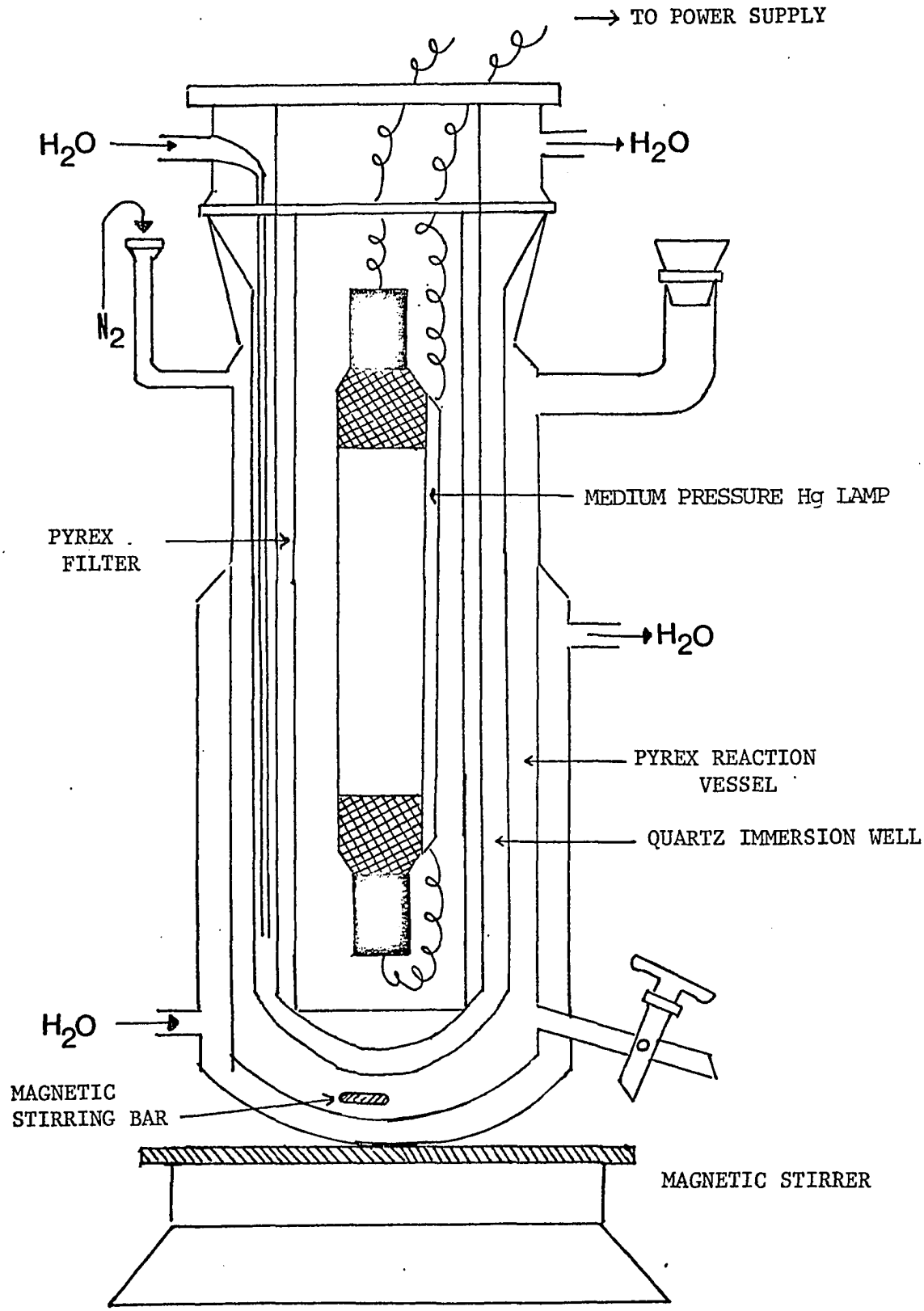


Fig 3:- PHOTOLYSIS SETUP

water. No nitrogen bubbling was done as the photoproduct formation was not found to be quenched by oxygen. In a typical preparative photolysis 120 mg of 6-acetyl uracil was dissolved in 500 ml of triply distilled water (con. 1.56×10^{-4} M). 2 ml from this was diluted to 5 ml and the uv spectrum was recorded. The solution was allowed to cool for 1 hour in the apparatus and was vigorously stirred with the help of a magnetic stirrer. The photolysis was initiated by firing the lamp and during photolysis at various intervals of time 2 ml aliquots were pipetted out and uv spectrum was checked as above. Fig. 4 shows the change in uv spectrum of 6-acetyl uracil with increasing period of photolysis. After about 75 minutes of photolysis the absorption peak at 295 nm due to 6-acetyl uracil completely disappeared with the appearance of a new peak with the λ_{max} centered at 270 nm. The solution turned faint yellow. The photolysis was terminated at this stage.

Isolation of the Photoproduct

After stripping off the H_2O the yellowish powder was dissolved in a minimum amount of hot 1:1 H_2O/ethanol mixture and the major photoproduct was isolated by preparative thick layer chromatography on silica gel - 60 PF 254 using 1,4-dioxane/H_2O (90:10) as the eluting solvent system. The band at R_f value 0.33 was scraped off and stirred with dioxane. After filtering off the silica

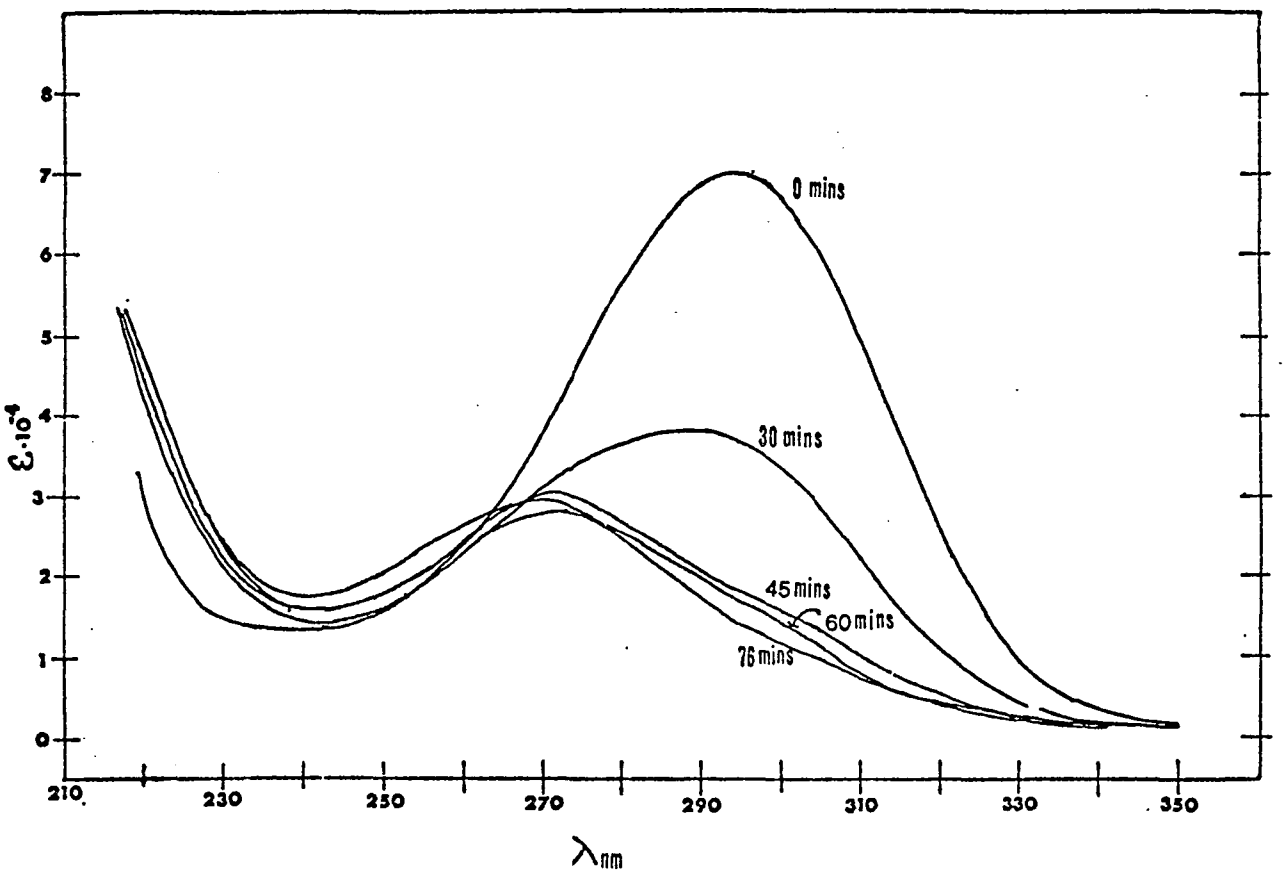


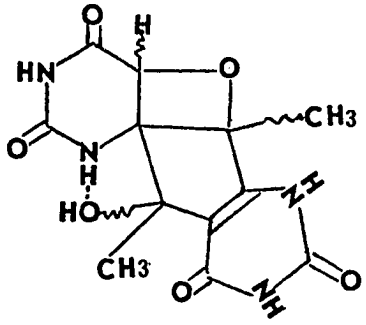
Figure 4. UV spectra showing the disappearance of 6-acetyl uracil accompanied by the formation of new photoproduct at various time lengths of photolysis.

gel the dioxane was evaporated to yield a white amorphous powder which was recrystallized from hot distilled water. Yield 60 mg. The compound did not melt below 300°C and above this temperature it turned brown and finally charred.

R_f values .52; .33 in solvent systems A and B. This compound was therefore more polar than 6- acetyluracil its precursor. Quantum yield was not determined but the photoreaction seems to have a high quantum yield with almost quantitative conversion to one photoproduct.

Structure of the Photoproduct

Based on our spectral data we assign the following structure to our photoproduct.



Mass-spectrum (Fig. 5) showed a molecular ion at m/e = 308 characteristic of dimeric species formed from 6-acetyluracil (m/e = 154). Also in the mass spectrum the next peak appeared at m/e = 291 (308-17) which could be due to loss of -OH from the molecular ion. This is typical of allylic alcohols.[4]

Elemental analysis calcd. for $C_{12}H_{12}O_6N_4$: C, 46.75; H, 3.9; N, 18.18. Found: C,46.55; H, 4.17; N, 17.93.

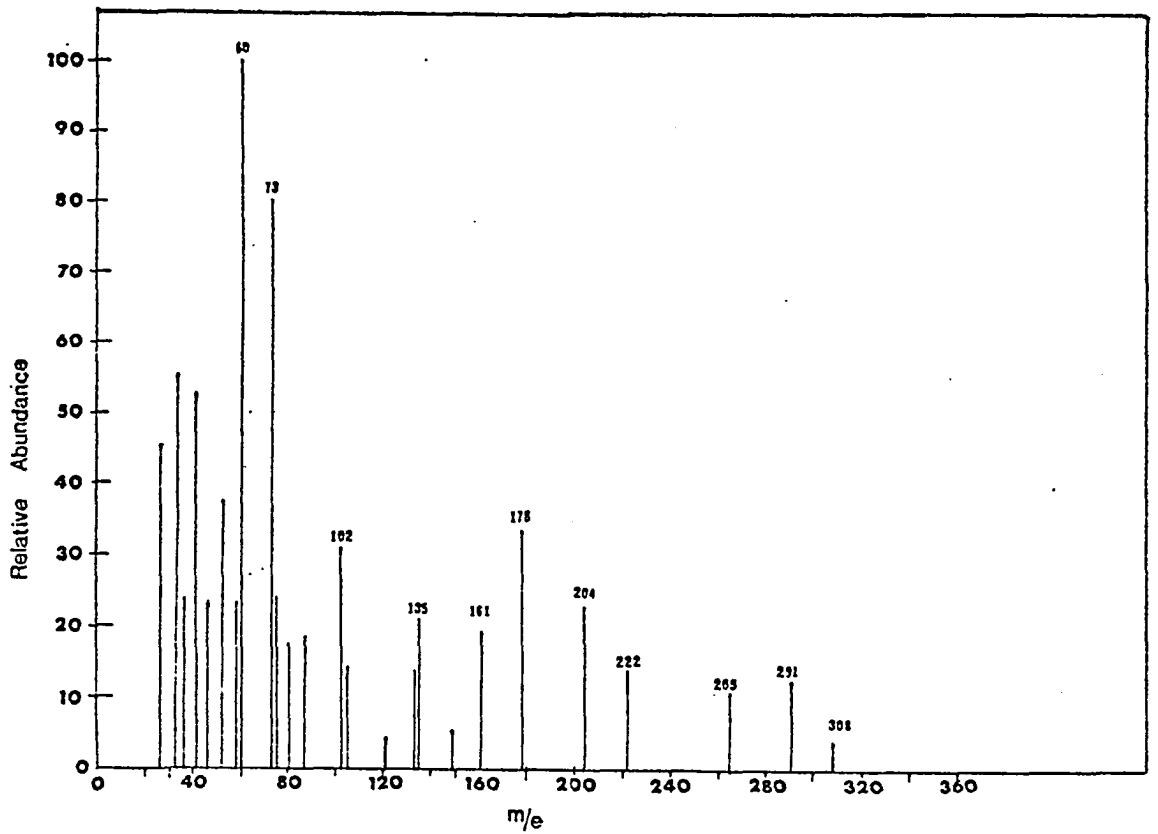
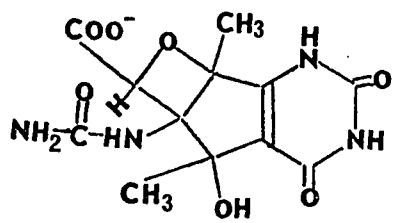


Figure 5. Mass spectrum of the photoproduct at 70 e.v. (only major fragmented ions are shown below m/e = 265).

Uv spectrum in H_2O displayed a sharp maximum at 269 nm with ε = 6500 below pH 5.5. Above pH 8 the uv spectrum had a λ_{max} at 291 nm with ε = 8600 (Fig. 6). These λ_{max} and ε values indicate the presence of a uracil type chromophore and an another uracyl type chromophore with a saturated 5,6 double bond. A positive test was obtained on paper spotted with photoproduct dissolved in H_2O which was sprayed with dil NaOH solution, dried and sprayed again with acidified p-dimethyl aminobenzaldehyde in ethanol. Thus it appears that in alkaline solution the formation of β-ureidopropionic acid derivative is taking place.[5]



Acidification of the alkaline solution to pH <5 regenerated the uv spectrum with λ_{max} centered at 269 nm. NMR spectrum (Fig. 7) in DMSO-d_6 gave proton signals characteristic of the above structure (δ values) 1.28 (3H, s); 2.3 (3H,s); 3.32 (1H, bs); 3.88 (1H,s); 6.48 (1H,bs); 7.7 (1H,s); 10.24 (1H,s); 10.96 (1H,s). Ref. DMSO-d6 signal at δ = 2.5 ppm. Adding few drops of D_2O removed the five one proton signals (Fig. 8) and only three signals at δ 1.28 (3H,s); 2.3 (3H,s); 3.88 (1H,s) appeared. Our

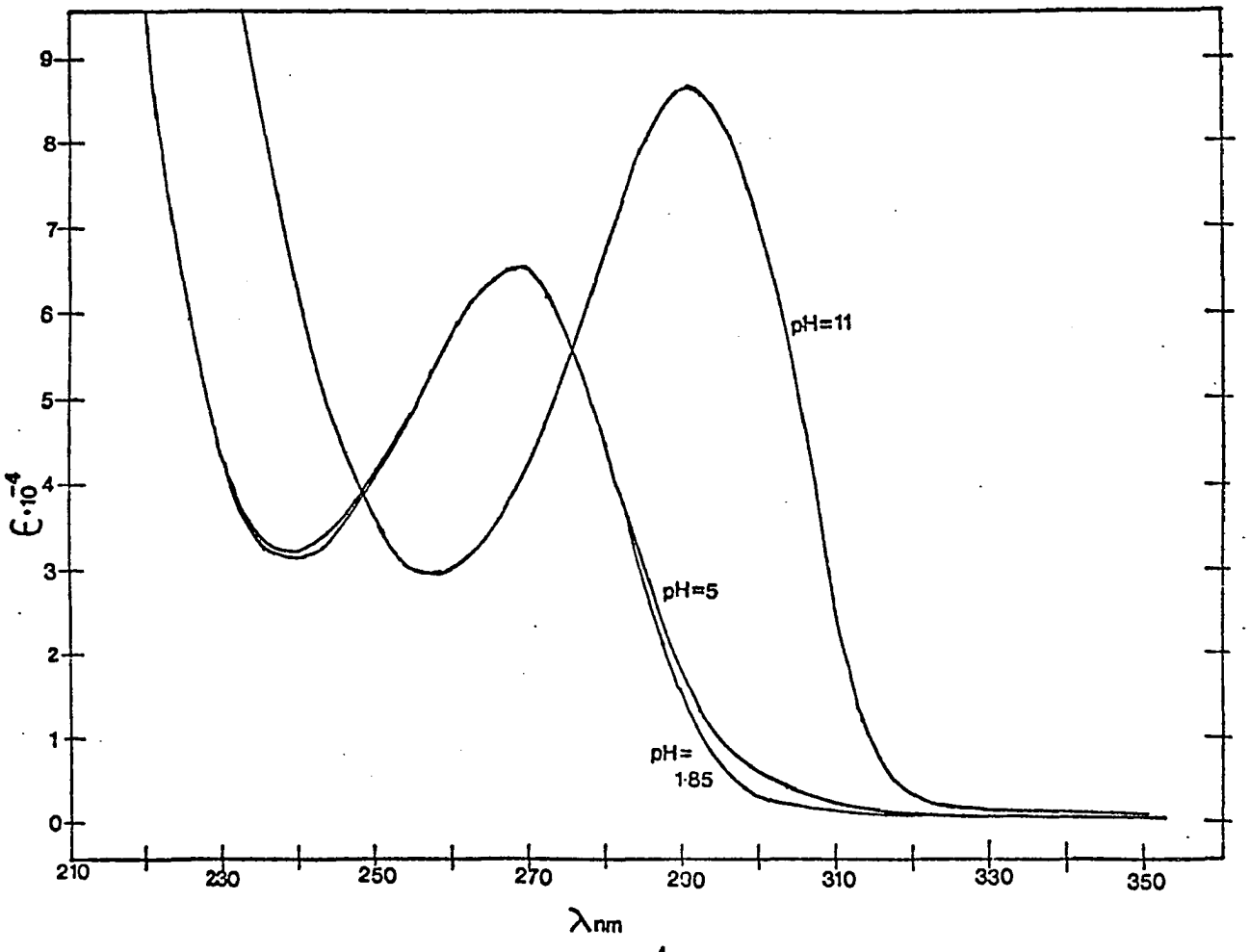


Figure 6. UV spectra of aqueous solutions (10^{-4} M) of the photoproduct at three different pH values.

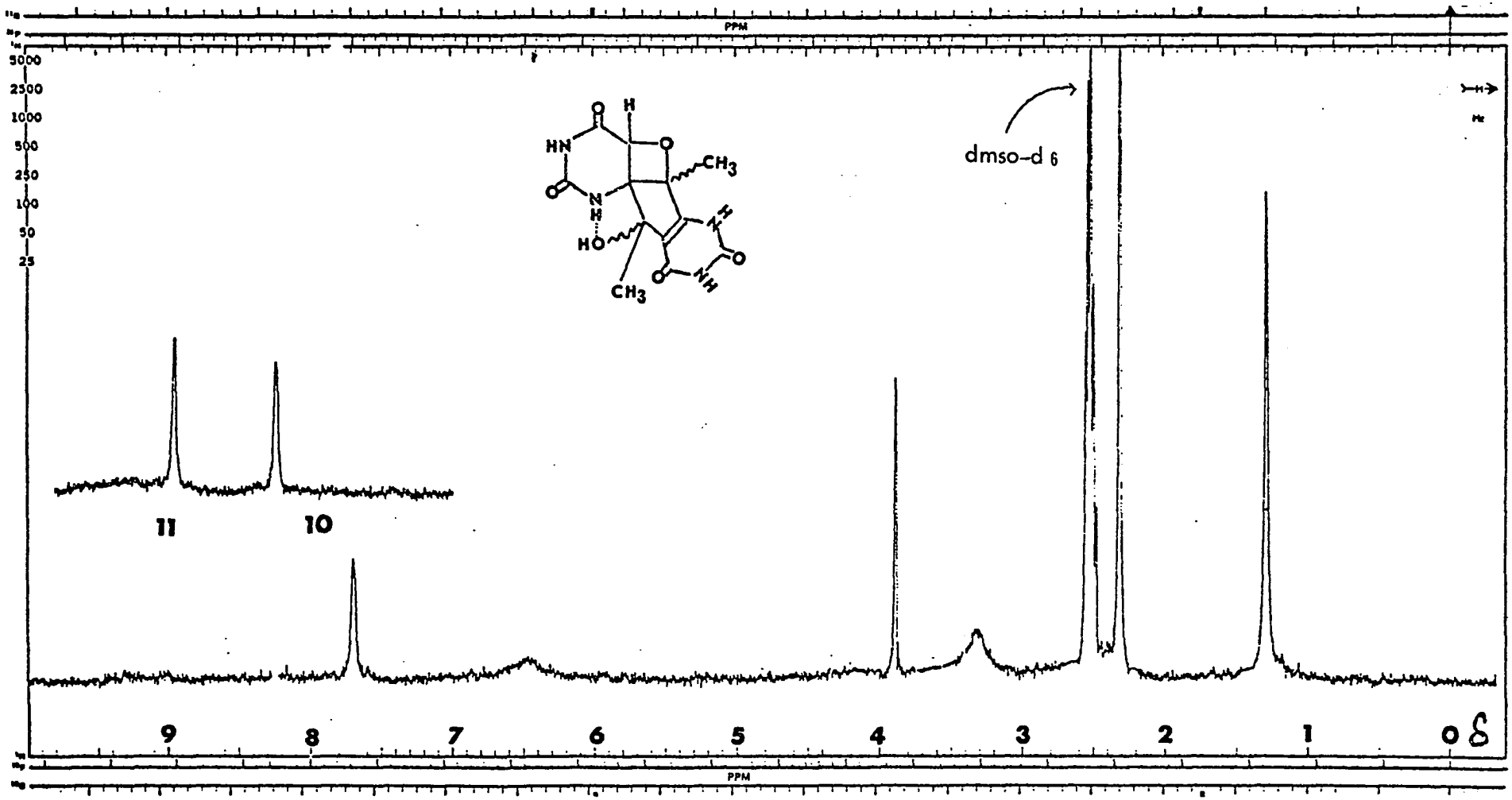


Figure 7. NMR spectrum of the photoproduct in $(CD_3)_2SO$ at 100 Mc.

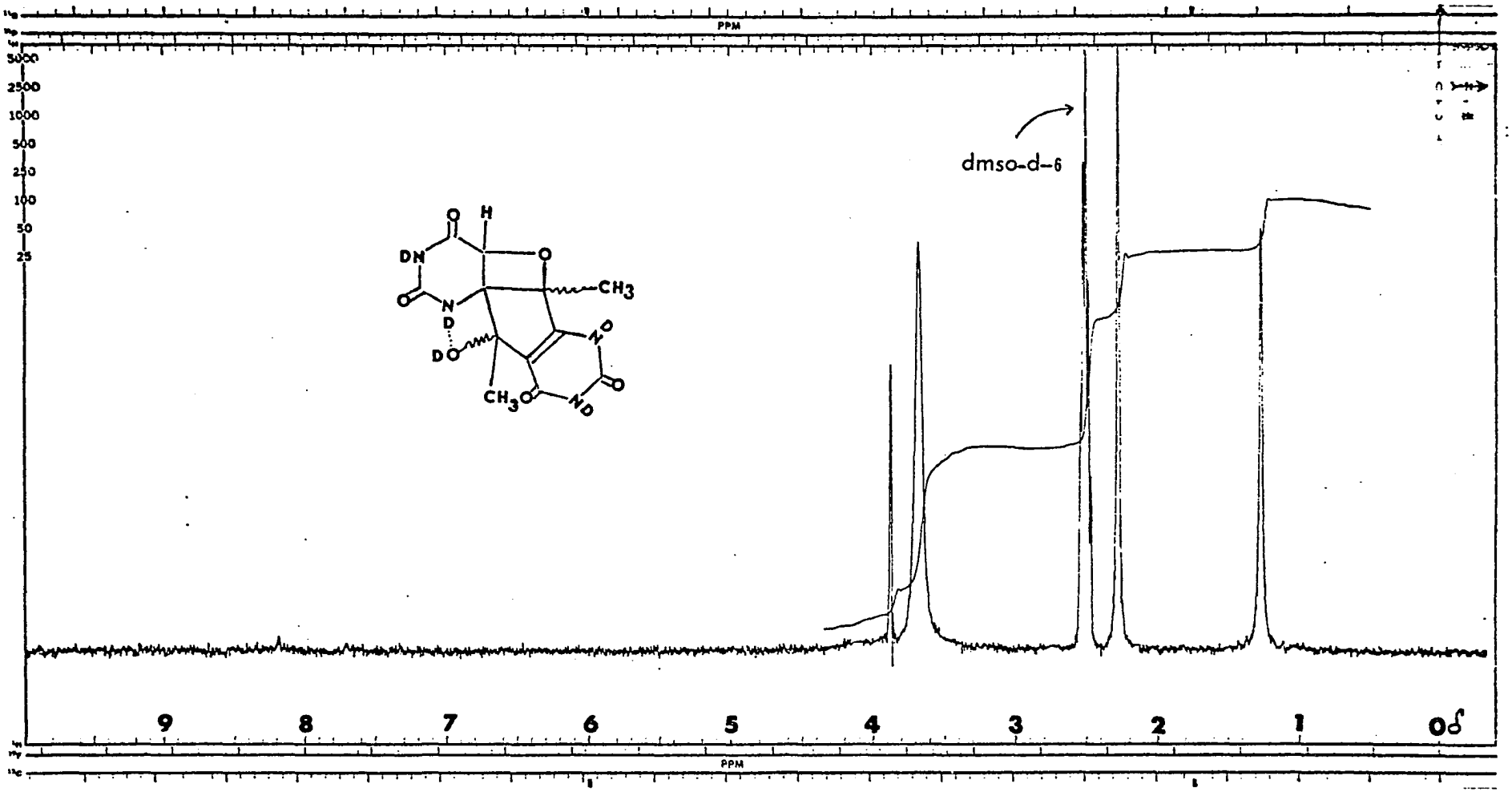


Figure 8. NMR spectrum of the photoproduct in $(CD_3)_2SO$ + D_2O at 100 Mc.

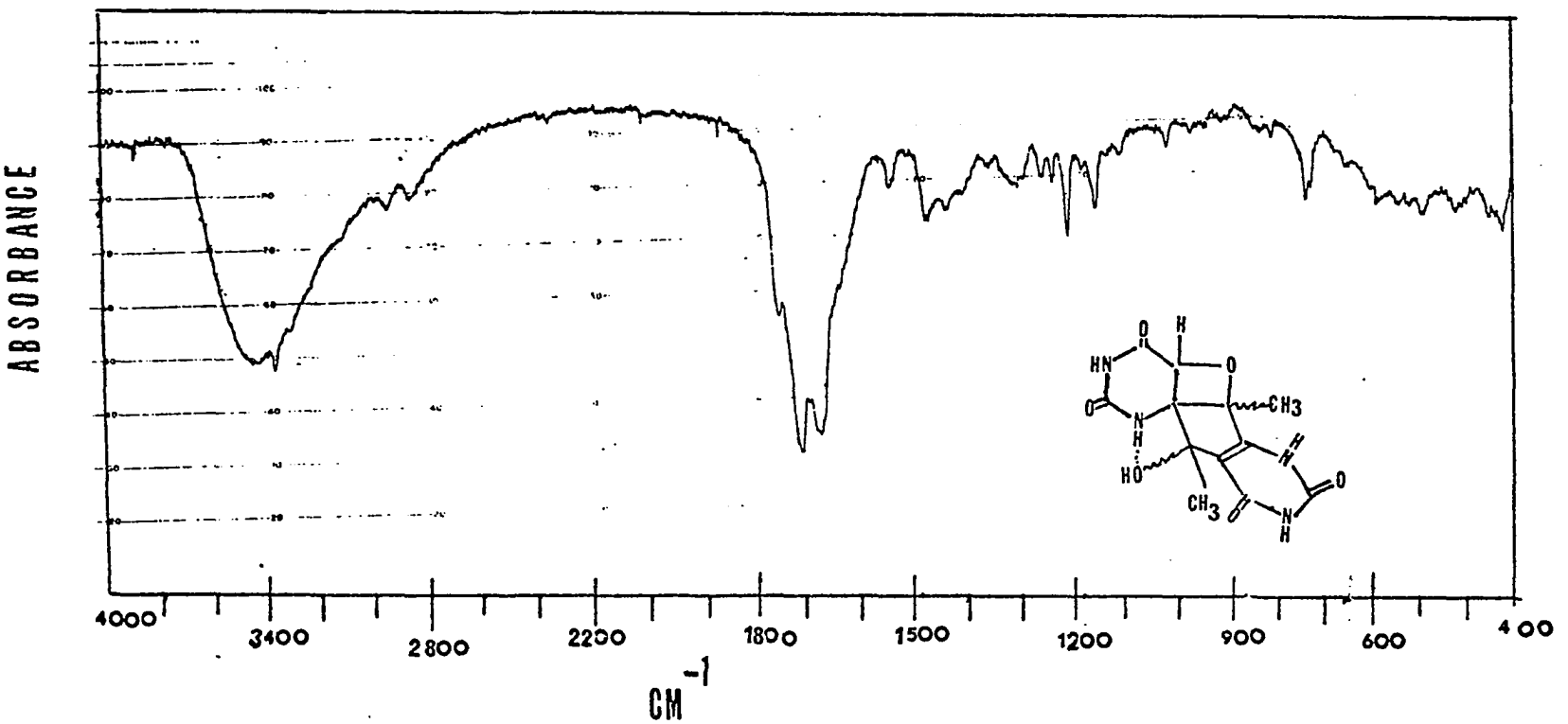


Figure 9. Infrared spectrum of the photoproduct in potassium bromide pellet.

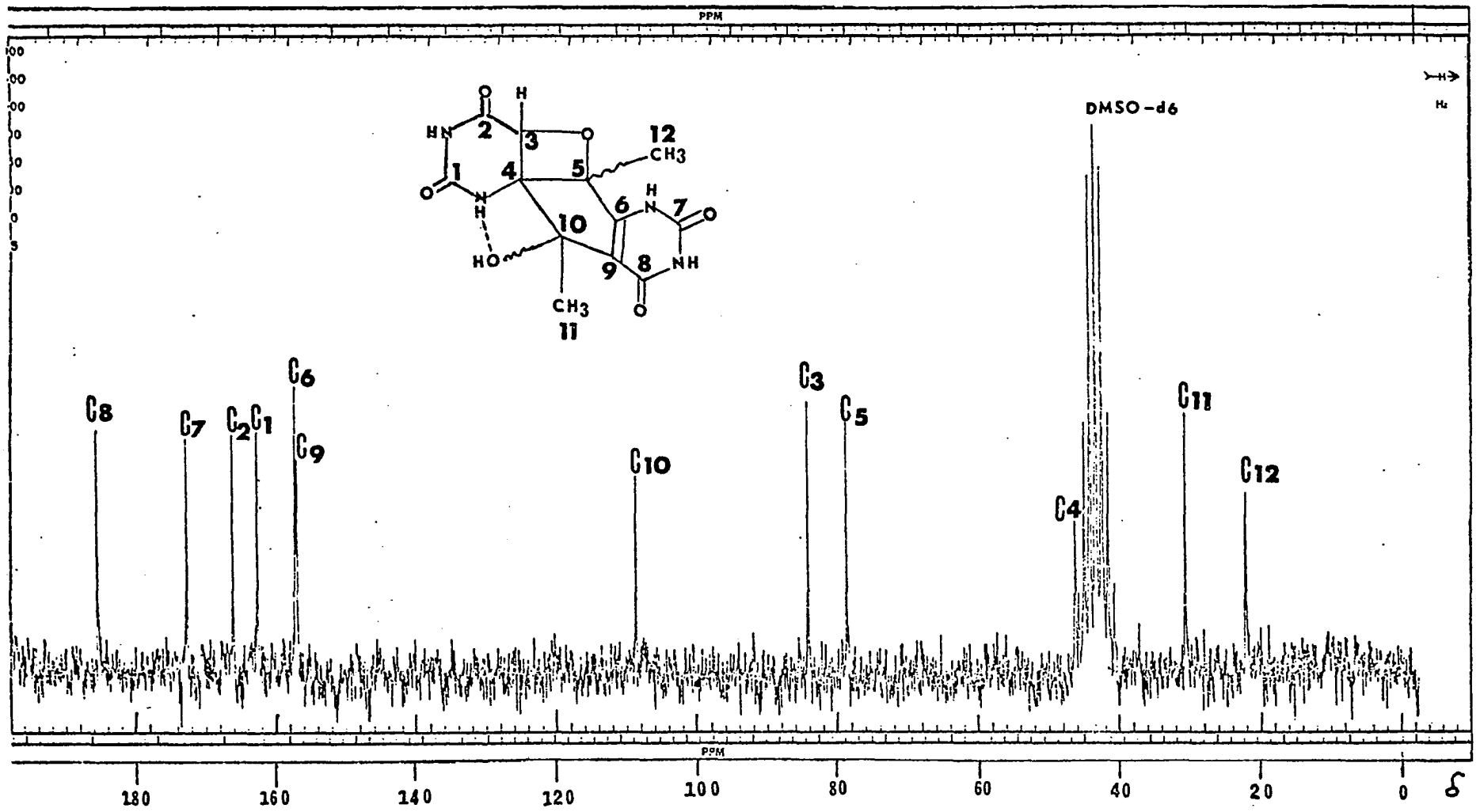


Figure 10. C^{13} NMR spectrum of the photoproduct in $(CD_3)_2SO$ at 100 Mc. [Ref. $(CD_3)_2SO$ signal at $\delta = 43.5$]

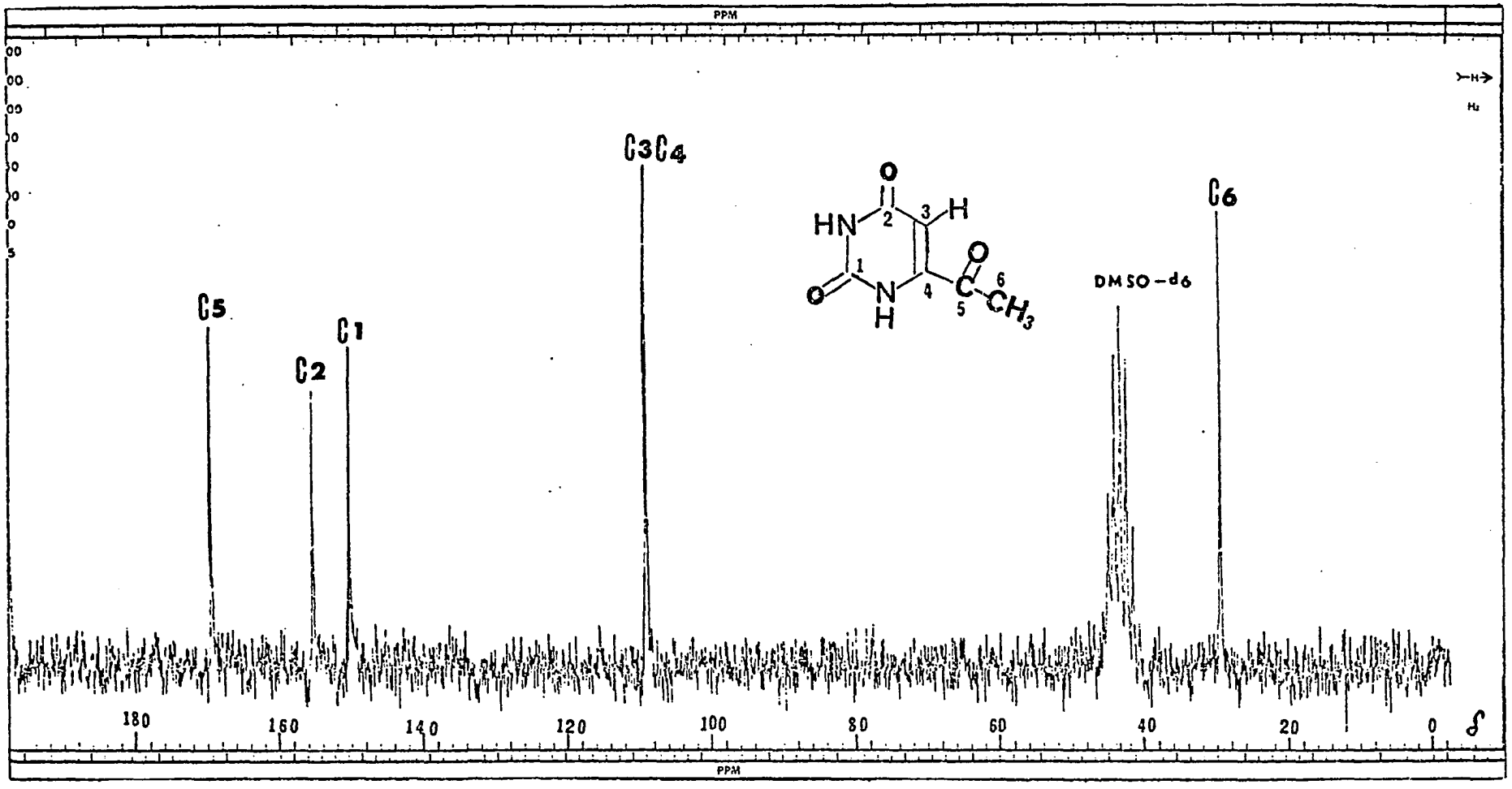
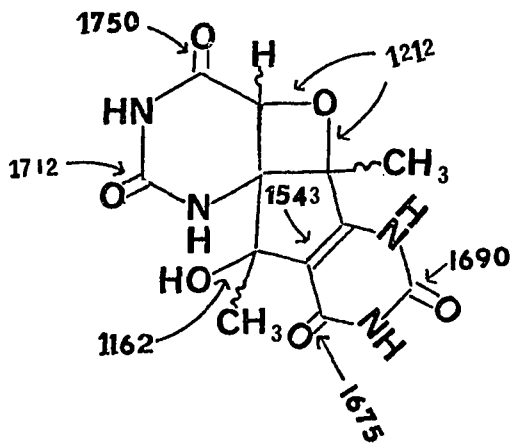


Figure 11. C^{13} NMR spectrum of 6-acetyluracil in $(CD_3)_2SO$ at 100 Mc.

structure thus accounts for all the five exchangeable protons (4 NH and one OH) in D_2O. IR (Fig. 9) showed characteristic frequencies at 1162, 1212, 1543. 1675, 1690, 1712, 1750 and a broad band in the region 3300-3500 cm^{-1}. The assignment of these absorptions can be made as follows.[6]

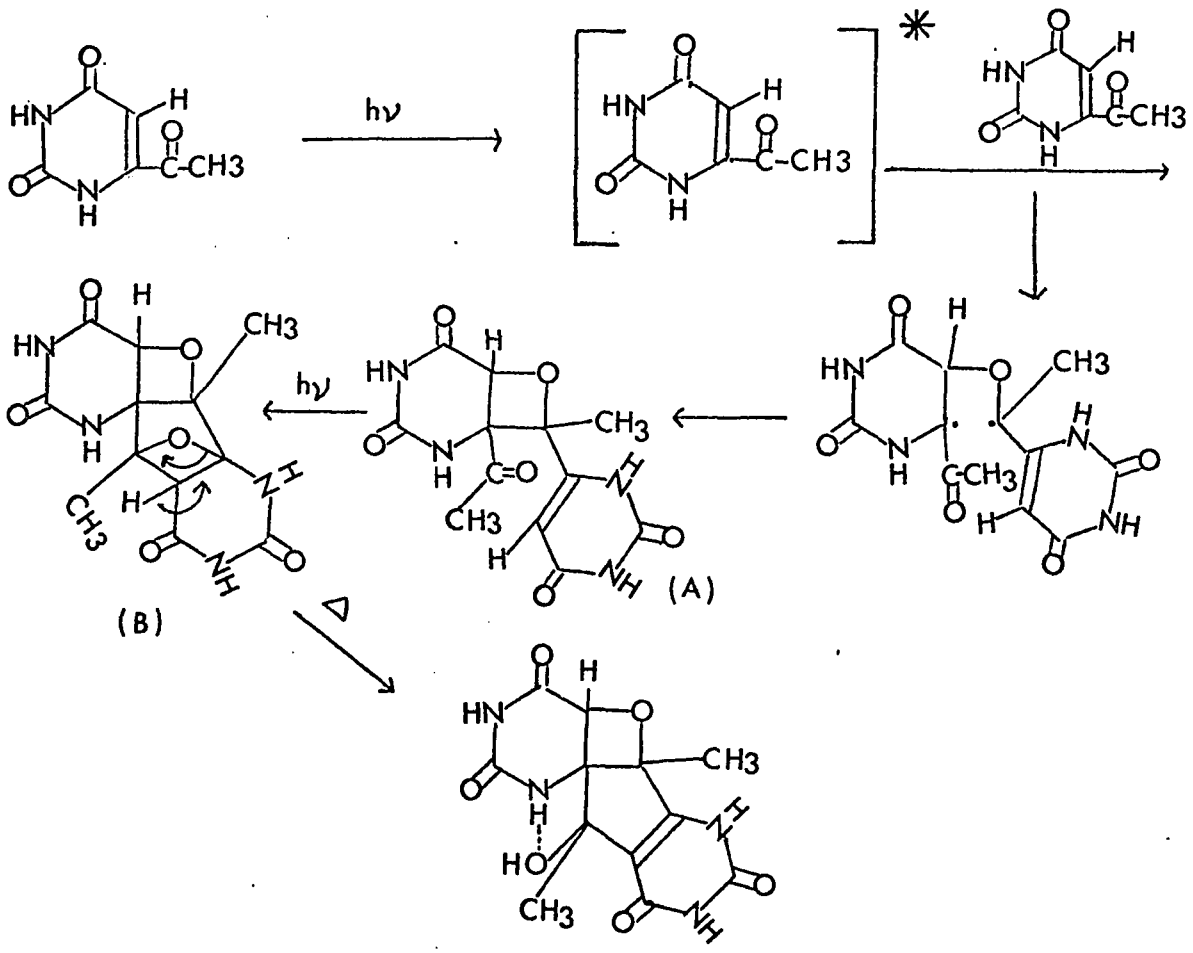


C^{13} NMR spectrum (Fig. 10) showed twelve carbon peaks. Our assignments of individual carbon atoms is based on approximately calculated chemical shifts and can be in error.[7,8] We have also included the C^{13} nmr spectrum of 6-acetyluracil for comparison (Fig. 11).

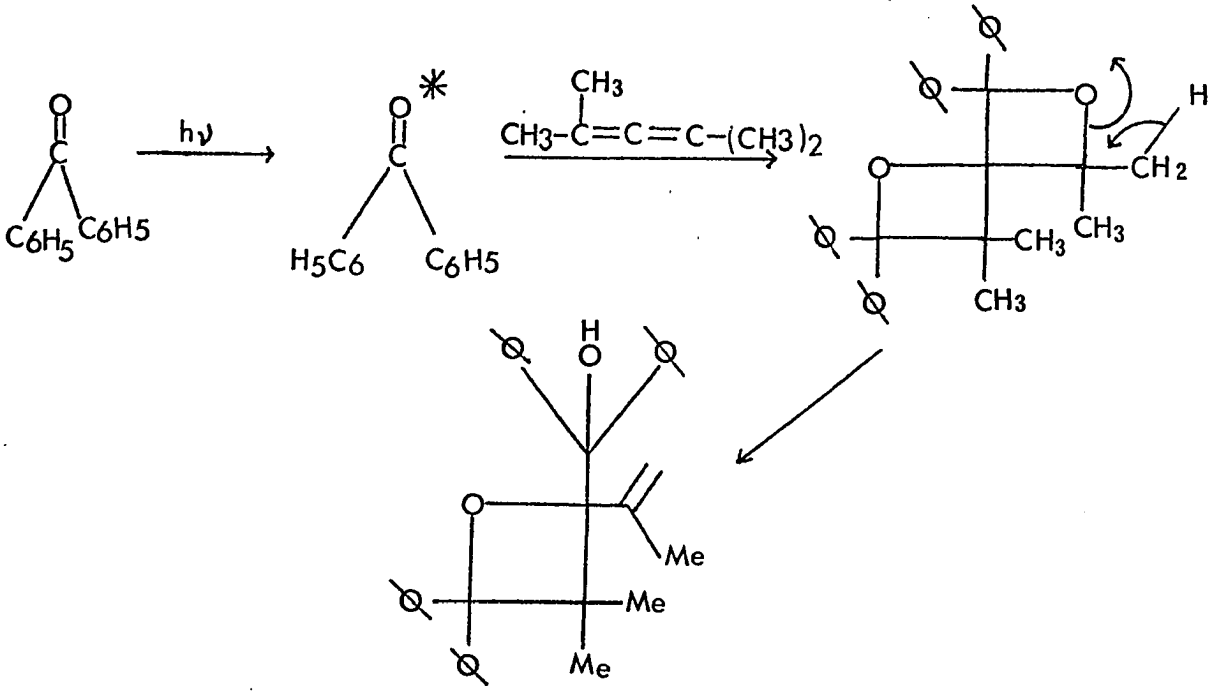
At present we are unable to determine the stereochemistry of the product at the four asymmetric centers.

Mechanism of photoproduct formation:- Oxetane formation is a typical photocycloaddition reaction of carbonyl group to double bond.. This reaction takes place either through an n,π^* singlet or triplet state of the carbonyl group.[9] In our case the reaction is probably occurring through n,π^* singlet excited state or a very short lived

triplet state as the rate of reaction was insensitive to the presence or absence of oxygen. We tentatively propose the following mechanism where the cyclo-addition of excited carbonyl group of one 6-acetyl uracil molecule to the 5,6 double bond of second 6-acetyl uracil molecule yields the oxetane type dimeric species A which is followed by second photocycloaddition of the remaining carbonyl group to the double bond in the adduct leading to the formation of a double oxetane type compound B. The loss of β proton with the formation of double bond is then accompanied by the ring opening in the second oxetane ring. The last process can be a simple thermal ring cleavage.



A similar type of reaction has been observed in the literature during the photocycloaddition of benzophenone to tetramethylallene.[9]



6-acetyl uracil thus displayed a very interesting photochemistry nonetheless owing to its failure to undergo expected photodimerization we could not use it in our energy transfer work.

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