SINGLE ELECTRON TRANSFER REACTION STUDIES BY OPTICAL AND ESR SPECTROSCOPY
(A CASE STUDY OF NUCLEIC ACID BASES)

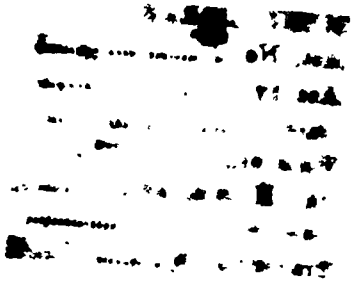
BY

SUCHANDRA BHATTACHARJEE
DEPARTMENT OF CHEMISTRY
SCHOOL OF PHYSICAL SCIENCES
1997

SUBMITTED

IN FULFILMENT OF THE REQUIREMENT OF THE DEGREE OF
DOCTOR OF PHILOSOPHY IN CHEMISTRY OF
NORTH EASTERN HILL UNIVERSITY, SHILLONG
INDIA.

Dedicated to my parents



declaration

The North Eastern Hill University
Month : June, Year : 1997

I SUCHANDRA BHATTACHARJEE, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any other research degree in any other University / Institute.

This is being submitted to the North Eastern Hill University for the degree of DOCTOR OF PHILOSOPHY in CHEMISTRY.

S. Bhattacharjee
SUCHANDRA BHATTACHARJEE

(HEAD)
DEPT.OF CHEMISTRY
NORTH EASTERN HILL UNIVERSITY

Dr.HARISH CHANDRA
SUPERVISOR

Acknowledgements

In the beginning I was just with enthusiasm alone ; I had taken up the venture. And they were right there all through to enable me to reach at this stage to write the opening page of my doctoral dissertation.

It is with great pleasure that I record my deep sense of gratitude to all, but I can't help going beyond for many of them.

It is my elation and privilege to express my sincere and profound gratitude to Dr. Harish Chandra, my supervisor who had introduced me into the fascinating field of ESR and had in painstaking moments, guided, inspired and persuaded me through the task, with an untiring zeal.

I am indebted to Prof. M.C.R.Symons, FRS, Department of Applied Chemistry, DeMont Fort University, Leicester, U.K for his valuable suggestions and financial assistance for my research work.

I am beholden to all the faculty members of the Dept. of Chemistry, NEHU for all suggestions and help I received from them.

I gratefully acknowledge the guidance, help and co-operation I received from the Head, Department of Chemistry, Prof. M.K. Mahanti and the Dean, School of Physical Sciences, Prof. S.N.Bhatt.

My heartfelt thank goes to Prof. M.K.Chaudhury, who was a great source of inspiration and Prof. H.Junjappa for the help I received from him during the tenure of my research work.

A deep note of appreciation is due to my research colleagues and friends : Nadeem, Guna, Jayashree and Wancy who stood by me through all odds to lend a helping hand. I am in dearth of words to express my gratitude to them. I also thank Mr. Dipak Sinha, Ms. Deepa Dey, Mr. Akhilesh Kr Gupta and all others who helped me.

A special mention also goes to the non teaching staff of the Dept. of Chemistry for the help and cooperation I got from them.

I am thankful to the Dept. of Chemistry and RSIC, NEHU for allowing me to use all the facilities available there.

I convey my gratefulness to the Staff, Computer Centre, NEHU for all the timely help I received from them.

I owe my thanks to the authorities of NEHU for their co-operation and help in all the administrative and technical aspects.

The Senior Research Fellowship of the Council of

Scientific and Industrial Research is gratefully acknowledged.

For all that I have done I owe it to the inspiration and love I got from my parents, brothers and my sister in law who had whole heartedly supported me and always stood by me even through the extremes of circumstances.

I am sincerely grateful to my parents in law for all the blessings, guidance and support they had always provided me with. I am also thankful to my sister in law for the encouragement I received from her.

Finally, my very special thanks are due to my husband, for he was a constant source of encouragement, enthusiasm and support and for bearing with patience all the troubles he had to face during the entire course. I feel short of words to express my gratitude to him.

I am and shall always remain obliged to every moment of strength I received from all members of my family, my supervisor and my friends.

S. Bhattacharjee

(SUCHANDRA BHATTACHARJEE)

List of Figures

List of Tables

Contents

CHAPTER - I

INTRODUCTION

1.1 NUCLEIC ACID BASES

" Two principles are necessary so that life shall succeed :
one consist of proteins, the other of nucleic acids ".

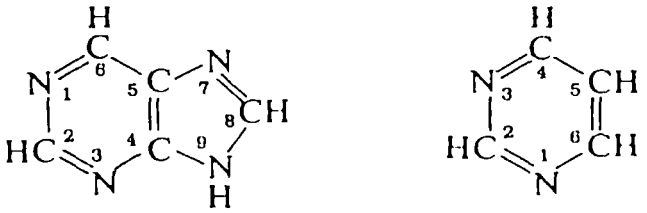
These sentences characterise very appropriately the key position of the two cell constituents which must be present for any living cell to function. While the importance of the proteins in cell metabolism has long been known, the role of nucleic acids has been appreciated only in recent times. Modern developments in the field of nucleic acid research, made possible by a variety of experimental techniques, have attracted the attention of scientists from many disparate disciplines.

The biological function of the nucleic acid may be tersely stated – they are responsible for the storage, in coded form, of the genetic information required for the synthesis of the enzymes and other proteins needed by the living cell, and for the direction of the process which translates the stored information into the amino acid sequence.

Nucleic acids are macromolecules, among them are the largest molecules known. Both forms of nucleic acids (DNA & RNA) are linear polymers of fundamental subunits called nucleotide. Each nucleotide contains ;

(1) A nitrogenous heterocyclic ring called the base. The

nucleotide bases are related either to the purine ring system or to the pyrimidine ring system.

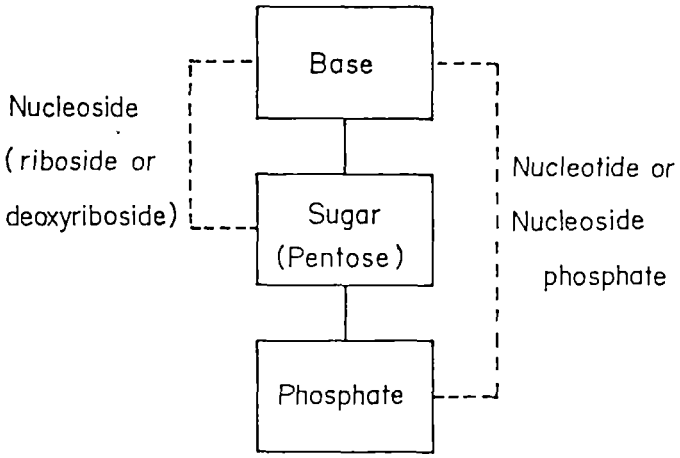


(a) (b)

(a) Purine, (b) pyrimidine

(2) A 5-Carbon sugar, which is D- ribose for RNA and 2-deoxy-D-ribose for DNA.

(3) A phosphate group , attached by an ester linkage to the sugar.



The bases of frequent occurence are five in number. The purine bases ; Adenine and Guanine, which consist of fused heterocyclic 5 and 6 membered rings, are common to both DNA and RNA. The pyrimidine bases Uracil and Cytosine which are single six membered rings are found in RNA, while Cytosine also occurs in DNA, Uracil is replaced by its 5-methyl

derivative Thymine

All the bases share definite aromatic characteristics, including a planar conformation. As a result of aromatic unsaturation the rings themselves and the bonds that lead to their substituent positions lie in a single plane. This gives rise to a plane of symmetry and as a result there is no sterioisomerism in the principle bases.

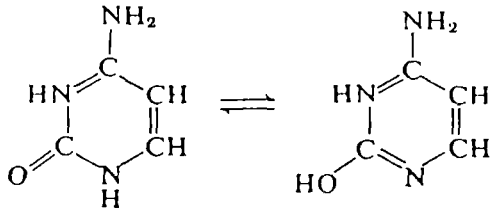
Most of the bases have ionisable groups e.g., NH2 & >C = O.

The - NH2 group can undergo ionisation [1] :

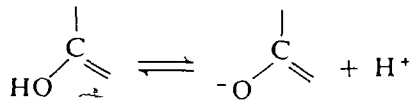
$$- NH2 + H^{+} \longrightarrow - NH3$$

with pK values much lower than for the corresponding ionisations in the amino acids. Because of these values there is no significant proportions of the bases in the charged form at pH 7.

The keto group can undergo rapid keto- enol tautomerism as ;



When they are in the enol form they can ionise ;



One of the most important aspects of the chemistry of nucleic

acid bases is their tautomerism. For instance, one of the most widely accepted theories of spontaneous mutation is based on the possible existence of bases in different tautomeric forms [2]. Every base which is a component of a nucleic acid can generally speaking exist in several tautomeric forms, the number of which depends on the number of exocyclic groups in the purine or pyrimidine ring. Heterocyclic hydroxy and amino compounds exists chiefly in keto and amino tautomeric forms [3,4]. Base tautomerism which does occur, is responsible for mutagenesis. Ionisation is a function of pH , in contrast to tautomerism, which is an innate property of neutral molecules [5].

The hydrophobic or apolar properties of the bases have a considerable role to play in the nucleic acid structure and function . The adhesion between the members of the stacked group is intensified by interchange between the electrons that circulate in the orbital above and below the planes of the ring. The functional specificity of nucleic acids and nucleotide co-enzyme is determined by the reactivity of the nucleoside components and in particular of the heterocyclic bases incorporated in them . The term reactivity must always be understood in its widest sense, to include not only interaction leading to the formation or rupture of covalent bonds, but also interactions of other type :

with neighbour bases in the same polynucleotide chain

or with the complimentary bases of another polynucleotide, proteins etc., [6].

The specific association of the purine and the pyrimidine bases in the nucleic acid is a property which provides for the basis for the storage , transmission and expression of the genetic information [7, 8] . Aromatic molecules can form dimer and polymer associates without any covalent bond . the stability of such associates results from van der Waals London and dispersion forces (dipole-dipole , dipole- induced dipole interactions etc.) and can involve charge transfer contribution according to the electron donor or electron acceptor character of the molecule [9]. The stability of the associates in solution is determined by two factors : interaction forces between bases themselves and by effects connected with interactions between the solvent and the free and associated bases [10] The interaction may be of two types ;

1. Interaction between the planes of rings of neighbouring bases in the same chain(interplaner or vertical and longitudinal interaction).

2. Interaction between the bases located in the same plane but in different chains (base pairing); this includes, in particular, hydrogen bonding.

1.2 SINGLE ELECTRON TRANSFER (SET) CHEMISTRY.

Fundamental Aspect.

Mechanisms of organic reactions are largely described as two-electron centered [11]. Electron movements are pictured as taking place two by two in the familiar curved arrow mechanism, and with rare exceptions[12], notions of one-electron organic chemistry did not enjoy much acceptance in the past. Although in inorganic chemistry " Single Electron Transfer" (SET) concept was well accepted, it was only in organic chemistry that some reluctance was there, may be due to lack of convincing evidences. Most classical Polar mechanisms have proved resistant toward re-evaluation in terms of electron transfer , but in the 1960's novel electron transfer processes made their way into the knowledge base of organic chemistry. In 1966 Kornblum [13] and Russell [14] independently provided details of the $S_{RN}1$ mechanistic pathway (" electron - initiated " radical chain mechanism of nucleophilic substitution) for the specific reactions that they were studying and in 1970 Bunnett [15a] discovered that such a pathway was also in effect in some cases of nucleophilic aromatic substitution (Scheme 1).

$S_{RN}1$ Mechanistic pathway

$$RX + Y^- \longrightarrow RX^{\bullet -} + Y^{\bullet} \quad (i)$$

$$RX^{\bullet -} \longrightarrow R^{\bullet} + X^- \quad (ii)$$

$$R^{\bullet} + Y^- \longrightarrow RY^{\bullet -} \quad (iii)$$

$$RY^{\bullet -} + RX \longrightarrow RY + RX^{\bullet -} \quad (iv)$$

Step (i) involves Single electron transfer from the nucleophile (Y^-) to the substrate RX. In step (ii) the radical anion ($RX^{\cdot -}$) dissociates rapidly to radical ($R^{\cdot}$), which then reacts with (Y^-) in step (iii) to form the product radical anion ($RY^{\cdot -}$) which in step (iv) serves as the one-electron donor in the radical chain process. Their preparative usefulness no doubt contributed strongly to the ready acceptance of the mechanism, which proved the way for related types of electron transfer catalyzed mechanism such as cycloadditions and oxidatively catalyzed aromatic substitution [15b].

What is Single Electron Transfer Phenomenon ?

Single Electron Transfer reaction is defined as one that is initiated by single-electron-transfer from the nucleophile to the substrate producing a radical intermediate. The fate of the resulting radical intermediate can then be involved in any number of events, one of which is described in the $S_{RN}1$ mechanistic pathway in scheme (1). The possible role of SET in organic reactions , as opposed to classical notion of electron-pair transfer , has attracted continuous and active attention in the past . An important step in this connection experimentally exemplifying such reaction pathways , has been the discovery of nucleophilic

substitution reactions proceeding via anion radical intermediates and taking place at benzylic carbon centers [13] or at aromatic carbon centers [15a]. On the other hand , the continuous development of organic electrochemistry , particularly in its mechanistic and kinetic aspects [16, 17] has been another source of interest and information for reactions triggered by SET. Since then many reactions between nucleophiles and electrophiles which were previously believed to follow polar mechanism, have now been recognised to proceed via initial 1e⁻ transfer and subsequent radicoloid steps.

Different Models for explaining SET.

Pross [15c] and Shaik [15d] have developed a configuration mixing model (CM) to compare these processes, [15e]. In the (D,A) nomenclature of the CM treatment picture (D = donor and A = acceptor), the ET step as coming about by the avoided crossing of the two crossing curves as shown in Fig.1

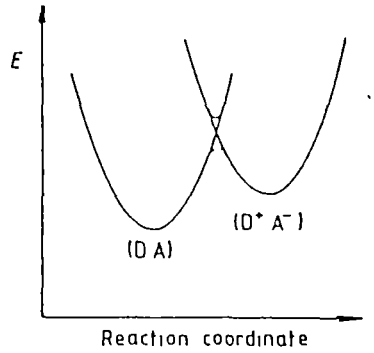


Fig-Potential Energy curves of (DA) and ($D^{\cdot -}A^{\cdot -}$) curves.

The curves are the plot of the potential energies of the

electron configuration for the precursor (DA) and the successor complex ($D^{\cdot+}A^{\cdot-}$). The movement along the reaction coordinate consist of solvation changes and geometric distortions(changes in bond lengths, bond angles etc.,) which increases the energy of (DA) until it has reached the same energy level as a similarly activated ($D^{\cdot+}A^{\cdot-}$) configuration. At this point ET transfer takes place. Pross has concluded that following factors should work in favour of ET processes : (i) Strong donor - acceptor pairing which will move the avoided crossing toward the initial state.

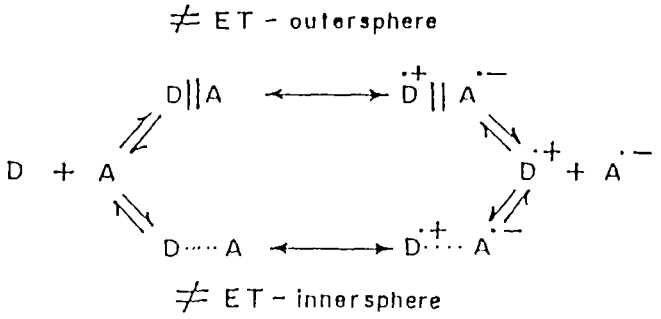
(ii) Steric interaction between D and A, which will decrease the probability of group coupling between $D^{\cdot+}$ and $A^{\cdot-}$.

(iii) Low $D^{\cdot+}$ - $A^{\cdot-}$, bond strength will decrease the likelihood of group coupling between $D^{\cdot+}$ and $A^{\cdot-}$ and

(iv) Strong delocalization of the radical centers of $D^{\cdot+}$ and $A^{\cdot-}$. In short , the CM treatment represents the most successful attempt so far to provide deeper insight into the difference between ET and polar processes in terms that are akin to the organic chemist's thinking. Recognition of SET pathway in organic reactions are growing enormously in many areas of chemistry , fundamental as well as applied ones [18, 19].

Marcus' Theory of SET.

Marcus [20] has done pioneering work on the theory of electron transfer mechanism and has proposed a very simple model in terms of outer-sphere and inner-sphere transfer mechanism. Scheme below summarises the ET mechanism for a donor D and an acceptor A.



The fundamental difference between the inner and outer sphere mechanism as far as transition state is concerned is that, in inner -sphere mechanism, donor and acceptor moieties maintain a substantial interaction in the transition state. The physical model underlying Marcus treatment of outer-sphere ET is indeed simple, in that donor (D) and acceptor (A) are approximated as two spheres of radii r_1 and r_2,

$$D + A \longrightarrow D^{\cdot +} + A^{\cdot -}$$

and charges z_1 and z_2, embedded in continuous medium of dielectric constant d. However, for organic molecules, the shapes of which are seldom spherical, ellipsoidal models have been used with some success [22]. The Marcus model is shown below :

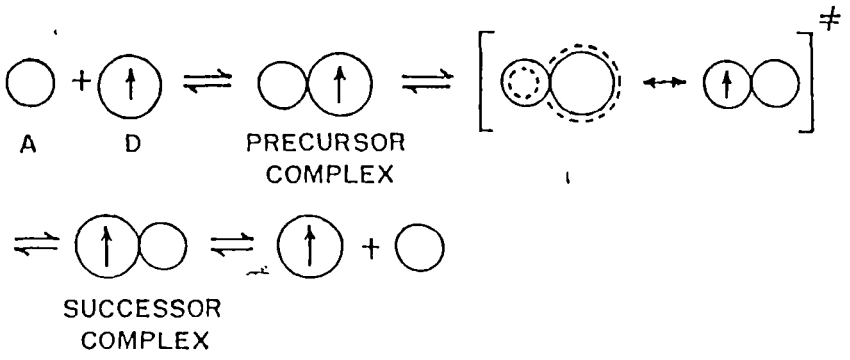


Fig - The Colliding Spheres model of the transition state of an outer sphere ET reaction.

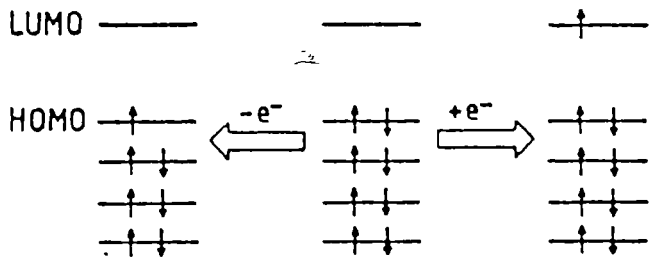
D, representing the large sphere with the arrow denoting the electron to be transferred. The two spheres diffuse together and form the precursor complex with the distance between the centers of the spheres usually taken to be $r_{12} = r_1 + r_2$. This complex is also denoted as collision or encounter complex. In order to reach the transition state . Franck-Condon principle requires, the energy levels between which the electron is to be transferred to be made equal to within $\pm$ RT, where R and T have their usual meanings.

This requirement is satisfied by increasing the energy of the system, until the energy levels match each other by bond and solvent reorganisation, associated with the bond (involves bond stretching and/or compression, angle deformation and torsional movements) and solvent (involves solvent induced changes in the electrostatic environment around the reactants) reorganisation energies. Bond reorganisation is symbolised by expansion of the smaller sphere and shrinking of the larger one in transition state, which is a resonance hybrid of the (DA) and $(D^{\cdot+}A^{\cdot-})$ forms.

After transfer of the electron, the transition state relaxes and transforms into the successor complex, which eventually dissociates into the two new species.

The Marcus-Hush theory of outer sphere electron transfer [23] is based on the Born-Oppenheimer approximation and thus relates the activation barriers to the nuclear reorganisation that accompanies electron transfer. Classification of organic electron transfer processes as well as qualitative and quantitative aspects of Marcus theory is very well described in details by Lennart Eberson [24a].

Molecular Orbital picture of electron transfer between two species with even number of electrons is as shown below :



Molecular Orbital representation of one electron oxidation/ reduction of closed shell molecule

The electron is transferred from the highest occupied molecular orbital (HOMO) of the reductant (R), to the lowest unoccupied molecular orbital (LUMO) of the oxidant ($R^{'}$). Solvent and bond reorganisation energies are symbolised by (S) and (b) respectively in order to match the energy of the two levels (as discussed earlier). In keeping with the

assumed very small bond distortion necessary for many organic molecules, solvent reorganisation (s) has been assumed to be more important for R.

In any of these kind of redox reactions, orbital symmetry plays a major role, as it must in all chemical reactions. The requirement for a net positive overlap between the electron donating molecular orbital and the electron accepting orbital, is a key part of the general theory of electron transfer. The ideas expressed by Pross and Shaik, are very important because they present an alternative to Marcus theory that is more comprehensible to organic chemists. Similar ideas were expressed by Kochi in Charge-transfer theory of electrophilic substitution and addition [24 b] reactions.

Taube's [25] definition actually covers most cases," The distinction is fundamentally between reactions in which electron transfer takes place from one primary bond system to another (Outer-sphere), and those in which electron transfer takes place within a single primary bond system (inner-sphere mechanism) ". Although the outer-sphere / inner- sphere terminology was coined originally for electron transfer reactions involving coordination complexes, it can be used profitably for organic processes after some extension of the definitions [21]. In outer sphere electron transfer, either no bond is cleaved or

formed within the time scale of the experiment, or in the opposite case, bond breaking and bond formation take place in separate steps, distinct from electron transfer step. Conversely, if all the three steps are concerted one will deal with an inner sphere electron transfer. An S_N^2 reaction may be considered as being formally equivalent to an inner-sphere electron transfer reaction, or even close to being truely equivalent in many instances.

The outer-sphere/inner-sphere terminology may also be used to characterise the way in which the reactants react, rather than to characterise the overall reaction. The Marcus theory is thus very well applicable to inorganic as well as organic systems and is a valuable tool in physical organic chemistry.

The methodology that are used to provide evidence for SET catalysed mechanism have been listed by Chanon and Tobe [26] These are :

(i) Detection of radicals by ESR Spectroscopy.

(ii) Stereochemistry

(iii) Formation of radical derived secondary products, including induced polymerisation of added monomers.

(iv) Kinetics including the use of "radical clocks"

(v) Isotope effects

(vi) Failure to confirm the a simple LFER (Linear free Energy relationship).

(vii) Comparison with compulsory electron transfer processes i.e., electrochemical processes that are as closely related as possible.

(viii) Photostimulation, particularly electron transfer catalysed reactions.

However some additional criteria are, appearance of charge transfer complexes which can be suspected to be precursors to fully charged separated species, the observation of chemiluminescence is an indication that odd-electron species are involved in a possible electron transfer mechanism. Medium effects are important for electron transfer reactions, as for other organic reactions, but are not very useful diagonistically, due to their unpredictable nature. A generally observed medium effects is that caused by extremes of acidity or basicity, in strongly acidic media electron transfer oxidation of organic molecules is favoured, both due to an increase in oxidation potential of the oxidant (caused by e.g., protonation of oxidising species or stripping of ligands from a metal complex) and kinetic and/or thermodynamic stabilisation of radical cations formed. The inverse situation seems to hold for electron transfer reduction in strongly basic media. Current experimental inquiries into solvent effects in electron transfer are, broadly ,of two types. The first involves measurements of time-dependent fluorescence stokes

shifts(TDFS) for chromophores forming suitable charge transfer excited states [27]. Such measurements probe the real time dynamics of polar solvent relaxation around a newly formed dipole. In-addition, measurements of ET rates , themselves either from photoexcited or ground states , can yield solvent dynamical information. The evidence for a SET pathway in the reaction of a nucleophile with a carbonyl compound involves the observation of a paramagnetic intermediate and kinetic data establishing that the rate constant for the disappearance of paramagnetic intermediate is within experimental error of the rate constant for the appearance of the product.

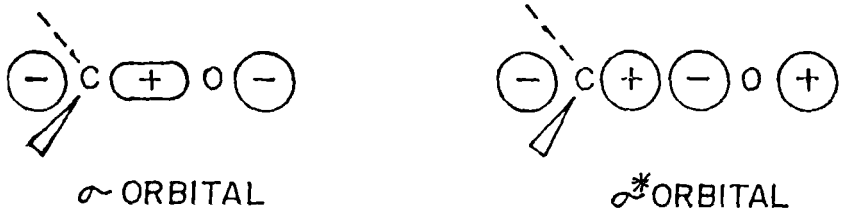
1.3 UV Spectroscopy

UV spectroscopy is the study of spectral transitions between the two quantized electronic states, described by molecular orbitals [28]. Five types of molecular orbitals are sigma, sigma*, pi, pi* and n non-bonding. Sigma orbitals are strongly bonding and essentially localised. Pi orbitals are more delocalised and consequently require less excitation energy than sigma electrons. Corresponding to each sigma and pi orbital there is a corresponding sigma* and pi* anti-bonding orbital. In molecules containing a heteroatom, the highest filled molecular orbital in the ground state is the n orbital. n orbitals are essentially localised on the

heteroatom and they have no antibonding counterpart. These orbitals are atomic in character. Some important transitions between these orbitals are ;

1. Sigma* <--- Sigma Excitations.

Sigma orbitals are of low energy with respect to n and pi orbitals and sigma orbitals are of high energy as compared to pi* orbitals. Consequently sigma* <--- sigma transitions corresponds to absorption at lower wavelengths of light. Both the sigma and sigma* orbitals are cylindrically symmetrical about the nuclear axis of the atoms forming the bond as shown below for carbonyl group ;

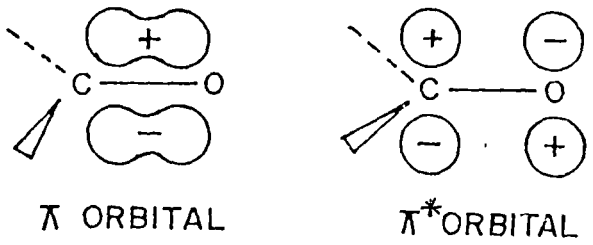


σ ORBITAL σ* ORBITAL

The sigma* orbital has a node between the atoms forming the sigma bond, which causes the bond to break when an electron is promoted to a sigma* orbital.

2. pi* <--- pi Excitation.

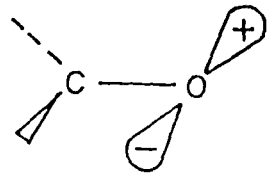
pi and pi* orbitals are usually linear combination of two p atomic orbitals and the bonding pi orbital is of lower energy than the anti bonding pi*. Both pi and pi* orbitals in the case of carbonyl group are shown below ;



π ORBITAL π* ORBITAL

In carbonyl group in the ground state, electronegativity of oxygen versus carbon, considerably displaces the pi cloud towards oxygen but in the pi* orbital the charge displacement occurs from oxygen to carbon. Both the pi and pi* orbitals possess a plane of anti symmetry, which coincides with the molecular plane.

3. pi* <--- n Excitations. For the carbonyl group, n electrons are considered in pure p atomic orbital localised on oxygen atom as shown below ;



P ORBITAL

The orbital is in the plane of the molecule and perpendicular to the pi orbital. In molecules containing oxygen, sulphur etc., the highest filled orbitals in the ground state are non-bonding and the lowest unfilled orbitals are pi*. It is thus expected that in such molecules the transitions , pi* <--- n will require the lowest energy and this transition would appear at longer wavelengths as compared to other transitions, e.g., pi* <--- pi. In the excited state the geometry is retained and the molecule continues to be essentially planer since two electrons remain in the bonding pi orbital.

Solvent effect:

The various properties associated with the solvents (e.g., dielectric, hydrogen bonding ability, etc.,) plays a very crucial role, in analysing the electronic spectra of the molecules. Some of the molecular properties which are influenced by the solvents and observed in the present investigation are described below ;

1. Assignment of electronic transition.

Assignment of electronic transition to a specific excitation is an important requirement for many type of studies. It is done by applying Kasha's rule . Spectra are recorded in solvents of varying polarities and polarity of the solvent affects mainly by two ways. Solute - solvent interaction can be either dipole-dipole or dipole induced - dipole interaction which in turn may relatively stabilise either ground or excited states. This will result in either decreasing or increasing the energy required for transition. The other way in which a solvent can influence the energy of the states is through its hydrogen bonding ability. Polar solvents may not form hydrogen bonds as readily with excited states as with ground states of polar molecules, and the energies of electronic transitions in these molecules will be increased by these polar solvents. On the other hand , in some cases the excited states may form stronger hydrogen bonds than the corresponding ground states. In such cases , a polar solvents would shift an absorption to longer

wavelength, since the energy of the electronic transition would be decreased. transitions of the pi* -- pi type are shifted to longer wavelengths by polar solvents.

(2) A non polar solvent does not form hydrogen bond with the solute, and the spectrum of the solute closely approximates what it would be in a gaseous state. In polar solvent, the hydrogen bonding forms a solute solvent complex, and the fine structure may disappear.

Charge transfer complexation.

This is the phenomenon observed when a molecule whose ionisation potential is low comes in contact with a molecule whose electron affinity is quite high and as a result of this interaction the molecular energy levels of both are perturbed. The magnitude of this interaction may be high or low depending upon the ionisation potential and electron affinity of the donor and acceptor respectively . A very low ionisation potential of the donor and a very high electron affinity of the acceptor will result in the complete transfer of an electron from the HOMO of donor to the LUMO of the acceptor giving rise to new band in the electronic spectra. Such a band is called Charge Transfer (CT) band [29 30] , which is the property of the new specie formed. In cases where the ionisation potential of the donor and the electron affinity of the acceptor are not favourable

for the complete transfer of electron only a perturbation of the energy levels of both donor and acceptor will occur. In charge transfer complexes the charge distribution is considerably different in the ground and the excited state. According to Mulliken, the wave function of the ground state of the molecular complex DA can, to a first approximation, be written as sum of the two terms :

$$\psi_N = a\psi_0(DA) + b\psi_1(D^+A^-)$$

Wave function relates to the hypothetical " No Bond " state of the system and the function relates to the state in which the electron is transferred from the donor to the acceptor (D A) a and b characterise the fraction of " no bond " structures and the structures with charge transfer in the ground state. The wave function of the complex in the excited state has the form

$$\psi_E = a^* \psi_1 (D^+A^-) - b^*\psi_0(DA)$$

Coefficients a, b, a*, b* are related to the orthogonality and normalising condition of the wave functions.

$$\int \psi_E \psi_N d\tau = 0$$

$$\int \psi_N^2 d\tau = \int \psi_E^2 d\tau = 1$$

Since charge transfer complexes are stabilised by electrostatic forces , they are bound to be affected by the polarity of the solvents. It may solvate the initial molecules and the intermediate complexes non specifically or it can form specific chemical bonds (Eg e.g. hydrogen bond,)

with the initial molecules and the intermediate complexes. If solvation or the ability to form these bonds is stronger in the intermediate complex than in the initial molecules, that particular solvent will accelerate the reaction : if on the other hand, the formation of these bonds is energetically more advantageous in the initial molecules than in the reaction complex, the solvent will delay the reaction. These effects may be so powerful that the direction of the reaction may also be changed ; for this reason, results obtained in one solvent must not be directly transferred to other solvent.

Isosbestic Points

It has been found that in the electronic spectra of multicomponent systems there are such wavelengths where the absorbance remains constant for all compositions of the system provided the overall concentration is fixed. Such points are called Isosbestic points or the isoabsorptive points [31]. The appearence of an isosbestic points are indicative of the presence of a charge transfer interaction between the donor and the acceptor present in the system. Observance of no charge transfer bands and only isosbestic points indicates only weak CT interaction. The shift in the isosbestic point with the relative change of concentration can be related to the different stoichiometry of the complex formed.

Charge transfer complexes play a very important role in single electron transfer reactions as these are precursors to electron transfer processes.

Tautomerism.

Nucleic acid bases can exist in several tautomeric forms, the number of which depends on the number of exocyclic groups in the purine or pyrimidine ring - heterocyclic hydroxy and amino forms and their corresponding tautomers . The phenomenon of tautomerism is an interplay of three factors. First during a change in tautomeric form the system of bonds of the molecule is reorganised. Second, the structure of the electron system in conjugated molecules is modified and, consequently, so also is their resonance energy. Third, the solubilising power of the molecule is altered, if the tautomeric equilibrium is examined in solution, where a shift may occur, the most interesting situation for the investigation of chemical and biochemical problems. The combined action of these factors determines the relative stability of the different tautomeric forms under the particular conditions concerned [32].

1.4 ELECTRON SPIN RESONANCE SPECTROSCOPY

This work presents the SET reaction studies of Nucleic

Acid bases by ESR spectroscopy employing the method of spin trapping. ESR spectroscopy is a technique for the study of species containing one or more unpaired electrons. The scope of the method includes the detection and characterisation of some transition-metal ions, cations, anions, organic free radicals, including biradicals and triplet states. Since this technique is now extensively used and well known the basic principle and analysis of ESR spectra are described; ESR is a magnetic resonance technique which achieves a response only from molecules with at least one unpaired electron. The signal obtained from the unpaired electron is a single line which reflects the net absorption of energy by the electron when the following "resonance" condition is met.

$$h\nu = g.\beta.H$$

Where $h\nu$ is the energy of absorbed photon, β is a constant for the electron , the Bohr magneton, H is the external field applied and g is the constant characteristic of the spin system (approx. 2.0 for organic free radicals). The absorption of energy by the electron corresponds to a change in sign of the electron spin or change in direction of the electron magnetic moment vector. If the unpaired electron experiences the field of another spin system, say a nucleus with a spin (I = 0), the magnetic field felt by the unpaired electron is slightly greater than , or smaller

than, the field experienced in the absence of nuclear spin system , depending upon the direction of perturbing additional field.

Thus for a spin (I) of $\pm$ 1/2 resonance now occurs at $H_1 = H - \delta H_1$ and $H_2 = H + \delta H_1$, where H is the perturbing field. Two signals or lines are observed. The coupling constant (a or A) is defined as the spacing between the observed lines, usually in gauss or militesla (mT). Since the original line due to unpaired electron is "split" into two lines , the spacing is also called a splitting constant or hyperfine splitting. If the unpaired electron experiences the field of more than one nuclear spin two possibilities arise :

(i) Either the interaction between each nuclear spin and unpaired electron is equal for all nuclei(equivalent),

(ii) or not equal for all nuclei(non equivalent)

For equivalent nuclear spins the system is considered to have a total spin of nI, where the number of lines are predicted by the expression (2nI + 1).

For non-equivalent nuclei the number of lines is predicted by the product of individual sets of equivalent nuclei : $(2n_1I_1 + 1).(2n_2I_2).....$, where n_1 is the number of equivalent nuclei with spin I_1, n_2 is the number of equivalent nuclei with spin I_2 etc. The intensity of lines from interaction with n equivalent nuclei are best obtained

from Pascal's triangle i.e the coefficients in the expansion of $(1 + x)^n$. The spacing between the lines are always symmetrically disposed about the centre of the spectrum (to first order approximation).

An ESR spectra is characterised by three parameters : the hyperfine splitting, the g factor and the line width. A fourth parameter, the electronic splitting, applies to triplets (species with two unpaired electrons which interact with each other), and will not be discussed here. The three important factors which determine the magnitude of interaction of the unpaired electron with the nuclear spin are :

(a) The magnitude of the magnetic moment and spin of the nucleus.

(b)(i) The S character of the orbital containing the unpaired electron (for orbitals with S character), and

(ii) The extent of "spin- polarisation" of the inner shell electrons (for essentially pure p or d orbitals).

(c) The spin density of the nucleus in question.

If all other factors remains constant , the magnitude of the splitting constant is directly related to the nuclear magnetic moment and inversely related to the spin, for e.g., for hydrogen atom, I = 1/2, A = 508 G [33], and μ = 2.793, and for deuterium atom I = 1, A = 78 G , and μ = 0.857. Thus 508/78 = (2.793/0.857)* [1/(1/2)] = 6.51.

The splitting constant is also related to spin density on the nucleus in question, since in delocalised systems the interaction between the electron and the nucleus must necessarily reflect the "time" spent in the vicinity of the nucleus. Thus the spin density on each carbon atom in cyclopropenyl radical must be one-third of unity.

Hyperfine splitting is also observed from nuclei which are bonded through one or more bonds to atoms bearing the unpaired electron e.g., , the hydrogen hyperfine coupling is 23 G for the methyl radical, 25 G for the ammonium radical and 26 G for the methyl group in the ethyl radical. A relationship between the hydrogen hyperfine splitting and spin density on a sp^2- hybridised carbon atom was first obtained from the ESR spectra of aromatic ions of known structure [34].

$$A_C^H = Q_C^H \rho_C$$

where A_C^H is the coupling constant of hydrogen attached to carbon, ρ_C is the spin density on carbon and Q_C^H is the proportionality constant relating the magnitude of hydrogen coupling constant to spin density on carbon. In organic radicals in solution , the orbital angular momentum of the electron is almost completely quenched, so that g factors are close to the value for the free electron (2.0023). However, differences from this value are observed , particularly when the unpaired electron is associated with

atoms which have unshared pairs of electrons e.g., g factor for $\dot{C}H_3$, $\dot{C}H_2OH$ and $\dot{C}H_2CHO$ are, respectively 2.0025 , 2.0031, and 2.0045 and, in general , hydroxyl- and carbonyl-substituted radicals have g- factors about 0.001 and 0.002, respectively, greater than the free spin value [35]. These differences , though numerically small; provides valuable information about the structure of a radical [36]. In short, the information obtainable from ESR spectra of free radicals is contained in ;

(a) the number and position of the spectral lines,

(b) the line width and

(c) the total absorption intensity.

Analysis of the number and positions of lines leads to the determination of the chemical and steric structure of the radicals and provides insight into the odd electron distribution. Line width may offer additional information on structure as well as on kinetics of reversible radical reactions. The concentration of the radicals is determined from the absorption intensity [36]. The direct detection and identification of short-lived free radicals by ESR is possible only if the radicals are produced in relatively high concentration in the ESR cavity by intense in situ irradiation or by rapid-mixing flow system. One of the indirect technique which is often used for the detection and identification of low concentrations of free radicals or

very reactive species in reacting system is " Spin - Trapping ". It was reported that nitroso compounds add organic free radicals to form persistent aminoxyls which are readily detectable by ESR spectroscopy [37, 38]. This work was extended by showing that aminoxyls are also formed by addition of short lived free radicals to nitrones [39, 40]. Shortly after this, the importance of this reaction as an analytical tool for detection and identification of short lived radicals was recognised[41-44] Later, Janzen and Blackburn [45] coined the expression "Spin Trapping"(ST) for a reaction where a short lived radical R· is scavenged by a diamagnetic compound, to form persistent radical adduct ST-R·, detectable by ESR.

R. + S.T --------> (ST-R.)

The diamagnetic scavenger is called "spin trap" (S.T) and the resulting persistent radical ST-R. is named as "Spin-adduct". The detection and characterisation of transient radical intermediates produced in reactions which are initiated by ET processes are essential to the mechanistic definition of those processes.

The necessity of such mechanistic understanding is becoming increasingly important in areas of science. Spin trapping with various nitroso and nitrone derived moieties has proven to be fruitful approach to the detection

and characterisation of transient radical intermediates ensuing from various substrates in solution , gas phase reactions [46] and in many other areas. An extensive compilation of spin adduct ESR data have been published by Buettner [47].

An immediate requirement of the technique is that the spin trap and spin adducts should be soluble in the medium of interest and that free diffusion of the spin trap to the location of free radical event is allowed.. Also the environment should permit high mobility of the spin adduct so that ESR spectra consists of pattern of sharp lines. The bulk and group electronegativity of the radical trapped determines the magnitude of the nitrogen and α -hydrogen coupling constants. Solvents effects change the nitrogen coupling constant (increases to larger values in protic solvents) and in turn the α - hydrogen coupling, it is advantageous to have spectra of nitroxides of " Known " structures available for comparison in the same solvent. In the spectra of nitroxides, splitting by hydrogen is often clearly resolved. When the hydrogen is attached directly to nitrogen, or is in the α-position, aH is frequently comparable in magnitude with aN. Since α- hydrogen splitting arise predominantly by a hyperconjugative mechanism, there is a pronounce angular dependence. Large values of aH are found with N- methyl nitroxides, and specially in certain

cyclic nitroxides where the C -H bond is correctly aligned with the p orbital on nitrogen. In general a relationship of the form

$$a_H = \text{constant} \times \cos 2\theta \text{ is obeyed}$$

The angle θ is the dihedral angle between C - H and the nitrogen P orbital, the constants depends on the solvent. Spin trapping is a kinetic method i.e the success of the spin trapping experimentaly depends critically on the rate conditions which exist in the system. For a favourable rate of spin adduct formation the rate of spin trapping must be much faster than the rates of other reactions of the radical. Moreover, the ideal spin trap would give spin-adducts which are perfectly stable and unreactive to other reagents in the environment, even free radicals. More than hundred different compounds have proved to be suitable spin-traps, however nitrones and α-nitroso compounds are preferred . An important pathway for the formation of spin adduct has been recently revealed by Lennart Eberson [48, 49] involving redox processes and is termed as " Inverted Spin Trapping " , because of the inverted electron configuration of the reagent pair, $Nu^-/ST^{\cdot+}$ Vs $Nu^{\cdot}/ST$ (eq. 1 and 2)

Proper Spin Trapping :

$$Nu^- \xrightarrow{-e^-} Nu^{\bullet} \xrightarrow{ST} Nu-ST^{\bullet}$$

Inverted Spin trapping as proposed by Eberson :

$$ST \xrightarrow{-e^-} ST^{\bullet +} \xrightarrow{NU^-} ST - NU^{\bullet}$$

PBN : - phenyl-N-tert.butyl nitrone,

MNP : 2-methyl-2-nitrosopropane.

correct citations ?

REFERENCES

(1) Yudkin, M.; Offord.R.; "Comprehensible Biochemistry" (Longman), (1973), 139.

(2) "Organic Chemistry Of Nucleic Acids", Part A, N.K.Kochetkov and E.I.Budovskii, Eds- Lord Todd and D.M.Brown, (Plenum press), London and New York (1991), p.134.

(3) Farguhar, E.L.; Downing, M.; Gill, S.J.; Biochemistry. (&): 1224 (1968).

(4) Gellart, M.; Lipsett, M.N.; Davies, D.K.; Proc. Nat. Acad.Sci. (USA), 48 : 2013 (1962).

(5) "Molecular Biology of Mutagens and Carcinogens", B.Singer, D.Grunberger, Plenum Press (1983), Plenum pub.Co., New York, 1983, p 27.

(6) "Organic Chemistry Of Nucleic acids," Part A, N.K.Kochetkov and E.I.Budovskii, eds. Lord Todd and D.M.Brown (Plenum), 1971, p. 121

(7) Hoogsteen, K.; (1959), Acta Cryst. 12,822.

(8) Hoogsteen, K.; (1963), Acta Cryst, 16, 907.

(9) Hirshfelder, J.O.; Curtiss, C.f.; Bird, R.B.; (1964) " Molecular Theory Of gases and Liquids " Wiley, New York.

(10) Organic Chemistry Of Nucleic Acids, Part A, N.K.Kochetkov and E.I. Budovskii, Eds Lord Todd and D.M.Brown (Plenum), 1971, p. 210.

(11) Lowry, T.H.; Richardson,K.S.; "Mech. and Theory in Organic Chemistry" , New York, Harper,(1981).

(12) Dewar, M. J. S.; Dougherty, R. C.; " The PMO theory of organic chemistry", New York,Plenum Press,(1975)

(13) Kornblum, N.; Michel, R. E.; Kerber,R.C., J. Am.Chem. Soc. 88, 5660, (1966)

(14) Russell,G.A.; Danen,W.C.; J.Am.Chem.Soc.88,5663,(1966).

(15) a. Kim,J.K.; Bunnett,J.F.; J.Am.Chem.Soc.,92,7463,(1970)

b. Chanon,M.; Bull.Soc.Chim.Fr.,197,(1982) , Julliard,M.; Chanon,M.; Chem.Rev.,83,425,(1983) Eberson,L.; J.Mol. Catal.,20,27,(1983).

c. Pross,A.;" Adv.Phys.Org.Chem.",21,99,(1985)

d. Shaik,S.S.;" Prog.Phys.Org.Chem.",15,197,(1985)

e. Pross,A.; Acc.Chem. Res.,18,212,(1985).

(16) Baizer,M.M.; Lund,H." Organic Electrochemistry",Dekker, New York,(1983).

(17) a.Saveant,J.M.; "Advances in Electrochemistry" Chapter.IV, 289. The Robert A.Welsh Foundation, Housten.

b.Saveant, J. M.; Bull. Soc. Chim. Fr., vol ? 225, (1988)

(18) Bilevich, K. A.; Okhlobystin, O. Y.; Russ.Chem.Rev.37, 1,(1968)

(19) Eberson,L.;" Advances in Physical Organic Chemistry", 18, 79,(1982).

(20) Marcus, R. A.; Ann. Rev. Phys. Chem.,15,155,(1964).

(21) Lexa, D.; Saveant, J. M.; Su, K. B.; Wang, D. L.; J. Am. Chem. Soc. 110, 7617, (1988).

(22) Kharkats, Yu. I.; Sov. Electrochem. (Engl.Trans.) 10, 588, (1974); 12, 1176, (1976).

(23) a. Hush, N. S.; J. Chem. Phys., 28, 962, (1958).

b. Hush, N. S.; Trans. Faraday Soc., 57, 557, (1961).

c. Marcus, R. A.; J. Chem. Phys. 24, 4966, (1956).

d. Marcus, R. A.; Ann. Rev. Phys. Chem. 15, 155, (1964).

e. Marcus, R. A.; Faraday. Discuss. Chem., 74, 7, (1982).

f. Marcus, R. A.; Sutin, N.; Biophys. Biochim. Acta, 265, 811, (1985)

(24) a. Eberson, L.;" Adv. Physical Organic Chemistry", 18, 79, (1982)

b. Fukuzumi, S.; Kochi, J. K.; J. Am. Chem. Soc., 103, 7240, (1981); 104, 7599, (1982).

(25) Taube, H.; " Electron Transfer Reactions of Complex Ions in solution", New York and London, Academic Press, (1970)

(26) Chanon, M.; Tobe, M. L.; Angew. Chem. Int. (Ed. Engl) 21, 1, (1982).

(27) For recent reviews see ;

a. Barbara, P.F.; Jarzeba, W.; Acc. Chem. Res. 21, 195, (1988)

b. Barbara, P.F.; Jarzeba, W.; Adv. Photochem. 1, 15, (1990)

c. Also see related discussions in , Bagchi, B.; Flemming, G. R.; J. Phys. Chem. 94, 9, (1990).

(28)Jaffe, H.H.; and Orchin, M.; Theory And Applications of Ultraviolet Spectroscopy, Wiley and Sons, Newyork (1962)

(29)Mulliken, R.S.; J.Am.Chem.Soc., 72, 600 (1960).

(30)Mulliken,R.S.; Chem.Phys, 19, 514 (1951).

(31)Cohen,M.D.; and Fischer,E.; J.chem.Soc. (1962) 3044.

(32)Organic Chemistry of Nucleic Acids, Part A, N.K.Kochetkov And E.I.Budovskii, Eds Lord Todd and D.M.Brown, (1971), p.136.

(33)Atkins,P.W.;Symons,M.C.R.;"The structure of Inorganic Radicals",Elsevier Publishing Co.,New York,N.Y.(1967)

(34)McConnell,H.M.; J.Chem.Phys.24,764,(1956) , Bersohn,R., ibid,24,1066,(1956), Weissman,S.I;ibid,25,890,(1956).

(35)Normann,R.O.C.;Pritchett,R.J.; Chem.and Ind.(London),2040, (1965).

(36)Symons,M.C.R.; "Adv.Phys.Org.Chem."1,283,(1963)

a. Bersohn, M.; Baird, J. C.; "An introduction to ESR", Benjamin, New York,(1966).

b. Carrington, A.; Mclachlan, A. D.; " Introduction to Magnetic resonance with application to Chemistry and Chemical physics", Harper International, New York,(1967)

c. Alger, R. S.; " Electron Paramagnetic Resonance Techniques and Application",Interscience,New York,(1968)

d. Scheffler, K.; Stagmann, H.B.; Elektronen spin resonanz, Springer, Berlin (1970).

e. Bielski,B.H.J.;Gebicki,J.M.; " Atlas of Electron spin

Spin Resonsnce Spectra", Academic Press,New York,(1967).

f. Wertj, J.E.; Bolton,J.R.; " Electron Spin Resonsnce, Elementary Theory and Practical Applications ", McGraw- Hill, Inc. (1972).

(37)Hoffman, A. K.; Henderson, A. T.; J. Am. Chem. Soc., 86,4671,(1961)

(38)Tudos, F.; Kende, I.; Berezsnich, T., Szolodovnikov, S.; Vojevodskii, V.; Magy.Kem.Foly.;69,371,(1963), Tudos,F.;Kende,I.;Berezhnykh,T.;Solodov,S.P.;Voevodskii, V.V.;Kenet.Katal.;6,203, (1965).

(39)Mackor,A.; etal, Tetrahedron Lett.,2115,(1966), Tetrahedron 23,4021,(1967)

(40)Iwamura,M.;Inamoto,N.; Bull.Chem.Soc.Japan.40,702,(1967)

(41)Janzen,E.G.;Blackburn, B.J.;J.Am.Chem.Soc.90,5909,(1968)

(42)Lagercrantz, C.; J. Phys. Chem.,75, 3466, (1971).

(43)Perkins, M. J.;" Adv. Phys, Org. Chem. ",17, 1, (1980)

(44)De Boer,Th.J.; Can.J.Chem.,60,1602,(1982) and references cited therein.

(45)Janzen,E.G.;Blackburn,B.J.;J.Am.Chem.Soc.,91,4481,(1969) For reviews see, Janzen,E.G.;Acc.Chem.Res.4,31,(1971), Janzen, E. G.;" Free Radical Biol.",4,115,(1980), Janzen,E.G.; Davis,E.R.;in " Free Radicals and Cancer " (R .A Floyd, ed.), Dekker, New York, 397, (1982).

(46)Janzen,E.J.; Creat.Detect.Excited State,4,83,(1976)

(47)Buettner,G.R; Free Radical Biol.Med.,3,259,(1987)

(48) Eberson, L.; J.Chem.Soc.Perkin Trans 2, 1807, (1992)

(49) a. Eberson, L.; J.Chem.Soc.Perkin.Trans 2, 171, (1994)

b. Eberson, L.; ibid, 1181, (1994).

CHAPTER - 2

REVIEW

REVIEW OF THE RADICAL INDUCED DAMAGE TO THE CONSTITUENTS OF NUCLEIC ACIDS.

It is widely believed that oxidative damage to DNA plays a crucial role in carcinogenesis. damage to DNA in cells has been found to be the major cause for mutation, cancer and cell deactivation under the influence of chemicals, uv light and high energy irradiation. It is known that ionising radiation [1,2] causes a number of deleterious effects ranging from reproductive cell death to mutagenesis and transformation. These effects are mainly due to lesions induced in cellular DNA, which is believed to be the prime targets for the action of ionising radiation, both via direct ionisation of the nucleotide bases [3, 4] and indirectly from secondary damage via reaction of $^{\bullet}OH$ (from water, 5-6). Over the period, much of the progress in this field has developed in parallel in two distinct areas that have remained essentially independent of each other. One is the realm of (aqueous) solution chemistry where product analysis and pulse radiolysis [7 8] have predominantly been applied and the other field is that of solid state (including frozen solutions) ESR [9-10]. It

is well understood that it is the radical intermediates which play a key role in the mechanism of the induced damage either chemically or through ionising radiation. Ever since the early days of radiation chemistry and biology it has been realised that the purine and pyrimidine bases are the most sensitive to radiation induced modification or destruction of the components of DNA, itself the most critical of the cellular targets [11-13]. Attempts to understand the radical chemistry leading from the primary ionisation event to the final non-radical products have involved the use of three general " tools " :

a. Product analysis studies on , mainly, the building blocks of DNA and model compounds for them [14-17].

b. Electron spin resonance in matrices, single crystals and liquid solutions (spin trapping and flow techniques) [18,21]. c. Time resolved methods (pulse radiolysis) with predominantly optical and conductance detection [22-25] again mainly on the constituents of DNA and their model compounds. The study related to Radiation induced damage to DNA by using various "tools" can be broadly divided into two parts. One comprising of the solid state and the other of the solution state.

2.1 SOLID STATE STUDIES :

Most solid state studies have centered upon Electron Spin

Resonance (ESR) and Electron Nuclear Double resonance (ENDOR) techniques. The applicability of information derived from the solid state studies to the in-vivo system is often questioned. But , first the state of DNA in vivo lies, somewhere between fluid and solid. To understand radiation effects in vivo, it is necessary to interpolate between the results from both liquid and solid samples. Second, solid state studies are informative about processes that would occur very rapidly in liquids, processes involving ionic intermediates, unimolecular events, and small highly reactive fragments.

PYRIMIDINE BASES

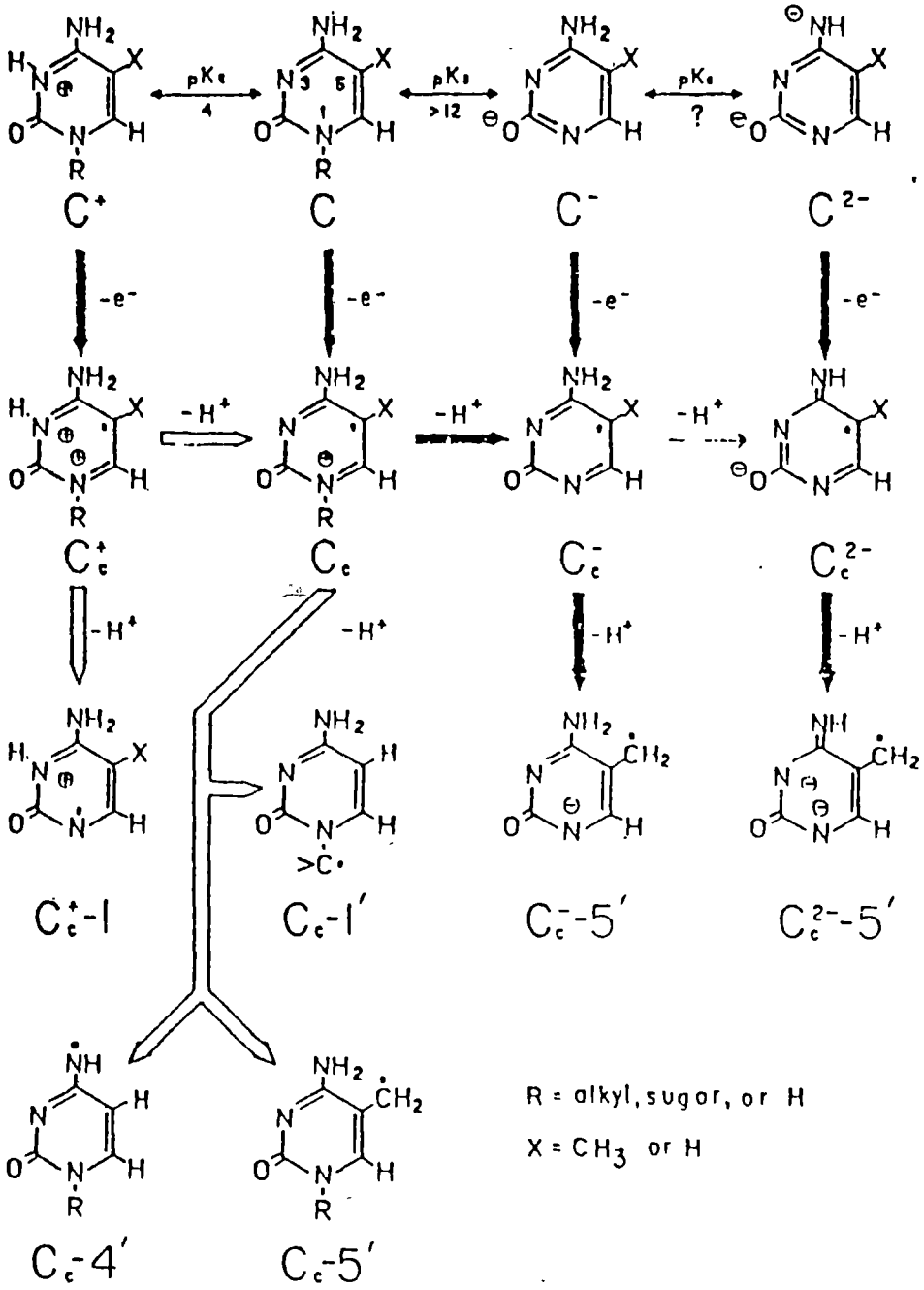
A. Electron abstraction : The loss of an electron by a pyrimidine base leaves a vacancy in the HOMO of the system. The resulting radical has been described most accurately as a π cation by Sevilla and co-workers [26] because that description leaves undesignated the net charge of the radical. The unpaired electron remaining in the HOMO has its highest density at C5. Marking off alternate atoms from C5, other sites of unpaired electron population can be anticipated : N1, N3 and the exocyclic groups at C2 and C4. Deprotonation is possible at position α or β to the sites of highest unpaired electron population in pyrimidine cations, C5 is largest and N1 is next largest [27]. The

most probable routes to deprotonation are shown in the following scheme,1;

It has been pointed out that the unsubstituted Uracil or Cytosine will undergo deprotonation at N1, and if there is a substituent at N1 deprotonation will occur from the substituent. In crystalline Thymine matrices , the cation deprotonates at N1 or the methyl group.Sevilla etal studied N1 substituted Thymine cation radicals in glasses, they obtained convincing evidence that the N1 and not N3 is the major source of nitrogen hfs. Although for all the pyrimidines studied the site of deprotonation is the N1,When there is a substitution at N1, the site of deprotonation is still keyed to N1. Deprotonation is, however, β to N1 and not α.

Anion addition : Anion addition is an alternative mechanism by which a pristine cation can return tc the parent charge state. Anion addition replaces the lost electron with two electrons, resulting in structures analogous to electron addition radicals. For example, OH^- addition to C6 of the pyrimidine cation gives a radical analogous to radicals formed by protonation of C6 of anion.

The addition of OH^- to Thymine cations has been demonstrated by Sevilla and Engelhardt.They suggest that the change in the H6 hfs, a_β hfs, comes from a change in the tortional angle. Their idea is that, at increased

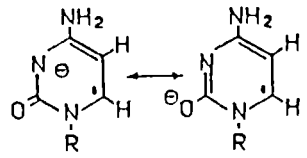

pKa 4
pKa >12
pKa ?
C+
C
C-
C2-
-e-
-H+
C+c
Cc
C-c
C2-c
-H+
C+c-1
Cc-1'
C-c-5'
C2-c-5'
Cc-4'
Cc-5'
R = alkyl, sugar, or H
X = CH3 or H

SCHEME - 1

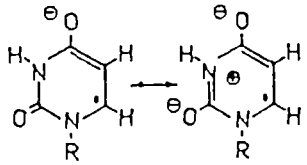
temperature, the OH group moves from a nearly axial to a more equatorial position. H6 must then become more axial and θ smaller.

B. Electron Addition :

Since the excess electron is in a π orbital, these ions are called π anions, regardless of the protonation state. The unpaired electron resides primarily at C6, C4 and C2. The position of highest spin density C6 , is emphasised in drawing the valence bond structures A and B. Excess negative charge is expected at N3 and O2 for Cytosine and O4 and O2 for uracil. In both Cytosine and Uracil radicals the other mesomeric structures carry the formal negative charge to C5.



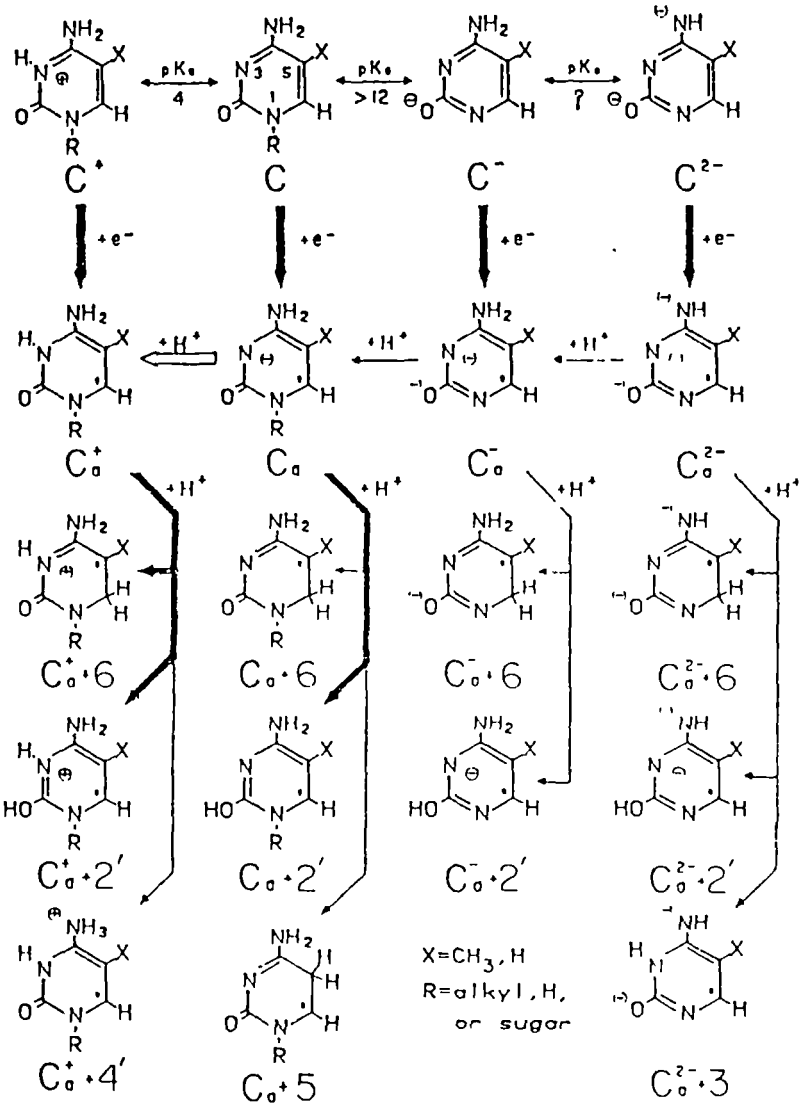
(I)



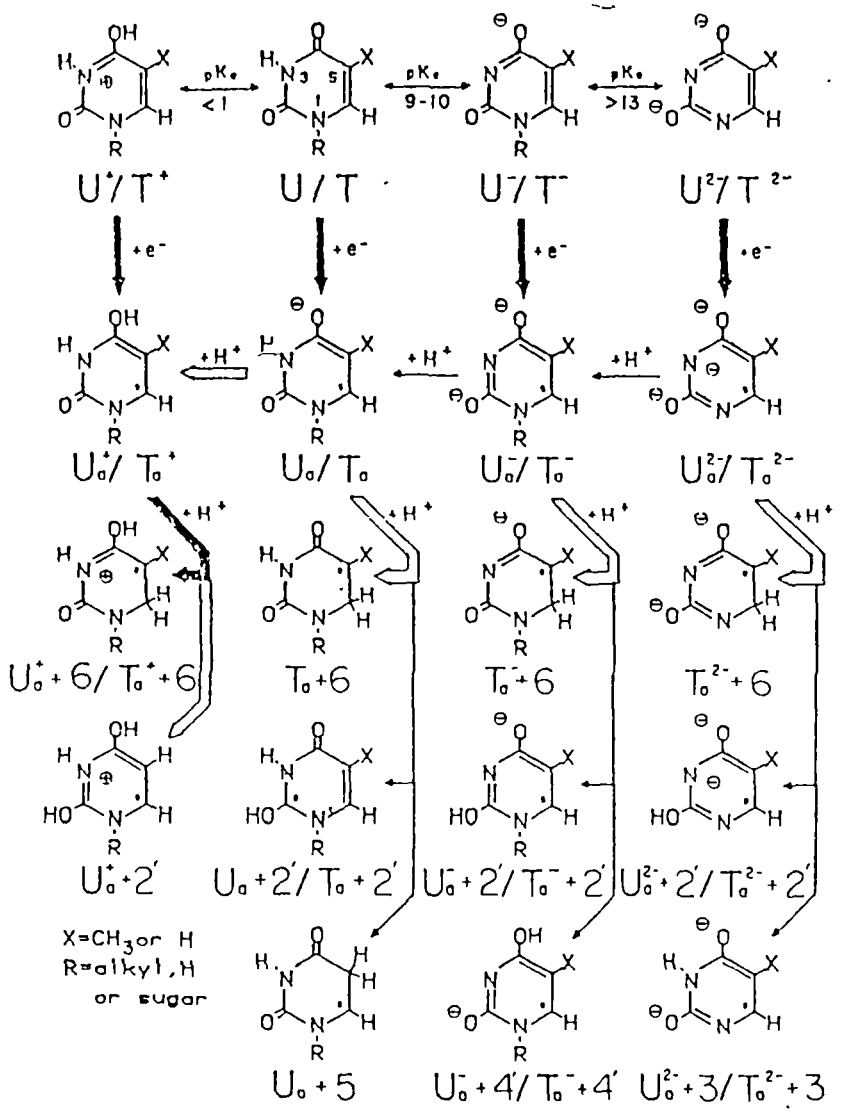
(II)

Protonation pathways are shown in the Schemes 2,3 . It has been demonstrated that protons are attracted to positions that have undergone the greatest increase in negative charge. Predicting the position requires the consideration of the source of proton. The proton could be donated by a hydrogen bond donor group , or it could be a "free" proton generated by decomposition of an electron loss centre.

Bernhard points that hydrogen bonded protons are most likely



SCHEME - 2



SCHEME - 3

to transfer to N3 of Cytosine anions and O4 of Uracil anions . In both cases, it is also possible to transfer a proton to O2; but O2 in crystals at least, does not usually participate in strong hydrogen bonds, thereby reducing the opportunity for this mechanism to operate. For the Thymine anion, it is unclear whether or not a proton transfers across an H bond to add at O4 or O2. It is known that the radical can acquire a matrix position at C6. This can definitely occur after protonation at O4 and probably also before protonation at O4.

He further points that "Free" protons will add either to O2 or to the C6 = C5 aromatic bond of the pristine anion. A probable site of addition is O2 if its lone pair orbital is not occupied in a strong H bond. For the Thymine it is the C6. And for Uracil and Cytosine, this position is unknown but it could be C5. If the pristine anion is already protonated at N3 in Cytosine, or at O4 in either Uracil or Thymine, addition of the free proton to the C6 = C5 bond will result in a net gain of H at C6. The resulting radical will have a positive charge.

Currently it is not clear whether any of the reported anions are in fact pristine anions. In every case protonation at the heteroatom allows alternatives. There is however, good indirect evidence which indicates that heteroatoms are readily protonated in the temperature region

77-300 K. Sevilla etal has provided ununivocal evidence for C6 as the main site of unpaired electron density.

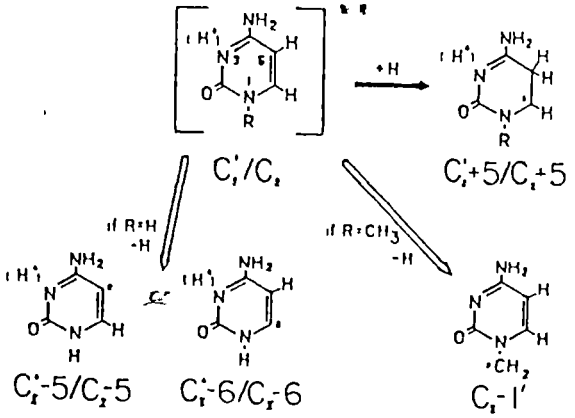
C. Excitation and Hydrogen Atom Reactions:

Superexcitation in unsubstituted Uracil or Cytosine appears to lead to dissociation of either the C6-H6 or the C5-H5 bond. However, if a methyl group is attached to N1, an H atom will be lost from the methyl group. The H atom thus produced adds preferentially (perhaps exclusively) to C5.Apparently, addition at C5 occurs whether or not a proton is at N3 in Cytosine and O4 in Uracil. The superexcited state of Thymine, substituted at N1 or not , will lose an H atom from the C5 methyl group. The resulting H atom will add to C6, specifically, whether O4 is protonated or not. The following Schemes 4,5 show these pathways for Cytosine and Thymine respectively.

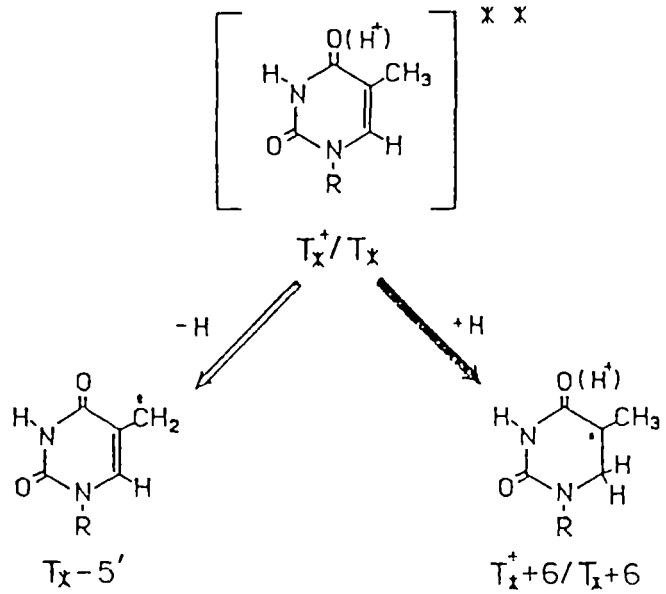
PURINES

A. Electron Abstraction:

Electron loss from Guanine or Adenine should lead to deprotonation or anion addition reactions: however, predicting the sites of deprotonation is difficult because the unpaired electron distribution is diffuse. Examination of the resonance possibilities for each of these forms shows that the unpaired electron is located not only on C2 and C4 but also C5,N1 and N3. ESR-ENDOR analysis shows that



SCHEME - 4



SCHEME - 5

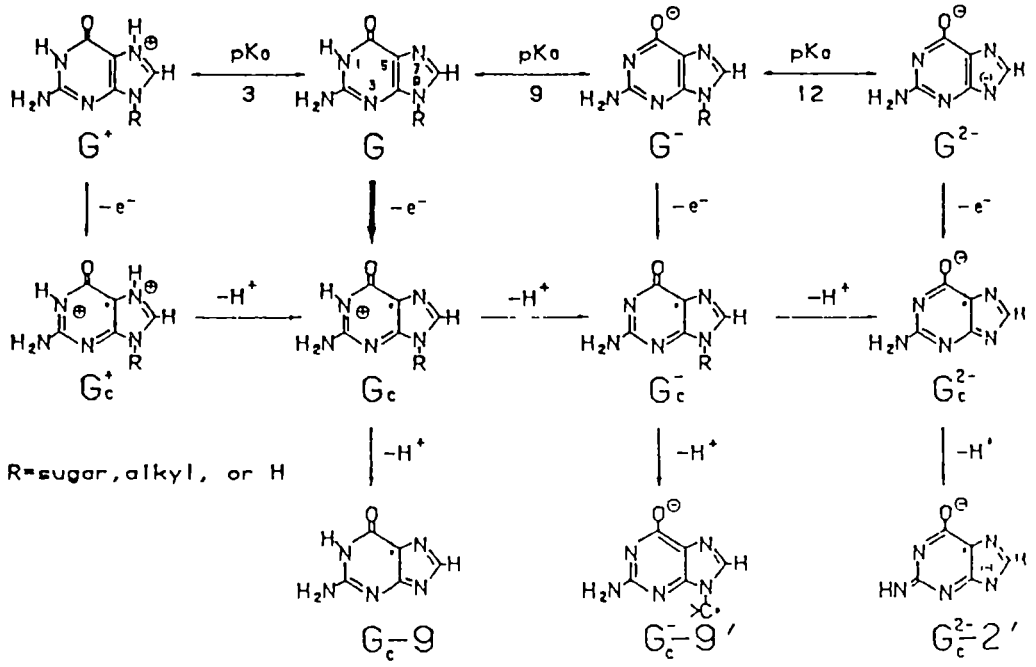
imidazole portion of the purine ring, the result would be to equalise the unpaired spin in the -C = C- double bond in contrast to pyrimidines.

Deprotonation of the cation in unsubstituted adenine occurs at N9 or N6' and in unsubstituted Guanine at N9, N1, or N2'. Substitution at N9, leads to a proton loss from that substituent at a position to unpaired electron density at N9 (schemes 6, 7). Return to the parent charge state can also occur by anion addition to C8.

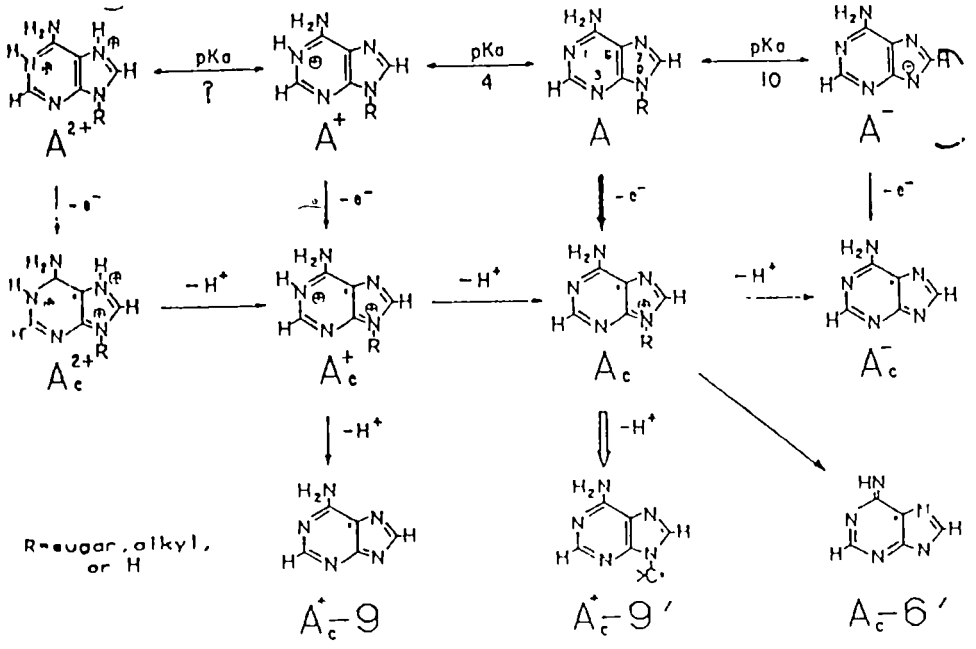
B. Electron addition:

The model for electron addition to Adenine, have shown that protonation via hydrogen bonds is most probable at N1 and N7, with N1 favoured over N7. Protonation via " free " protons occurs at non-hydrogen bonding orbitals, usually N7 or N3 and at the carbons C8 or C2, depending on the prior protonation state at N7, N3 and N1. Protons add preferably to C8. This event is more probable, or at least the resulting radical is more stable, if a proton is on N7 or N3. A proton on N1 stabilises the C2 addition radical and possibly promotes protonation at C2 of the pi anion. Conversion between the C2 and C8 H adducts depends on the protonation state of the radical as shown below in the scheme 8.

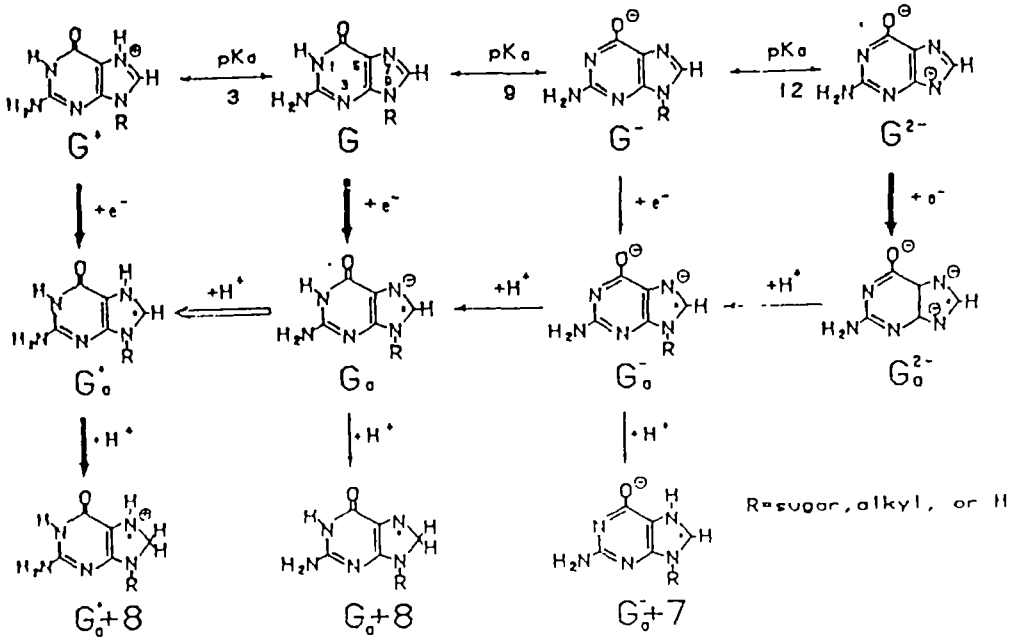
C. Excitation and Hydrogen Atom Reactions:



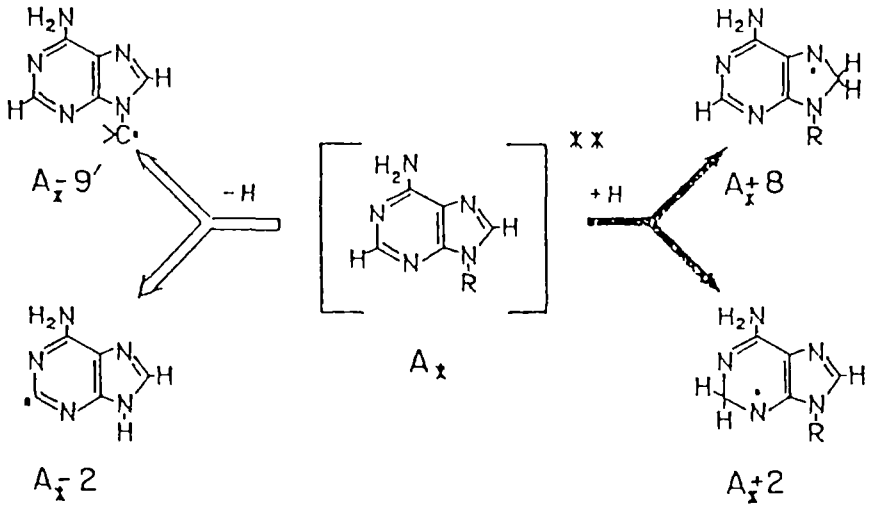
SCHEME - 6



SCHEME - 7



SCHEME - 8



SCHEME - 9

The site of H atom attack is C8 and/or C2 in Adenine and C8 in Guanine. Accordingly a substituent on N9 is a good source of H atoms. In the absence of an N9 substituent, the C2-H bond of Adenine is a site of homolytic dissociation. For Adenine, the expected reactions are shown in Scheme 9.

DNA MACROMELECULE

DNA exposed to the direct effects of ionising radiation undergoes chemical changes that originate primarily from sites that have either lost or gained an electron. Determining the chemical nature and the distribution of the damaged sites is a central problem in the field of radiation biology.

The most widely accepted model, at the present, is that at temperatures of 77K and below , electrons are trapped predominantly (or exclusively) at Thymine and holes are trapped at Guanine [29]. But difficulties with the $T^{\cdot-}/G^{\cdot+}$ model of direct damage in DNA have been apparent for some time. Graslund etal in their work on fiber DNA were unable to eliminate C as a site that traps electrons, in addition to T [30]. Sevilla etal, studying dinucleoside phosphate , concluded that the pyrimidines are more electron affinic than purines but could not exclude the possibility that cytosine's affinity was comparable to Thymine.In summary, there is evidence that electron attachment to the pyrimidine

bases is more probable than to the purine bases, but the relative distribution among the four bases is unknown.It has been shown through ESR that Cytosine is also a dominant site of Electron trapping [31].

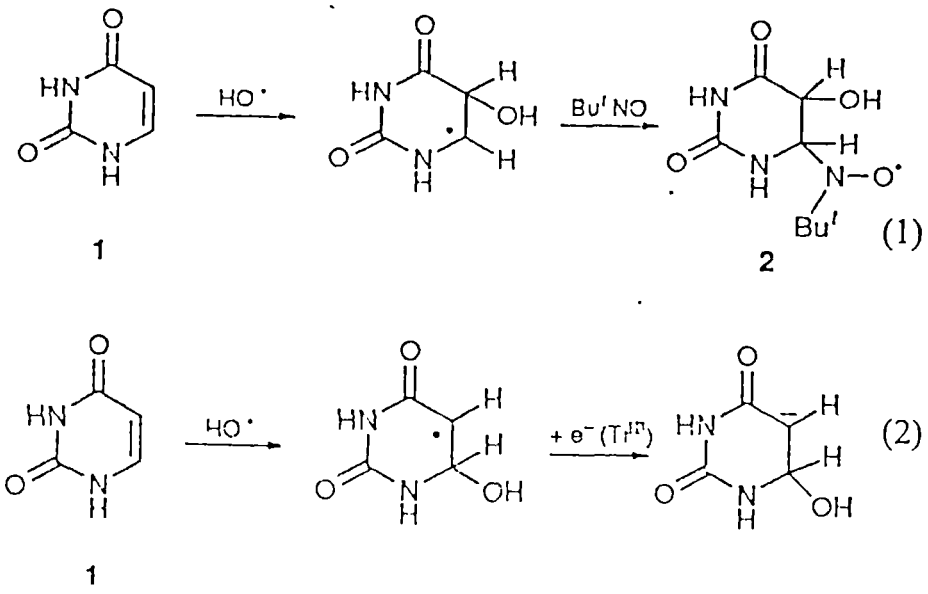
In summary it can be said that ESR studies of irradiated solid DNA and its constituents at 77 K have suggested the formation of one electron deficient (a radical cation) and one electron rich (a radical anion) center on the bases but not on the phosphate [32, 33]. There is a general agreement that the positive charge migrates to and is localised in Guanine. Earlier it has been suggested Thymine as a final location of negative charge [32]. However recent studies have questioned this assignment and proposed Cytosine instead [34, 35].

2.2 SOLUTION STATE STUDIES :

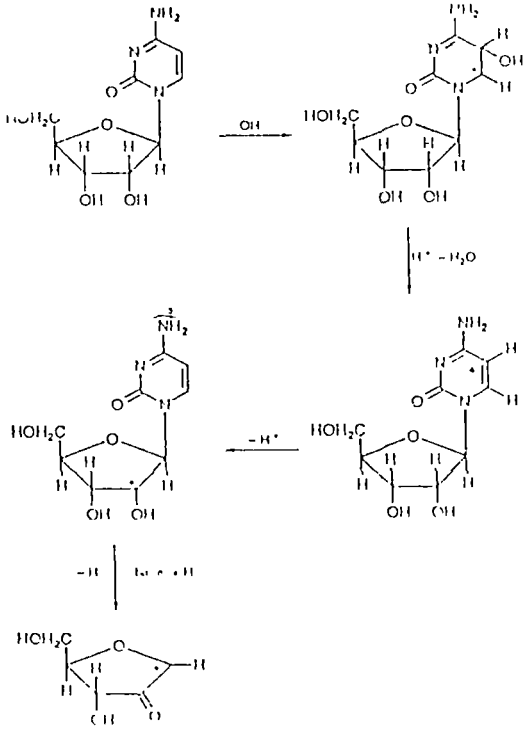
Since ionising radiation absorption is not specific to special residue(s) of the molecule, chemical transformation (radical cation formation and solvated electron) can occur in principle , on any constituent of DNA (nucleobases, sugars or phosphates) [36]. DNA lesions are partly generated also by indirect effect where the radiation is absorbed by the solvent (water) in close proximity to form the oxidising OH radical and the reducing hydrated electron ($e^-_{aq.}$) and $H^\bullet$ [37].

A. Reaction with OH$^{\bullet}$:

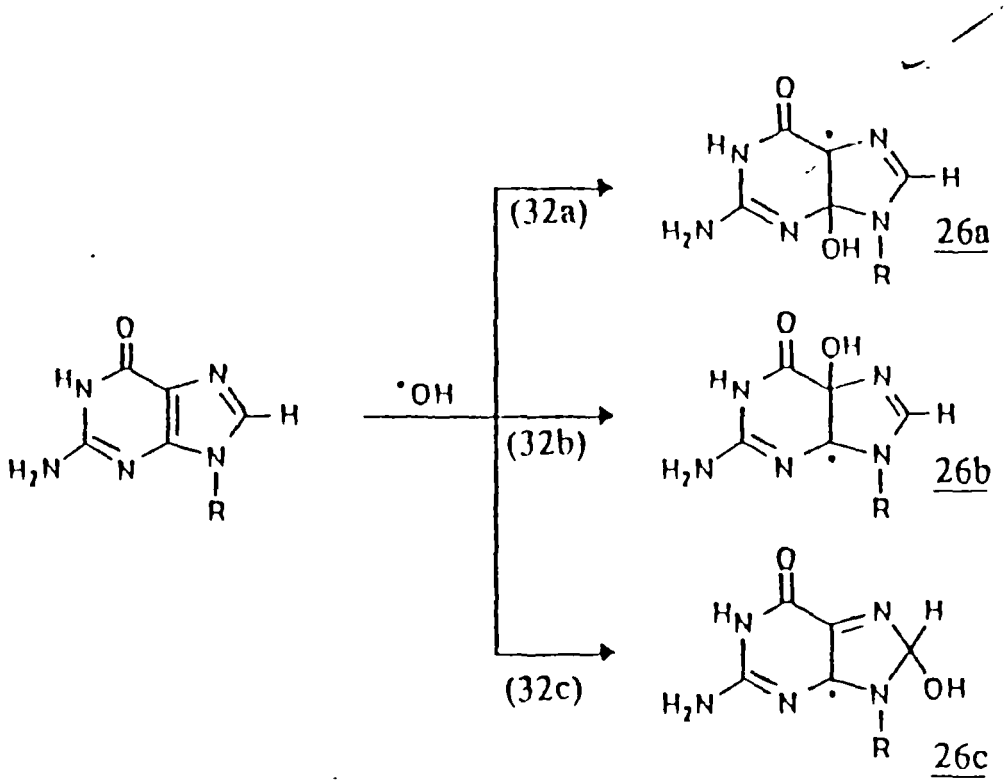
It is generally accepted that $^{\bullet}OH$ radical is the most reactive of them, and has been shown to react [38-41] with pyrimidine such as uracil, thymine etc.,. The reactions are generally accepted to involve additions to the heterocyclic systems i.e., the major site of initial damage, induced by both $^{\bullet}OH$ and direct ionisation is the nucleotide base, which then results in the transfer of damage from the base to the sugar phosphate [42-48]. The mechanism of these transfer processes are not clear, though under certain circumstances, base radical cations may be involved [49- 51]. It has been demonstrated that the hydroxyl radical combine with the 5,6 double bond of the pyrimidine base moiety to form C5-yl and C6-yl radicals and abstract hydrogen atom from the sugar moiety to form carbon centered radicals [52]. The $^{\bullet}OH$ induced C5-yl and C6-yl radicals are regarded as precursors of base damage [53]. However, the mechanism of transfer of the site of radical attack on the pyrimidine to the sugar ring , as a prelude to fragmentation via phosphate loss is not clear, though under certain circumstances radical cation may be involved [54] followed by rapid transfer of the radical centre to C2' in the ribose ring. The reaction of $^{\bullet}OH$ with pyrimidine bases may be summed up as shown in scheme 10 ;



SCHEME - 10



SCHEME - 11



SCHEME - 12

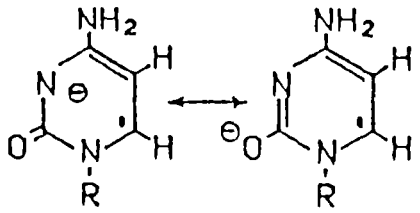
and with the pyrimidine nucleosides as, scheme 11 ;
while addition of 'OH with purine bases may be as, scheme 12 ;
whereas with purine nucleosides, the initial site if attack may be transferred from the base to the sugar in a similar way depicted for pyrimidine bases.

B. Reactivity with e^-_{aq}.:

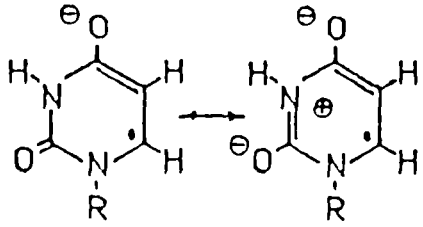
The purines have a very high intrinsic reactivity with e^-_{aq} [40]. This property is endowed with the electron deficient pyrimidine. All purines react as neutral bases with e^-_{aq} with second order rate constants, essentially independent of their individual structure. In the past there have been only few attempts aimed at elucidating in detail the nature and further reactions of purine electron adducts. Notable among them are the investigations of Moorthy and Hayon [55], Sevilla etal [56] and Hissung etal [57]. They found that the electron adducts change with pH in a way that was interpreted in terms of protonation equilibrium of the e^-_{aq} adducts. Hissung etal were able to demonstrate by conductance and optical measurements that the electron adduct is mono protonated (i.e it is a neutral radical). This conclusion has been confirmed by Visscher etal [58, 59]. From ESR spectroscopy of purine radicals, it is known that the electron adduct to the adenine moiety (the " pristine anion ") gets protonated (even at 4K) [60-62] on a nitrogen and upon warming , the reaction is followed

(via paths not very well understood) by a rearrangement that results in protonations at carbon 2 or 8 of the purine system.

For the pyrimidine bases, the unpaired electron resides primarily at C6, C4 and C2. Excess negative charge is expected at N3 and O2 for cytosine and at O4 and O2 for uracil [60],



(I)



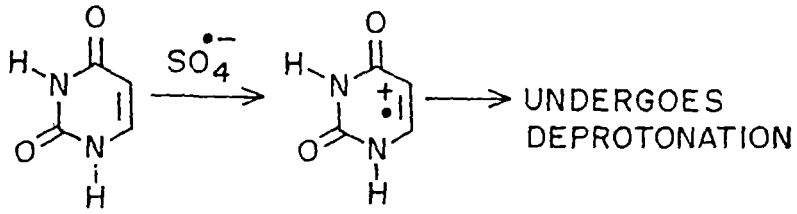
(II)

For protonation pathways, it may be mentioned that the protons are attracted to positions that have undergone the greatest increase in negetive charge. If a proton is available at time of addition and protonation will be fast if the required activation energy can be obtained. Since, the excess electron is in a pi orbital, these ions are called pi anions, regardless of protonation states

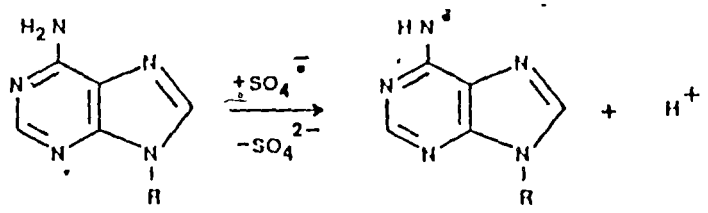
C. Electron abstractions :

Reactions of $SO4^{\cdot -}$, $Br2^{\cdot -}$(the secondary radicals) are reported in literature [53-61, 63-71]. Fujita etal have also used other secondary oxidising radicals in addition to these, they are $Cl2^{\cdot -}$, $(CNS)2^{\cdot -}$ etc. [72]. With the

pyrimidine system the electron abstraction can be shown to occur as,



Although sites of deprotonation are many e.g., when the Thymine moiety is ionised deprotonation is possible from N1 [73] N3 and from the methyl group at C5 [40] and also hydration at C5-C6 [46,74,75]. Deprotonation can thus in short occur at positions α or β to the sites of highest unpaired electron population. For the purine bases, electron loss leads to deprotonation or anion addition reactions, however, predicting the sites of deprotonation [40] is difficult because the unpaired electron distribution is diffuse. the purines have odd numbered rings , so the simple picture for pyrimidines, that of unpaired electron density at alternate ring positions, no longer applies. However, examination of the electron population should be located at C2, C4, C5, N1 and N3. Steenken ~~et al~~ have shown the reaction of $SO_4^{\bullet -}$ with adenine to proceed as ;



In addition N9 has also been shown to be a site of deprotonation . If there is a substituent at N9, a proton can be lost from that substituent at a position β to unpaired electron density at N9.

These damages produced in the heterocyclic bases are then transferred to the sugar leading to strand breakage - one of the major lesions to cause damage to DNA. Care has been taken to give proper credit for the works of other authors in the literature. The author would like to apologise for any ommission which may have occured by oversight or error in judgement.

REFERENCES

(1) Huchchinson,F., Cancer Research,(1996) 26,2046.

(2) Painter,R.B., In Radiation Biology in Cancer Research,eds. Meyn,R.E.; Painter,H.R.; Raven Press,New York, 1980.

(3) Graslund,A.; Ehrenberg, A.;Rupprecht,A.; Strom,G.; Biochim.Biophys.acta,1971,264,172.

(4) Boon,P.J.; Cullis,P.M.; Symons,M.C.R.; Wren,B.W.; J.Chem.Soc.,Perkin Trans.2 (1984) 1393.

(5) Eiben,K.; Fessenden,R.W.; J. Phys.Chem. (1971) 75, 1186.

(6) Cattterall,H.; Davies,J.Michael; Gilbert,C. Bruce J.Chem.Soc. Perkin Trans II, (1992) 1379.

(7) Scholes,G.; In "photochemistry and Photobiology of Nucleic Acids" Wang,S.Y.; Ed.: Academic Press : New York(1976) p-521.

(8) Huttermann, J.; Kohnlein, W.; Teoule, R.; : Ed.Bertinchamps,A.J.; " Effects of Ionising Radiation On DNA "; Springer Verlag :Berlin (1978)

(9) Box,H.C.; "Radiation Effects. ESR and ENDOR Analysis ": Academic Press : New York(1977)

(10) Bernhard,W.A.; Adv.Radiat.Biol (1981) 9 .199.

(11) Scholes,G.; In Effects of Ionising Radiation on DNA; Bertinchamps,A.J.;Hutterman,J.;Kohnlein,W.; Teoule,R.;Eds,; Springer : Berlin, (1978) pg-153.

(12) Bertinchamps,A.J.;Huttermann,J.; Kohnlein,W.; Eds.Effects of Ionising Radiation on DNA ; Springer: Berlin (1978)

(13) Von Sonntag,C.; The Chemical basisof Radiation Biology ; Taylor and Francis, London (1987).

(14) Von Sonntag,C.; Int.J.Radiat.Biol. (1984) 46,507.

(15) Cadet,J.; Berger.M.,; Int.J. Radiat.Biol. (1985), 47, 127.

(16) Cadet,J.; Berger,M.; Shaw.A.,Mechanism of DNA Damage and Repair: Simic,M.G.; Grossman,L.;Upton.A.D.,Eds.; Plenum : New York (1986) Pg 69.

(17) Von sonntag,C.;Schuchmann, H.P.;. Int. J.Radiat.Biol, (1986), 49, 1.

(18) Box,H.C.; Radiation Effects,ESR and ENDOR Analysis ; Academic: New York (1977)

(19) Sevilla,M.D.;In Excited States in Organic Chemistry and Biology ;Pullman,B.;Goldblum,N.; Eds,;Riedels Dordrecht, (1977) P 15.

(20) Symons,M.C.R.; J.Chem.Soc.,Faraday Trans 1,(1987) 83,1.

(21) Close,D.M.;Nelson,W.H.;Sagstuen,E.; In Electronic magnetic Resonance of the Solid State, J.A.Weil.; Eds.; Can.Soc.Chem, Ottawa,1987,; Pg 237.

(22) Neta,P.;Adv.Phys.Org,Chem., (1976), 12, 223.

(23) Myers,L.S.; In Free Radicals in Biology ; W.B.Pryor.,Ed, Academic ; New York,1980,vol 4,Pg 95.

(24) Greenstock.C.L.; Isr.J.Chem. (1984), 24, 1.

(25) Von Sonntag,C.; Radiat.Phys.Chem. ? ? ?

(26) (a)Sevilla,M.D.; Paemal,Van C.; Photochem. Photobiol. 15 (1972), 407.

(b) Sevilla,M.D.; Paemal,Van.C.; Zorman,Z.; J.Phys.Chem., 76 (1972), 3577-3782.

(27) [illegible] W.A.; Advances in Radiation Biology, Vol. 9, p. 212.

(28) Kar,L.; and Bernhard, W.A. ; J.Chem. Phys., 73 (1980), 3625-3634.

(29) Cullis, P.M.; Podmore, I.d.; Lawson,M.; Symons, M.C.R. Dalgarno,B.G.; McClymons; J.Chem.Soc. Chem.Comm, (1989) 1003-1004.

(30) Graslund, A.; Ehrenberg, A.;Rapprecht,A.; and strom,G.; Biochim.Biophys.Acta.254,172-186 (1971).

(31) Bernhard,W.A.; J.Phys.Chem.,(1989) 93,2187-2189.

(32) Symons, M.C.R.; J.Chem. Soc.Faraday Trans 1(1987) 83, 1-11.

(33) Hutterman, J.; In Radical Ionic Systems: Lund, A., Eds- Dodrecht Kluwer, (1991)p. 435-462.

(34) Farragi,M.;Broitman,F.; Trent,J.B.; and Klapper, M.H.; J.Phys.Chem(1996)100,14751-14761.

(35) Barner, J.; Bernhard, W.A.; Mercer, K.R.; Radiat.Res., (1991),90,974-978.

(36) Faraggi,M.; Broitman,F; Trent,J.B.; Klapper, M.H.; J.Phys.Chem.(1996),100,14751-19761.

(37) No 13 and the references therein.

(38) No 11 and the references therein.

(39) Catteral,H.; Davies,M.J.; and Gilbert,B.C.;; J.Chem.Soc.Perkin Trans.2(1992) Pg 1379.

(40) Steenken,Steen. Chem.Rev.(1989) 89,503-520.

(41) Fujita,S.; and Steenken,S.; J.Amer.Chem.Soc.(1981),103,2540-2545.(1981)

(42) O'Neill,P.; and Fielden,E.M.; Adv.Radiat.Biol.(1993) 17,53.

(43) no 31

(44) Hazra, D.K.; Steenken,S.; J.Phys.Chem(1987),91,425.

(45) Novias,H.M.; Steenken,S.;. J.Phys.Chem.(1987),91,425.

(46) O'Neill,P; Davies,S.E.;. Int.J.Radiat.Res. (1987),52,577.

(47) Deeble,D.J.; Schuchmann,M.N.; Steenken,S.; Von Sonntag,C.;J.Phys.Chem.(1990), 94, 8186.

(48) Bansal,K.M.; Fessenden,R.W.; Radiat.Res.(1978) 75, 497.

(49) Frolinde Schulte,D.; Simic,M.G.; Gorner,H.; Photochem.Photobiol.(1990),52,1137

(50) Hildenbrand,K.; Behrens,G.; Frolinde Schulte,D.; Hirak,J.N.;J.Chem.Soc.Perkin Trans. 2(1989)283.

(51) Frolinde Schulte,D.; Hildenbrand,K.; in Free Radicals in synthesis and Biology, Ed.,F.Minisci, NATO ASI series, Kluwer Academic Publishers, Dordrecht 1989,335.

(52) Kuwabara,Minikora Radiation Phys.Chem. Vol 37, 5-6, Pg 691-704(1991)

(53) No.13

(54) No.39.

(55) Moorthy,P.N.; Hayon,E.; J.Amer.Chem.Soc.(1975) 97,3345

(56) Sevilla,M.D.; Failor,R.;Clark,C.;Hairoyd,R.A.;Pattei,M.; J.Phys.Chem.(1976),80,353.

(57) Hissung,A.;Von Sonntag,C.;Veltwisch,D., Amus, K.D.; Int.J.Radiat.Biol. (1981),39,63.

(58) Visscher, K.J.; de Haas,M.P.; Loman,H.; Vijnovir,B.; Warman,J.M.; Int.J.Radiat.Biol.,(1987), 52 ,745.

(59) Visscher,K.J.; Hom,M.; Loman,H.; Spoelder,H.J.W.; Verberne,J.B.; Radiat. Phys.Chem. (1988) 32, 465.

(60) Bernhard, W.A.; Adv.Radiat.Biol(1981), 9, 199.

(61) Huttermann,J.; Ultramicroscopy, (1982), 10, 25.

(62) Close,D.M.; Nelson,W.H.;Sangstuen,E.; ; In Electronic Magnetic Resonance of the Solid State. J.A. Weil, Ed.; Can.Soc. Chem. ; Ottawa 1987, Pg 237.

(63) Javanovic,S.V.; Simic,M.C.; J.Phys.Chem.(1986) 90,974-978.

(64) Myers,L.S.Jr; Hollis,M.L.; Theard,L.M.; Peterson,F.C.; A.Warnick,; J.Amer.Chem.Soc,(1970) 92, 2875-2882.

(65) Wilson,R.L.; Wardman,P.; Asmus,K.D.; Nature, 1974,252,323.

(66) Hayon,E.;Simic,M.J. ; J.Amer.Chem.Soc.(1973),95,1029-1035.

(67) Vieira,A.J.S.C.; Steenken,S.; J.Phys.Chem. (1987) 95, 4138-4144.(1987)

(68) O'Neill,P.; Davis,S.,; Int.J.Radiat.Biol, (1987), 52,577-587.(1987)

(69) Buxton,G.V.;Greenstock,C.L.; Helman,W.P.;Ross,A.B.; J.Phys.Chem.Ref.Deata,(1988) 17, 513-886.

(70) Candeias,L.P.; Steenken,S.; J.Amer.Chem.Soc. (1989), 111, 1094-1099.(1989)

(71) Neta,P.; Huie,R.E.; RossA.B.; J.Phys.Chem.Ref.Data, (1988) 17,1027-1284.(1988)

(72) Fujita,S., Int.J.Radiat.Biol.(1984) 45, 371-377.

(73) Sevilla,M.D.; van Paemal; (1972) Photochem. Photobiol. 15, 407-409.(1972)

(74) Schuchmann,H.P.;Deeble,D.J.; Oibrich,G.; Von Sonntag,C.; Int.J.Radiat.Biol.(1987), 51, 441.

(75) Behrens,G.;Hildenbrand,K.;Frolinde Schulte,D.;Herak,J.N.; J.chem.Soc.Perkin.Trans. II, (1988),355.

OBJECTIVE

OBJECTIVE

Damage to DNA has been a major area of research. This damage is primarily responsible for cancer, aging, cell deactivation etc.. Although, a lot of work on damage related studies have been done, the real cause of cell deactivation is still not precisely known and debate is going on. These studies have been mainly confined to DNA and its constituents, in the solid state or solution state, mainly the aqueous state. These studies have been undertaken at ;

(i) at 77K or even at 4K ,

(ii) Using a powerful source of irradiation e.g., γ-rays, pulse radiolysis, photoexcitation etc.,

(iii) Reaction with strong oxidising agent e.g., $SO_4^{\cdot -}$, $OH^{\cdot -}$ etc.,

(iv) in aqueous phase.

Inspite of so much of work, the mother nature has illuded the scientific community and kept up to herself the greatest secret of all - the cause of cancer. (We) , therefore, felt strongly motivated to undertake the study with a different approach with a hope that our findings, no matter how trivial they may be, might help towards a wider understanding of the problem. We, therefore, confined our work ;

(i) at ambient temperature, because the human body is at that temp. Any damage at this temp. will be relevant to the

system in vivo.

(ii) Not employing any powerful irradiating source of radical generation. Because the percentage of cancer deaths due to radiation exposure is very low.

(iii) By creating such conditions where initially only one electron is transferred and the chain of reactions begins.

(iv) By employing spin trapping technique to trap the short lived intermediates.

(v) Non-aqueous phase. It is known that some very fast two electron reactions in aqueous phase can proceed in two steps involving one electron reaction in non-aqueous solvents. Moreover, the non-aqueous phase provides an ideal environment which mimics the interior environment of double helix of DNA.

(vi) If an electron transfer occurs under these conditions, does it follow an " Inner - sphere or Outer - sphere " mechanism.

The present project has been executed in two parts. First part deals with the electronic spectroscopic studies of the nucleic acid bases in non-aqueous solvents. In order to understand the role of any molecule in single electron transfer processes in a given media, it is necessary to understand its optical properties in those solvents. Literature survey revealed that so far the uv spectra of these base molecules in non-aqueous solvents has not been

studied so far. Therefore, it became imperative to study the uv spectra and its associated properties in solvents of different polarity. It is also accepted that for a molecule to participate in electron transfer processes, the primary requirement is that a molecule should form a charge transfer complex with that molecule to whom electron is to be transferred. UV spectroscopy is a simple technique through which these informations can be obtained satisfactorily.

The second major part of the project is the study of single electron transfer reactions by ESR (employing spin trapping technique).

CHAPTER-3

EXPERIMENTAL

EXPERIMENTAL

CHEMICALS

All the chemicals used were of the highest available quality. All the solvents used in the spectral measurements were of spectrograde quality. For ESR measurements solvents were dried by standard procedures. The substances used, if required, were purified by repeated recrystallisation to a sharp melting point. Their purity was confirmed by recording IR and NMR spectra. For UV spectroscopic study, the spectral transmissions were checked against highly purified water.

UV STUDIES

All UV measurements were carried out with Beckman DU 650 spectrophotometer (resolution 0.1 nm and band width 1 nm). Matched pair of quartz cells with path length 1 cm was used. The wavelength calibration of the instrument was checked against Holmium oxide filter and intensity calibration was checked against standard solution of K_2CrO_4 in 0.1N NaOH. For quantitative measurements standard solutions of the order of 10^{-3} M were prepared and necessary dilutions were made. For qualitative measurements solutions were prepared by stirring them vigorously, centrifuging and filtering them through micropore filters to obtain a homogeneous solution. Spectrograde solvents were used . The spectra which were

recorded under deareated condition were prepared by bubbling nitrogen gas in the UV cell through a needle pierced through a self sealing septum. All solutions were prepared only prior to recording the UV spectra. Second derivative plots were obtained by a software programme supplied by Beckman. In order to check the authenticity of the results some of the UV spectra were recorded on Cary 2300 spectrophotometer, and were found to be identical.

ESR STUDIES

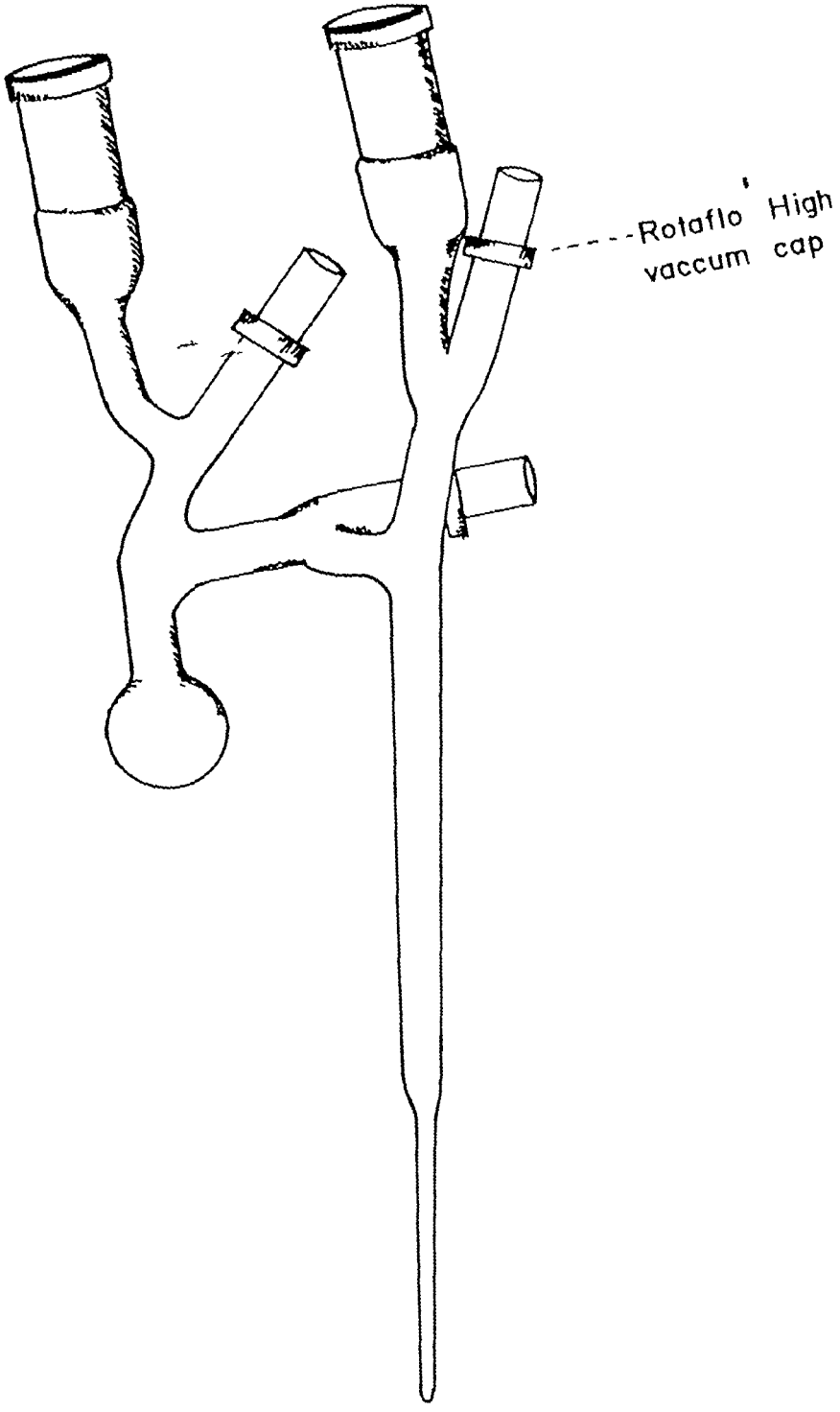
First derivative ESR measurements were recorded on Varian E-109, X- band spectrometer, with 100 KHz field modulation. A 9.6 GHz, microwave frequency generator was used. All ESR measurements were made at room temperature (20 ± 2°C). Field calibration of the ESR Spectrometer was frequently checked with standard samples of di-tert.butyl nitroxide or standard marker supplied by Varian.

In spectral recording, optimum level of microwave power was used to avoid saturation effect. In some cases spectra were recorded deliberately at low microwave power, where spectral overlapping was intense. Best level of modulation amplitude was selected to avoid line broadening. In most of the spectra field sweep±20 G or ± 25 G was chosen. Some spectra were always recorded at higher field sweep to see

any specie with a very different g value. In early stages of the reaction when reactants were just mixed, scanning time was low to detect any short lived specie, otherwise in most of the measurements it was 4 - 8 mins. Where secondary or tertiary hyperfine splitting was very low, a component of the spectra was scanned at a small field range with slow scan speed. A best combination of receiver gain and time constant was chosen to achieve good signal to noise ratio. Solvents with low dielectric constant were preferred, unless required to study the higher dielectric effect on the reaction mechanism. The total volume in the cell was no more than 0.5 ml. All hyperfine measurements reported are an average of more than one set of values and their accuracy are within the range of $\pm$ 0.2 G. g values were calculated with respect to DPPH as standard marker and the accuracy is within $\pm$ 0.0003.

EXPERIMENTAL DETAILS.

All the solvents used were spectrograde quality and were dried by the usual procedure. Their purity was checked by IR and UV. All the ESR experiments were carried out in a specially designed cell of Quartz, as shown in the diagram. All reacting solutions were thoroughly degassed by repeated freeze and thaw cycle to a vacuum of 0.02 torr. Since in



ESR CELL

our system the intermediates are, free radicals and ionic species , the removal of oxygen was essential. Only degassed solutions were mixed and scanning was started immediately. The concentration of the substrates were of the order of ca. 10^{-3} molar. Different batches of spin traps supplied were used and the results were essentially same. Low concentrations of the spin traps and substrates were used to minimise the participation of secondary reactions. After mixing, the cell was left in the cavity of the spectrometer to avoid accidental exposure to stray light. In some cases spectra were recorded even after 24 hours to see the growth of any specie which could be helpful in interpreting the mechanism. All experimental observations reported are fully reproducible.

COMPUTER SIMULATION.

All the spectral assignments were confirmed by simulating the spectra using the parameters obtained from the experimental spectra, by an in house developed simulation programme.

CHAPTER - 4

RESULTS & DISCUSSION
UV
SPECTROSCOPY

UV SPECTROSCOPIC STUDIES OF NUCLEIC ACID BASES.

The functional specificity of nucleic acids and nucleotide coenzymes is determined by the reactivity of the nucleoside components and, in particular, of the heterocyclic bases incorporated in them. It is well accepted that all the processes e.g., damage , repair or mutagenesis etc., which ultimately affects the DNA or RNA molecule, begins from the heterocyclic base. In other words, heterocyclic bases are the primary site of attack. If one wants to study any particular process (e.g, Single Electron Transfer processes) affecting ultimately DNA or RNA it is only logical to begin with the study of such affects on the individual bases, which constitutes the building blocks , and then extrapolate them to the macromolecules. It must be emphasised that for carrying out any experiment under any specified condition or environment, it is of utmost importance that the molecular state of the compounds under these conditions be determined first. In our opinion ,the electronic spectroscopic studies would provide satisfactory information about the molecular state of the compound.

As mentioned in the objective, we have undertaken the single electron transfer reaction in the non-aqueous environment. In order to see the feasibility of SET, charge transfer complex formation is a positive indication, as CT complex is

a potential precursor to an ET act , thermally as well as photochemically initiated [1]. For studying charge transfer complexes, however, it is essential to know the electronic transitions of the substrates involved in the same environment. The uv study in different solvents besides telling the nature of electronic transitions, tells about the role of hydrogen bonding, the dielectric effect which may affect the polarity of the orbital and the transitions state, association (self association , CT formation etc), and tautomerism etc., that might occur. We feel that the path to the objective of the present work would be paved through a systematic approach to the problem using UV spectroscopy as the initial step and a prelude to the ESR studies.

Experiments described here have been carried out in non-aqueous solvents that may in some way mimic the interior environment of a double stranded polynucleotide chain. Insolubility of these bases restricted such studies in the past. We however, managed to dissolve these bases as described in the experimental section. The bases and their corresponding nucleosides studied , were the following ;

1. Guanine - Guanosine .
2. Adenine - Adenosine .
3. Cytosine - Cytidine.
4. Thymine - Thymidine.

5. Uracil - Uridine.

Some very interesting and new results have been observed and are being presented here. The work described here have been broadly divided in three categories for the systems mentioned above ;

A. UV transitions and their associated features.

B. Associations ; (a) self-associations and (b) base pairing

C. Charge transfer complexation studies.

A. SPECTROSCOPIC TRANSITIONS.

UV spectra for all the naturally occuring bases and their respective nucleosides have been recorded in different solvents. The spectra reported are purely qualitative in nature. The second derivative plots were used to mark the positions of the bands. The recording of spectra in solvents of varying polarity has been advantageously used as a diagnostic tool to identify the nature of the transitions, e.g., with the increase in the polarity of the solvent, pi --> pi* transitions undergo bathochromic shift (red shift) while n --> pi* undergo hypsochromic shift (blue shift) - Kasha's rule.

In pi -- > pi* transitions, the ground state of the molecule is relatively non-polar, and the excited state is often more

polar than the ground state. As a result, when a polar solvent is used , it interacts (stabilises) more strongly with the excited state than with the ground state, and the transition is shifted to longer wavelength (bathochromic shift, lower energy).

In n --> pi* transitions, the ground state is more polar than the excited state. In particular, hydrogen bonding solvents, interact more strongly with unshared electron pairs in the ground state molecule than they do in the excited state molecule. As a result, an n --> pi* transition will have its absorption maximum shifted to shorter wavelength (hypsochromic shift, higher energy) as the hydrogen bonding ability (polarity) of the solvent increases. One should note that an n --> sigma* transitions would be affected in the same way as an n --> pi* transition.

GUANINE / GUANOSINE

GUANOSINE

Fig.1a shows the uv spectra of guanosine in solvents of different polarity in the scan mode. Except in n-hexane and dichloromethane, two bands centered around 250 nm (fairly well defined) and at 280 nm (broad) are observed. However, the second derivative plots reveal four bands

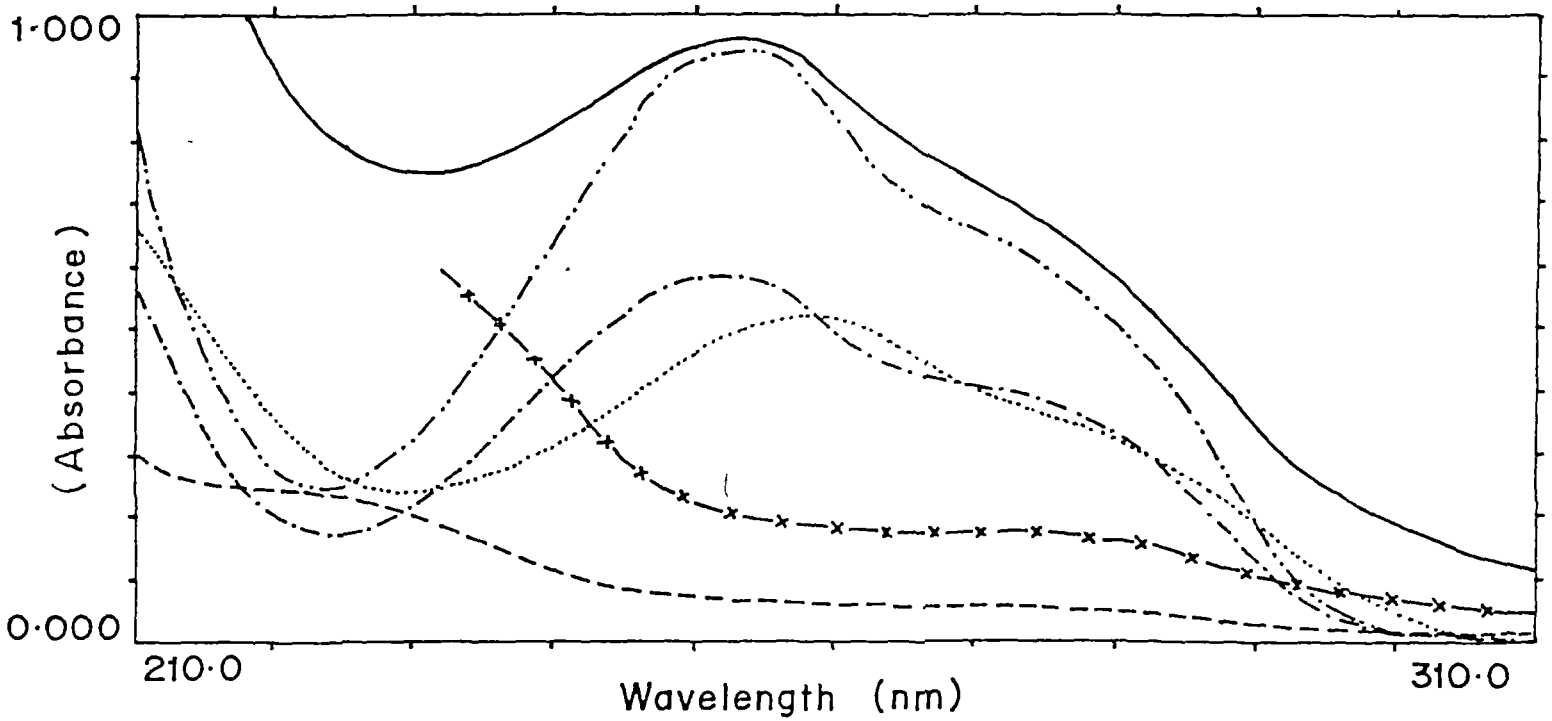
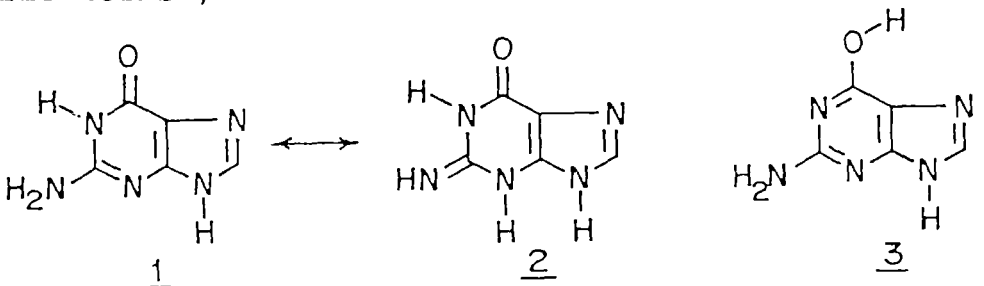


Fig. 1a. UV Spectra of Guanosine in solvents of different polarities.-•-•-•-, water ;•••••, methylcyanide ;-••-••- methanol ; x-x-x-x, dichloromethane ; ——, 1,4 dioxan ;---- n-hex

centered around ; band I 248 nm , band II 255 nm , band III 275 nm, and band IV 284 nm, Fig.1b. In dichloromethane only band III and band IV are observed Fig 1c. In n-hexane band III and band IV are observed along with a new band at ca. 225 nm, Fig 1d. The uv spectra of the individual bases in aqueous media is reported and our results (scan mode)in aq. media are in good agreement. The uv spectra of the different batches of the bases were identical.

The hyperchromic effect : The diminution of the band or the appearance of a new band is postulated as due to some kind of association or charge transfer interactions. However, we did not observe blue or red shift with the change in the polarity of the solvent. The high intensity of the bands suggest that the transitions are fully allowed. The positions of the respective bands in different solvents are given in Table I . guanosine can exist in the following tautomeric forms ;



1 2 3

Since there are two chromophores (carbonyl and amino group) and both are known to exhibit their electronic transitions, it is therefore, logical that both of them should show

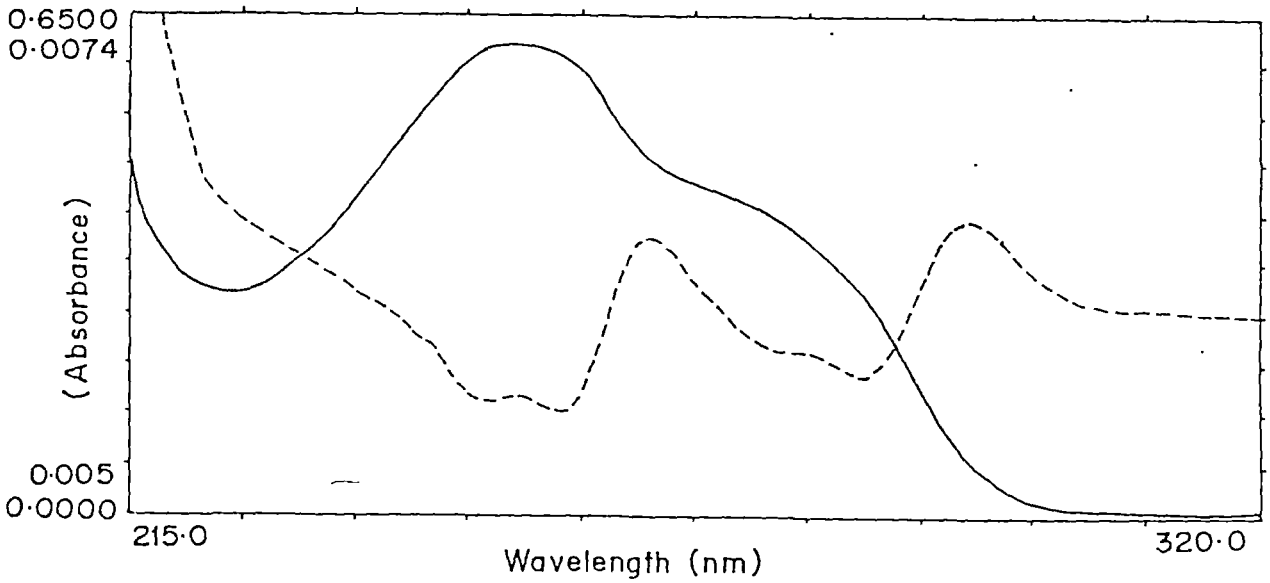


Fig. 1b. UV Spectra of Guanosine in Tert.Butanol.

—— ,scan mode ;-------, second derivative mode.

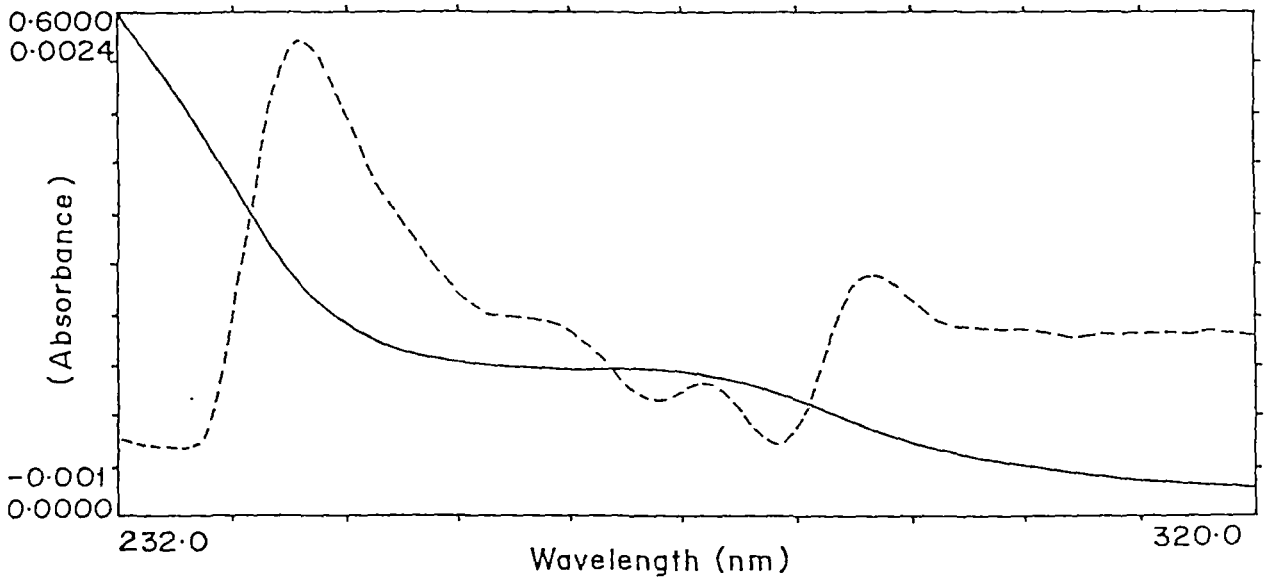


Fig. 1c. UV Spectra of Guanosine in dichloromethane.

—— , scan mode ;- - - - - , second derivative mode.

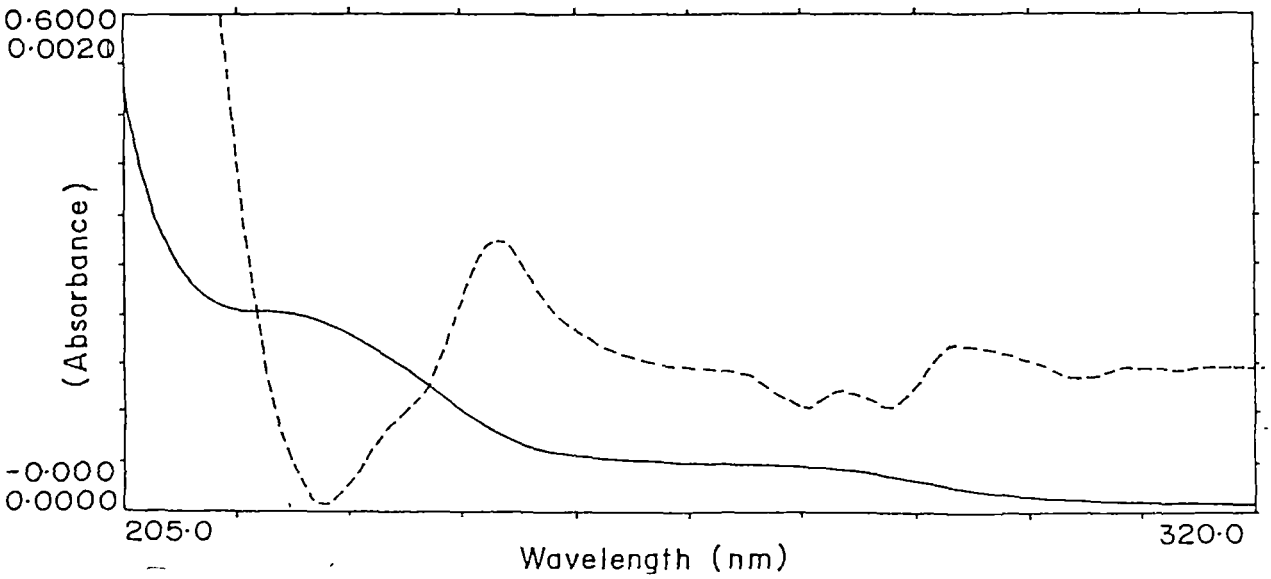


Fig. 1d. UV Spectra of Guanosine in n-Hexane.

—— , scan mode ; ------ , second derivative mode.

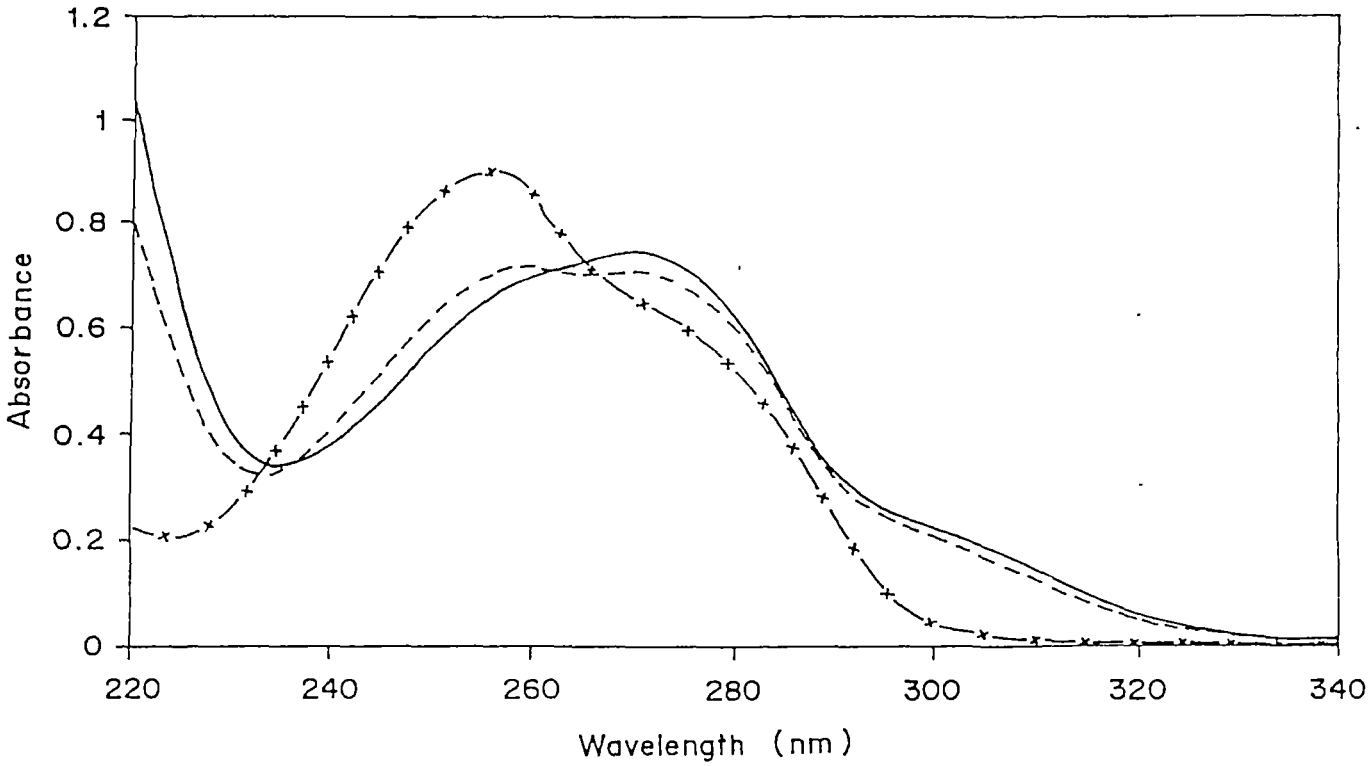


Fig. 1e. Effect of incremental addition of NaOH to a solution of Guanosine in Water. —x—x— in water (pH=6.9), ----, (pH=7.3) ——— (pH=7.7).

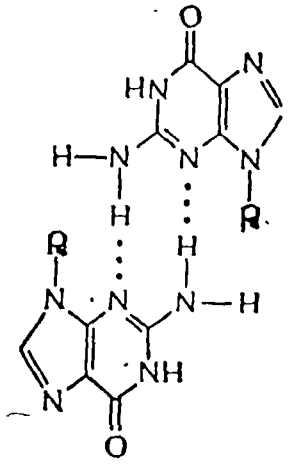
pi-->pi* transitions. In aniline , pi--pi* transitions occurs at 234 nm and in benzaldehyde it is at 250 nm in water. Extending this analogy to our system, we are inclined to postulate band I (due to amino group) and II (due to keto group) due to structure I ; band III (due to imino group) due to structure II ; band IV (due to enolic group) due to structure III. However, structure II would be thermodynamically less favoured than structure III, therefore, band III in all probability corresponds to structure II. The amino - imine tautomerism is reported to occur in adenine The transformation energy , ΔH for adenine is 1.477 eV while for guanine's keto - enol tautomerism it is 0.185 eV [2]. The lower value for guanine suggests that this process is certainly more facile than amino-imine transformation, but we see no reason why it should not occur in guanine too, may be with lesser ease. Careful look at the band III in non- hydroxylic and non-polar solvents (n- hex. and dichloromethane) shows that this band is better defined in non-hydroxylic and non-polar solvents than in strongly hydroxylic and polar solvents. This suggests that the chromophore responsible for this transition is susceptible to dipolar and hydrogen bonding effect. Imine would be more prone to such effects than enol. This lends further credence to our postulation of band III as due to Imine (structuure II). We assigned band IV as due to enolic chromophore on the

basis of a simple and fairly convincing experimental evidence. To a solution of guanosine in water (pH = 6.9) a small drop of NaOH solution was added (pH increased to 7.3 and 7.7), the decrease in the intensity of the band II was observed with a corresponding increase in the intensity of IV, further smaller addition produced the similar effect. This suggests that NaOH stabilises the basic specie present in the system , viz., the enolic specie. The effect observed with Band I and III was negligibly small. This observation points out that the difference in the basicity of the chromophores responsible for band II and IV is much more as compared to the difference in the basicity of the chromophores responsible for band I and III. Therefore, if band II corresponds to keto group,then band IV should correspond to enolic form, Fig 1e. Inspite of our best efforts, we could not locate band at higher wavelength with very low intensity, which could be assigned as due to n--pi* transitions. This is not surprising as it is expected to have a minor absorption band at a longer wavelength and could be largely masked by the stronger pi--> pi* transition.

B. ASSOCIATION.

The keto form and the amino form has the natural predominance in highly polar and hydroxylic solvents

(water, methanol etc.,) while in non-polar and non-hydroxylic solvents (n-Hex. dichloromethane etc.,) it is the enolic and imine forms. In going from polar and hydroxylic solvents to non-polar and non-hydroxylic solvents the band I and II due to amino and keto form respectively disappeared altogether while band III due to imine form and band IV due to enolic form showed little effect. The second derivative plot of uv spectra in n-hexane, Fig. 1d revealed a new band at ca. 226 nm . Such properties exist in aromatic molecules ; many artificial dyes have a tendency towards self association and this phenomenon is characterised by "magnificent" spectral effects: appearance of new and specific absorption or florescence band, self quenching of bands, formation of gels etc.[3]. Further during an IR investigation of dimer formation of the bases it was pointed out by Pitha etal [4] that such a phenomenon may be accompanied by appearance of new bands sometimes outside the measured range, but should be accompanied by the disappearance or dimunition of the bands of the free species. We postulate that the disappearance of well defined bands in solvents of lower polarity is due to self-association, through H-bonding as shown below ;



It may be mentioned here that in hydroxylic and polar solvents, hydrogen bonding between solute and solvent, decreases the self-association energy, resulting in an increased number of monomer units. Conversely, the opposite effect should be observed in non-polar and non-hydroxylic solvents. We put this to test.

To the solution of guanosine in dichloromethane, an incremental amount of methanol was added and with each addition a hyperchromic effect was observed Fig.1f. After attaining a maximum value ,intensity started decreasing due to dilution. The increase in intensity around λ max was nearly fourfold. This suggests strongly that guanosine in dichloromethane is certainly not in a monomeric state but in a dimeric state (may be open as well as cylic) [5] and may be even in a polymeric state (?). With the increasing addition of methanol a stronger hydrogen bond between solute and solvent molecules pulls the solute molecules apart and spectra observed is identical to the one in pure methanol. Such kind of behaviour was observed with cyclohexyluric acid

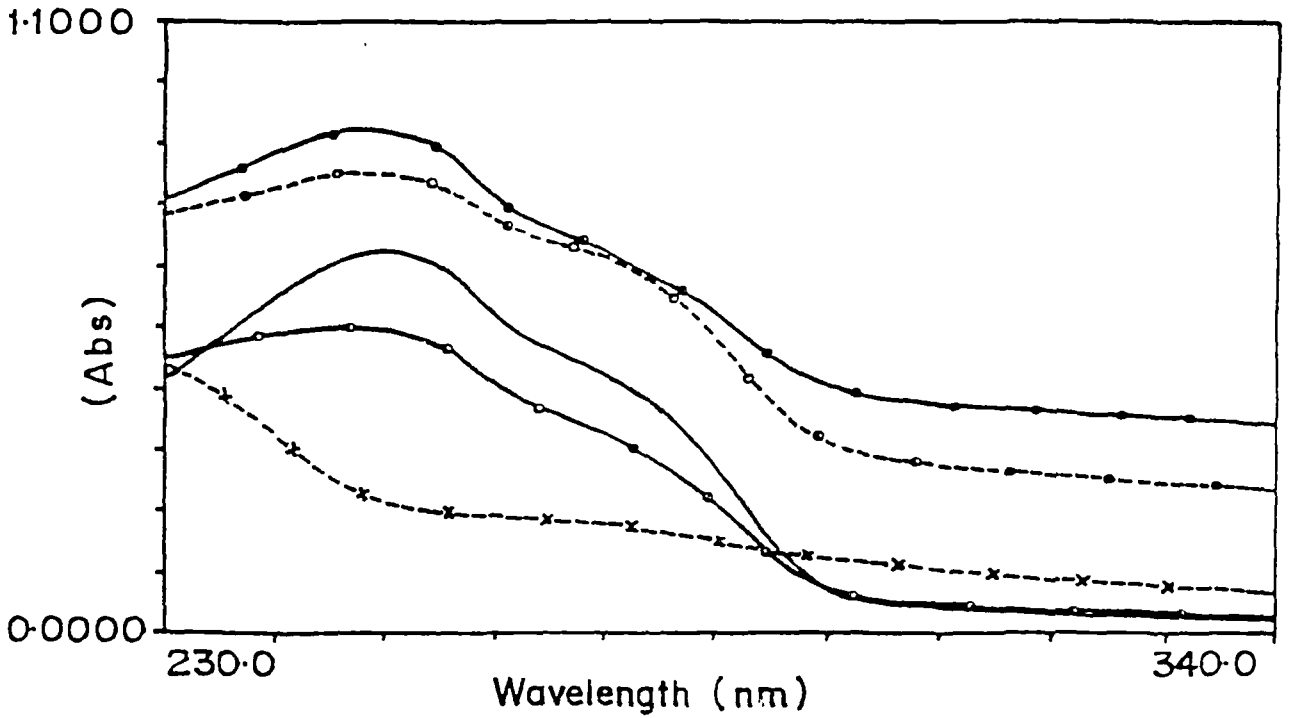


Fig. 1f. Effect of incremental addition of methanol on the UV spectra of Guanosine in dichloromethane.

x---x , Guanosine in CH_2Cl_2 ; o-o-o, 10 % (v/v) CH_3OH added ; ——, 40 % (v/v) CH_3OH added ; —•—•— 80 % (v/v) CH_3OH added ; --•--•-- 100 % (v/v) CH_3OH added. Methanol was added in both the reference and the experimental cell.

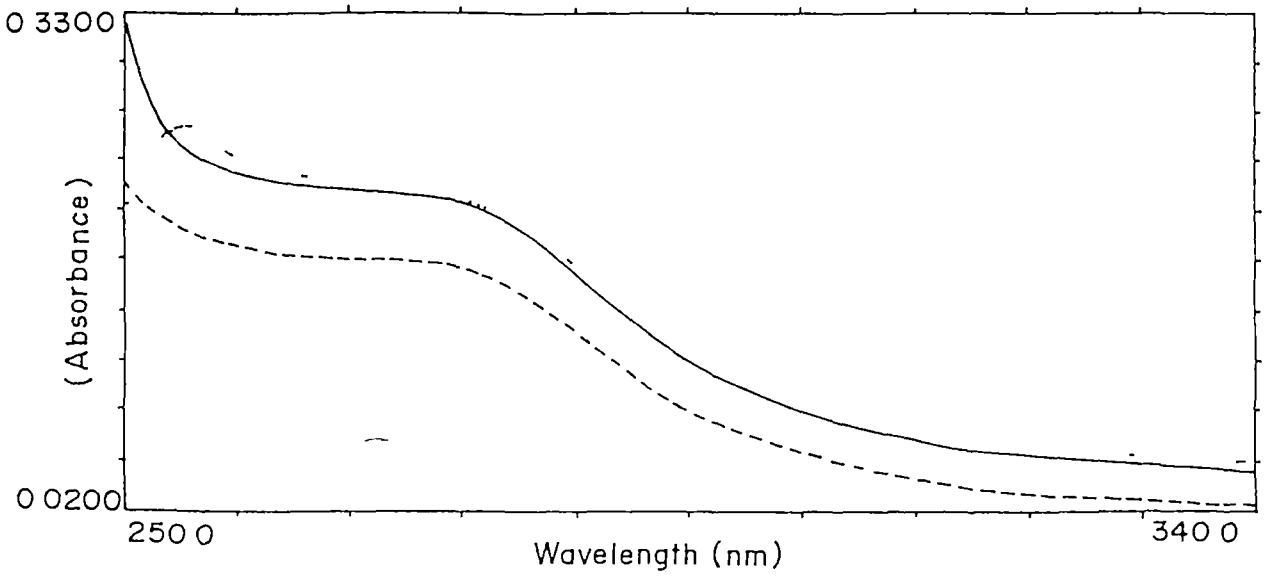


Fig. 1g. Effect of incremental addition of DMSO on the UV Spectra of Guanosine in Dichloromethane ---- Guanosine in CH_2Cl_2; —— 1% (v/v) DMSO added; .....2% (v/v)DMSO added.

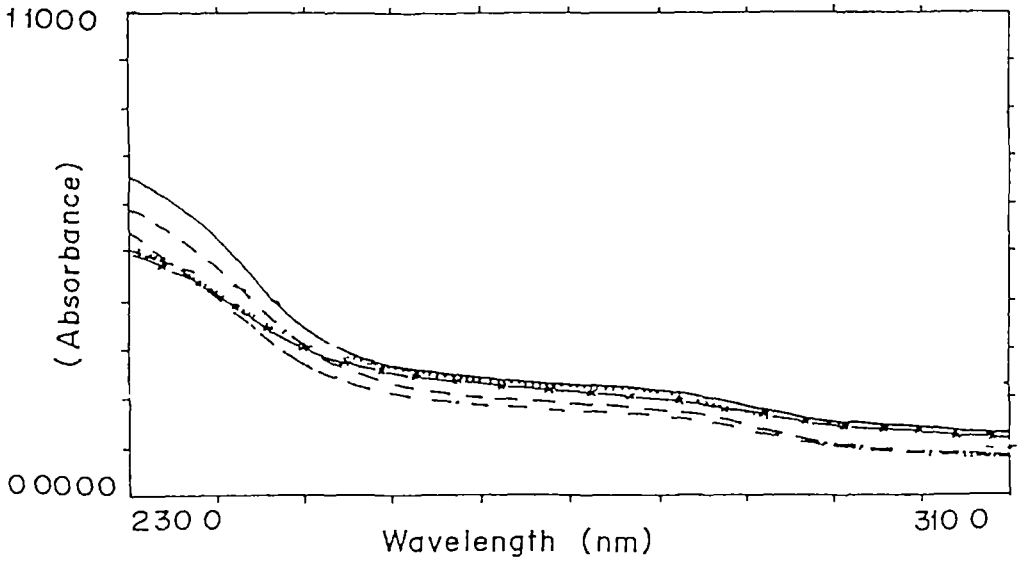


Fig. 1h. Effect of incremental addition of methylcyanide on the UV spectra of Guanosine in dichloromethane.

—— Guanosine in CH_2Cl_2; ..... 10% (v/v)CH_3CN added; -x-x- 40% (v/v)CH_3CN added; - - - 80% (v/v)CH_3CN added; ——— 100% (v/v)CH_3CN added

by IR study [6] . Similarly, to a solution of guanosine in dichloromethane when small amount of dimethylsulfoxide was added, the band at ca. 250 nm was revealed, fig 1g. In a similar experiment, incremental amount of methylcyanide was added to the guanosine solution in dichloromethane, spectra identical to the one in pure methylcyanide was not restored only gradual fall in absorbance was observed, due to dilution, fig 1h. This indicates that hydrogen bonding with hydroxylic solvent is so strong that it breaks the associative bonds.

GUANINE

Fig. 2a shows the UV spectra of guanine in solvents of varying polarity and the band positions are given in Table II.Unfortunately, the bands in guanine are not as well defined as in guanosine. It seems that solvent strongly affects the bands. We were further handicapped by the lower solubility of guanine as compared to guanosine. Hence, higher intensity spectra could not be obtained. A representative plot Fig.2b in water in scan mode with its corresponding second derivative plot is shown for clarity. It appears that in guanine too, we observed four bands. In polar and strongly hydrogen bonding solvents we could mark four bands but in non-polar and very weakly hydrogen bonding solvents only band III and IV are clear. Using the same

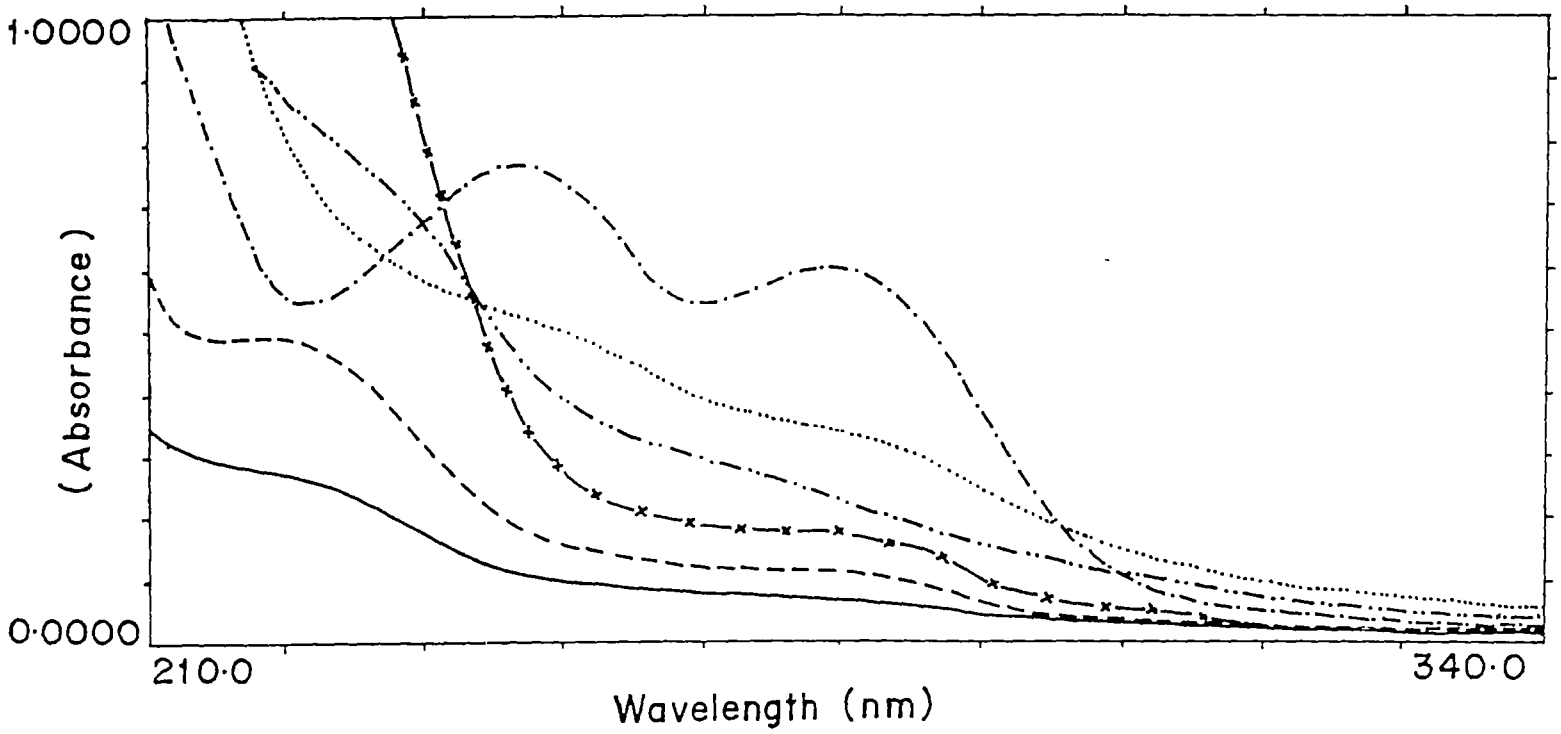


Fig. 2a. UV Spectra of Guanine in solvents of different polarities.
—·—·—·—, water ;– – – –, methylcyanide ;·····, methanol ;
—x—x—, dichloromethane ;—·—·— 1,4 dioxan ; —— ,n-Hex.

arguments as we used for guanosine, one can say that in guanine too, two similar type of tautomeric equilibrium exists. Band I and Band III can be assigned to amino-imine tautomers, and band II and IV due to keto and enol forms respectively. In methylcyanide, only band III and IV are clear and a new band appears at ca. 226 nm, which we have assigned as due to self-association. Similar behaviour is observed with n-hexane (with an additional band at ca. 226 nm, fig.2c) and dichloromethane. In 1,4 dioxan the situation is bit complicated. The well defined nature of band I and II has disappeared and a band appears in the lower region at ca. 233 nm which we tentatively assign as due to dimer (but we are not in a position to explain a strong red shift as compared to n-hex.).

B. ASSOCIATION

The uv results in different solvents as just mentioned above shows that in guanine too similar effects were observed as in the case of guanosine,rather a stronger self association was found to take place. In methyl cyanide a band at ca 226 nm was also observed,though it is strongly polar but weakly hydrogen bonding. The stronger self-association of guanine was confirmed by doing a similar experiment as we did with guanosine. To the guanine solution

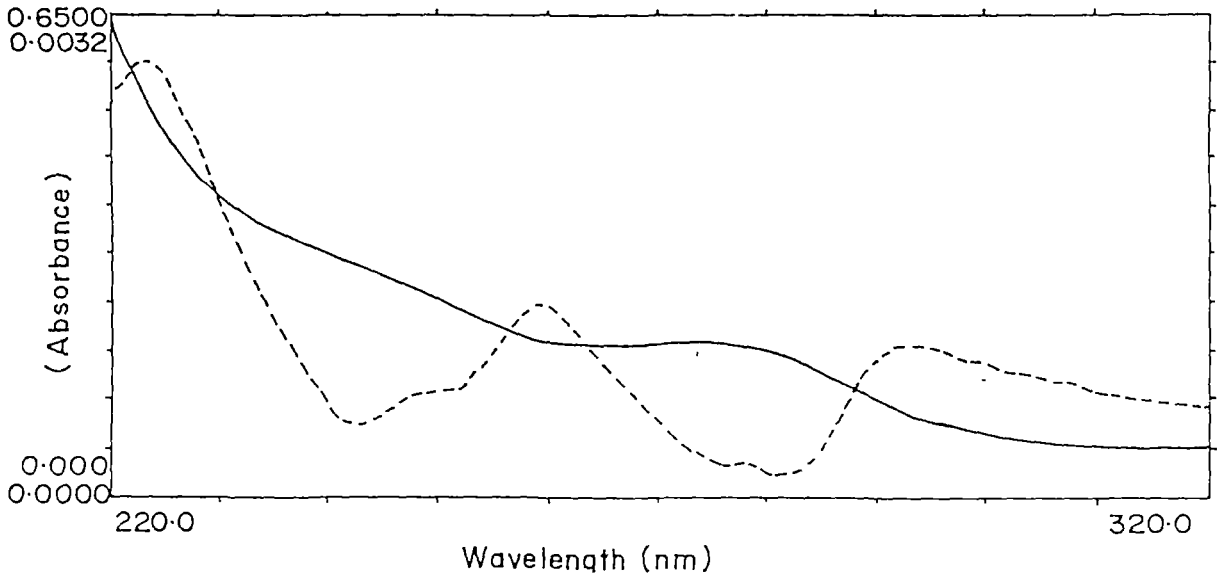


Fig. 2b. UV Spectra of Guanine in water.

_____, scan mode ; – – – – – –, second derivative mode.

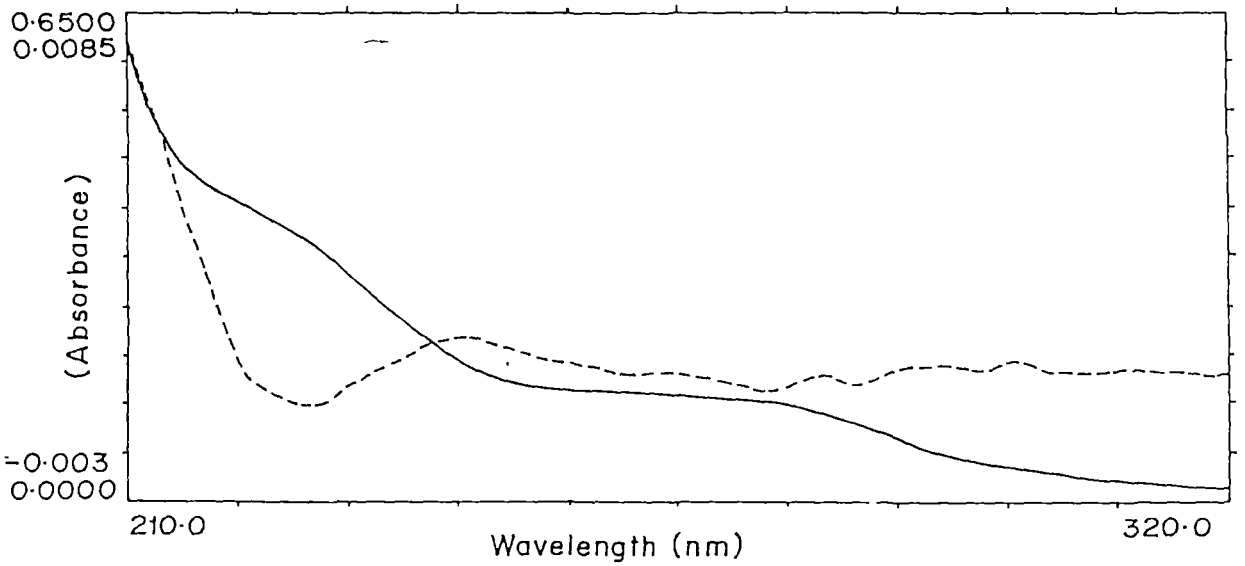


Fig. 2c. UV Spectra of Guanine in n-Hexane.

_____, scan mode ; – – – – –, second derivative mode.

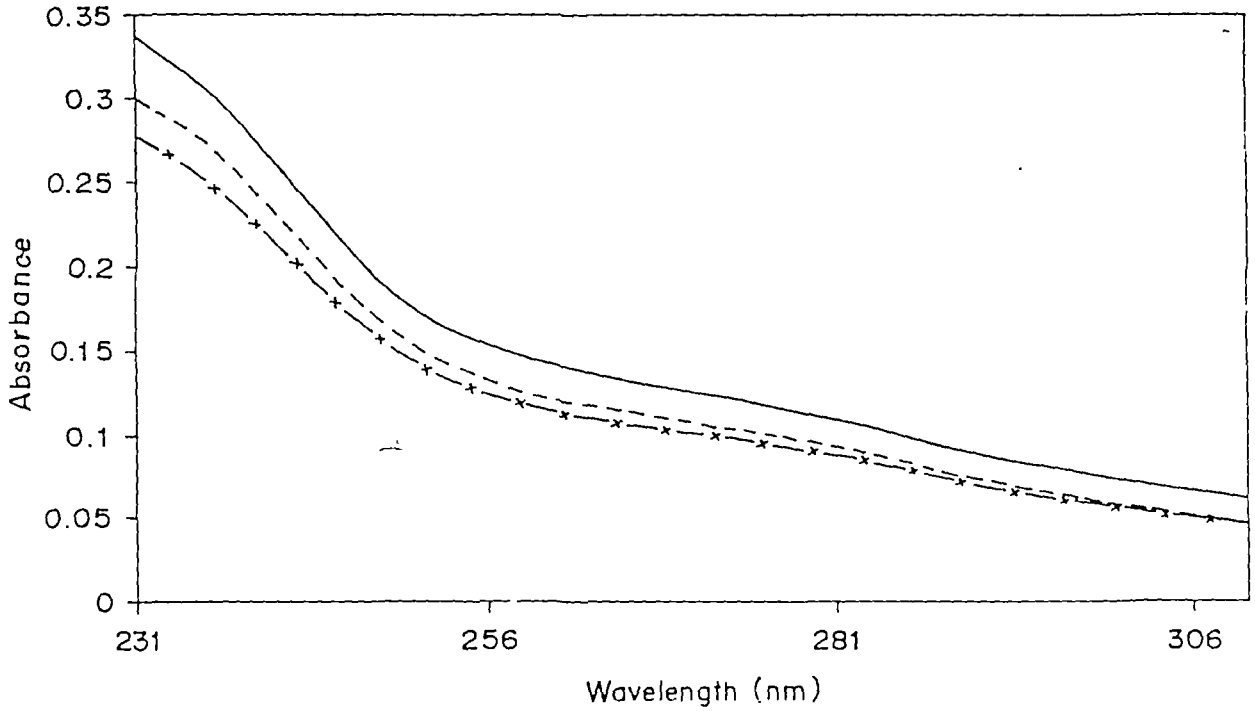


Fig. 2d. Effect of incremental addition of methanol on the UV spectra of Guanine in dichloromethane.——Guanine in CH_2Cl_2, _____10%(v/v) CH_3OH added,-x-x-x- 40% (v/v) CH_3OH added.

TABLE I. Band Positions of Guanosine in Solvents of Different Polarities (calculated from the second derivative plot).

SOLVENT	BAND I	BAND II	BAND III	BAND IV	SPECIAL BAND
H_2O	248	255		283	
CH_3CN	248	256	277	284	
CH_3OH	249	256	276	283	
$(CH_3)_2CHOH$	248	256	275	283	
$(CH_3)_3COH$	248	255	276	283	
DIOXAN	250	256	275	284	
CH_2Cl_2			274	283	
n-HEXANE (n-HEX)			274	283	225

TABLE II. Band Positions of Guanine in Solvents of Different Polarities (calculated from the second derivative plot).

SOLVENT	BAND I	BAND II	BAND III	BAND IV	SPECIAL BAND
H_2O	244	253	276	283	
CH_3CN		254(w)	274	283	226
CH_3OH	248	253	276	283	
DIOXAN			275	283	
CH_2Cl_2			275	283	
n-HEX			275	283	226

Note : Error limit is $\pm$ 1 nm.

in dichloromethane when an incremental amount of methanol was added, to our surprise spectra due to monomeric guanine molecules in methanol could not be restored, only decrease in absorbance due to dilution occured , Fig. 2d. This indicates that in the case of guanine, once the self association has occured, it could not be broken by employing strong hydrogen bonding solvents. This can be explained on the basis that the incorporation of sugar moiety in the molecule reduces the basicity, which manifests in lowering the electron donating capability of the molecule and hence weaker self association in guanosine. It deserves mention that the same tautomeric equilibrium is operative in both guanine and guanosine as seen from their band positions. Our experimental results do not support the tautomerism proposed by some workers in the imidazole ring [7]. Had this been the case, both compounds would have shown different band positions, as the H at N (7) or N (9) is replaced by sugar in the case of guanosine leaving no scope for any tautomerism in imidazole part of this compound.

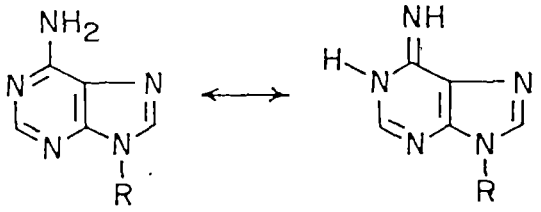
ADENINE \ ADENOSINE

Adenine and Adenosine both belong to the same class of purine bases as guanine and guanosine . The only difference between adenine and guanine is the absence of a carbonyl functional

group.

ADENOSINE

Fig.3a shows the uv spectra of adenosine in solvents of different polarity, and band positions are calculated from their respective derivative plots and given in Table III . As a representative plot Fig. 3b and 3c ,the spectra in 1,4 dioxan and n-Hexane in scan mode and its second derivative mode respectively is shown. In highly polar and hydroxylic solvents three bands were observed. Band II is very well defined, band III and IV could be revealed only through their derivative plots. The major chromophore in this molecule is amino group. The tautomeric equilibrium between amino - imine group for adenine is reported in the literature [8a,b]. Out of these three bands, two can be assigned due to their two tautomers as ;



1 2

The question arises, the remaining one is due to what ? Mason [9] has observed two bands at 261 nm and at 267 nm for adenine and he assigned the strong band at 261 nm as due to polarized long axis, while weak band (as shoulder) at 267

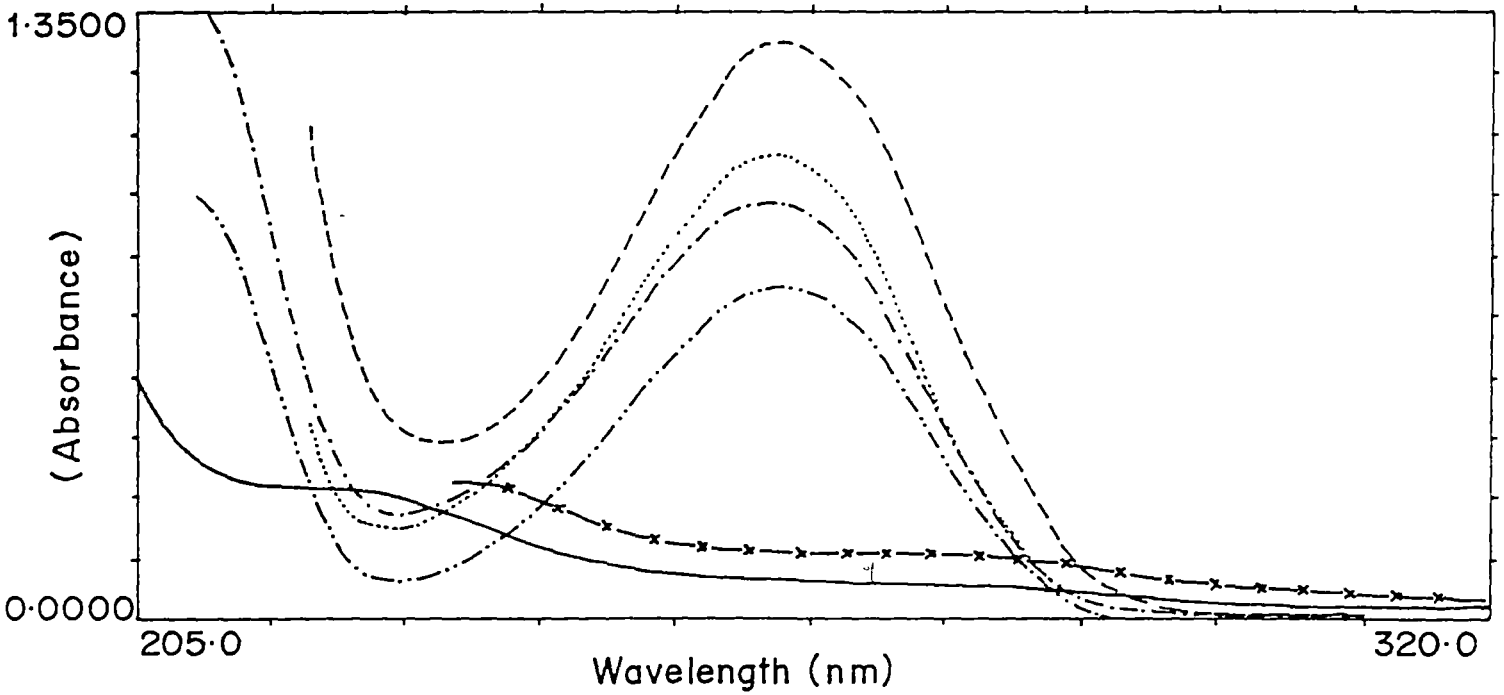


Fig. 3a. UV Spectra of Adenosine in solvents of different polarities....., water ;-·-·-methylcyanide;-··-methanol ; -×-×-×- , dichloromethane ;---, 1,4 dioxan,——— , n-Hex;

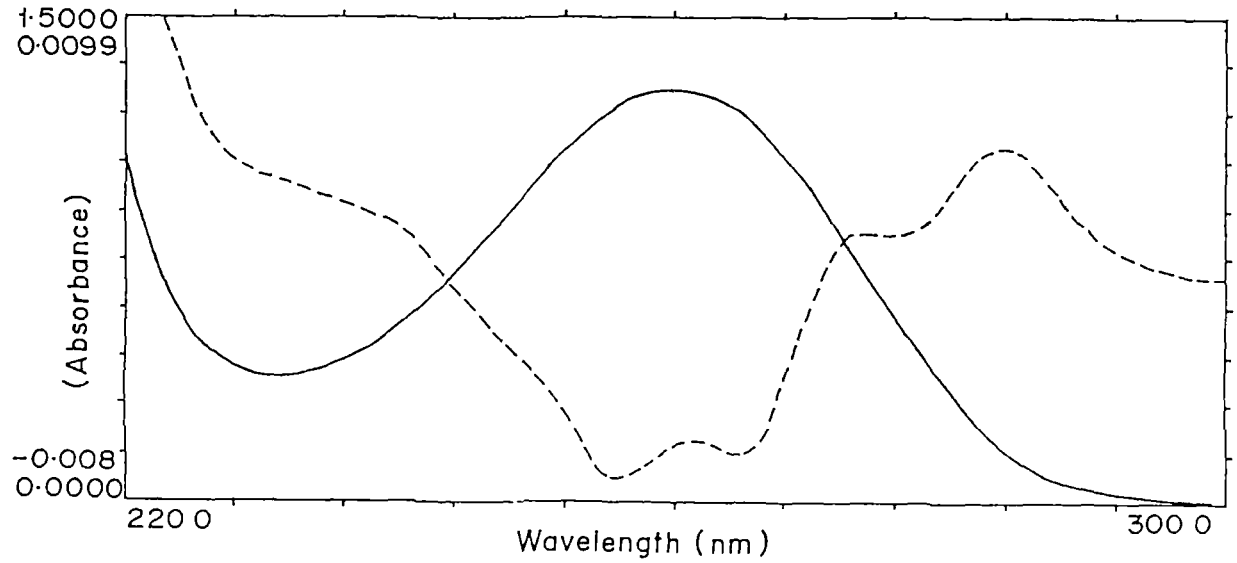


Fig. 3b. UV Spectra of Adenosine in 1,4 dioxan.

——, scan mode; ------, second derivative mode.

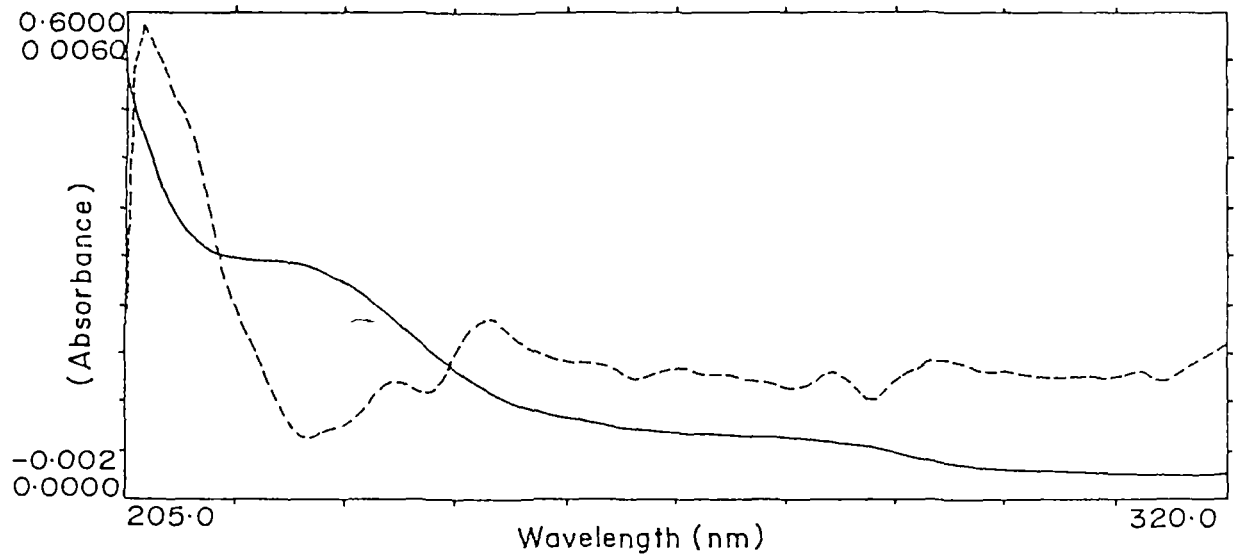


Fig. 3c. UV Spectra of Adenosine in n-Hexane.

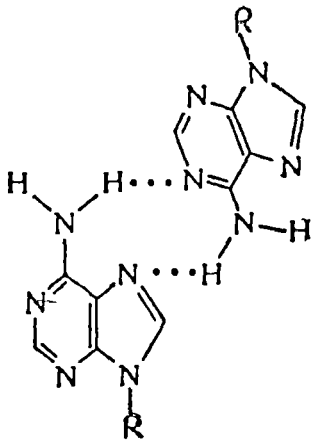
——— , scan mode ; ------ second derivative mode.

nm due to polarized short axis, while Stewart etal [10] has reversed the assignment and he has further remarked that 267 nm band could not be resolved from the 261 nm band in the case of adenosine. We , however, with the help of the second derivative plot could resolve the bands for adenosine too. It can thus in conclusion be said that the band at 261 and 267 is due to the two polarised axis of the amine form while the one at 278 is due to its corresponding tautomer imine. Since the concentration of the imine form is very low, bands due to its two polarised axis were not expected .

B. ASSOCIATION

In n-hexane only one band is observed at 225 nm which is due to its dimer as assigned for guanine and guanosine. In dichloromethane in the scan mode no well defined band was observed while the second derivative plot revealed two bands. The disappearance of band at ca. 260 nm was due to dimer formation, similar effect as that in the case of guanine and guanosine. To test for the extent of association that might have occured, to a solution of adenosine in dichloromethane incremental amounts of methanol was added and the band due to the monomeric adenosine was restored and the resultant spectra was identical to the spectra in pure methanol indicating a weak association. The association is

of the type;



ADENINE

Fig. 4a shows the uv spectra of adenine in solvents of different polarities. Only in highly polar and hydroxylic solvents, three bands were observed and their positions as calculated from their respective derivative plots are given in table no. IV . As a representative plot Fig.4b the spectra in scan mode and its second derivative mode in water is shown. It shows band I very well defined, while band II (shoulder) and band III are revealed only through their derivative plots. The tautomeric equilibrium between amino - imine group for adenine is reported . Out of these three bands, band I at ca. 260 nm and band II ca. 269 nm are due to long and short axis polarised bands and band III due to its corresponding imine tautomer. We would like to point out that for adenine, both short as well as long axis bands are better resolved.

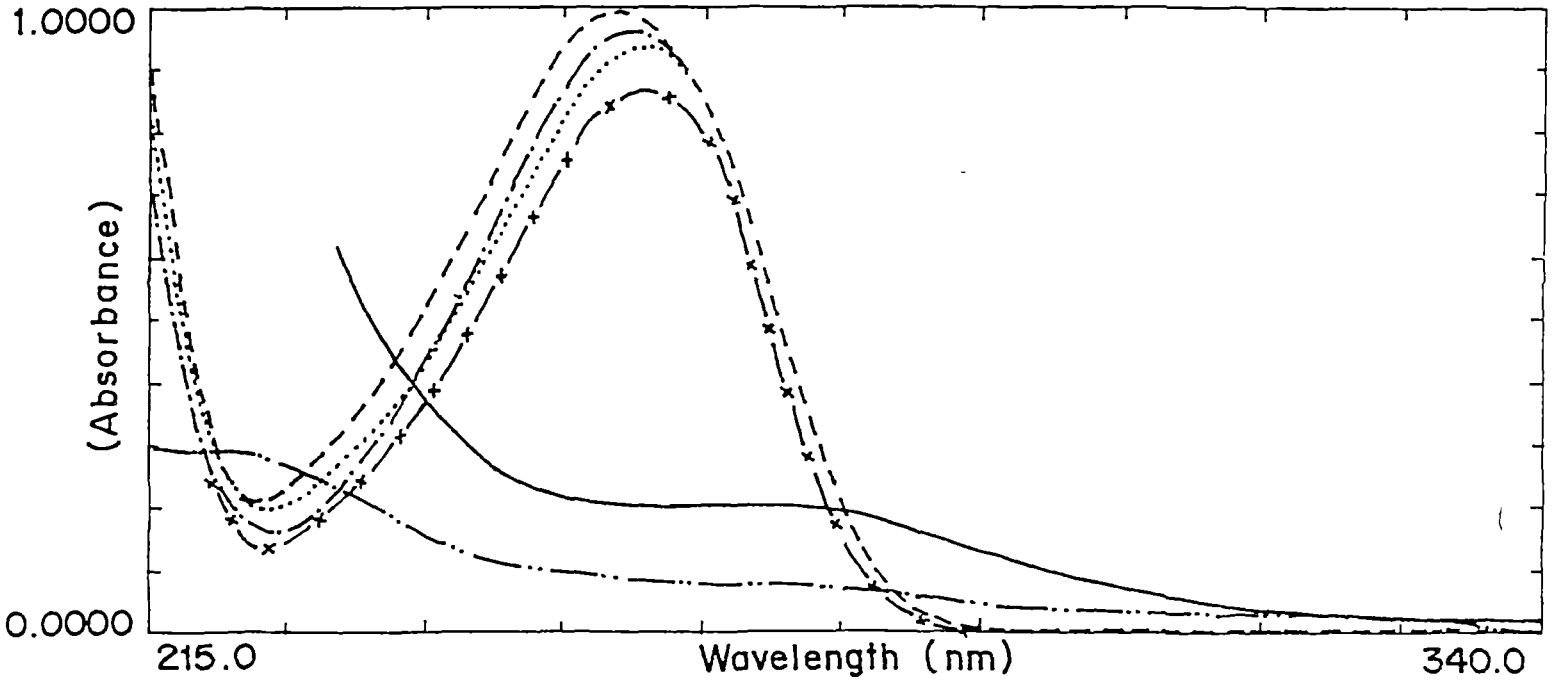


Fig. 4a. UV Spectra of Adenine in solvents of different polarities. ·····, water;- - - , methylcyanide; x—x , methanol — , dichloromethane;—·—, 1,4 dioxan;—···—, n-Hexane.

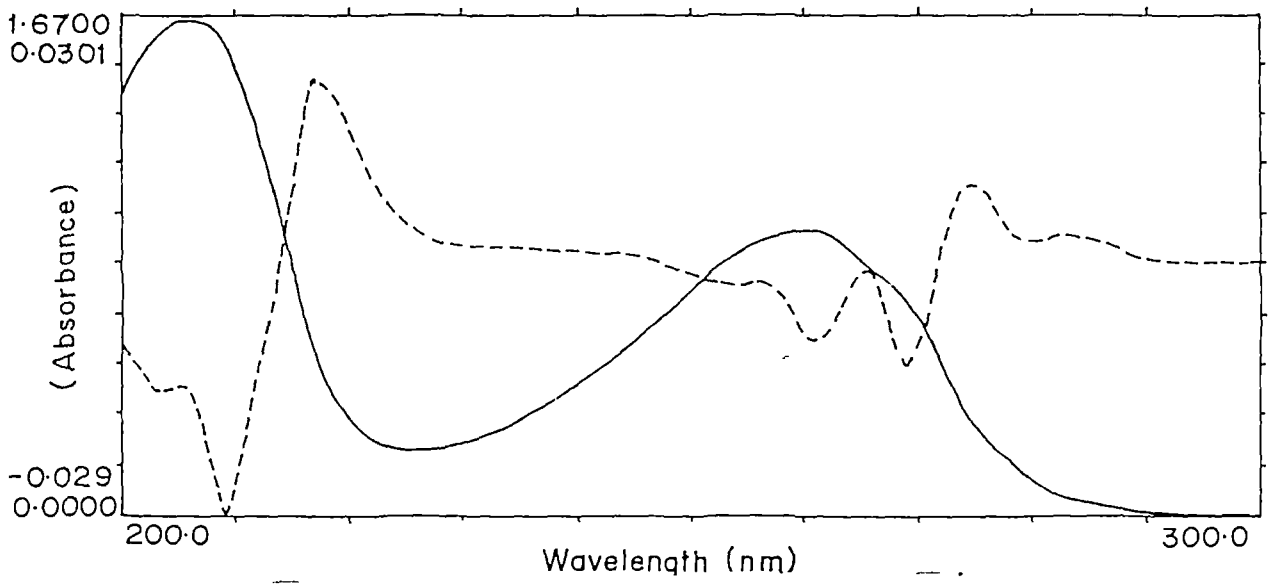


Fig. 4b. UV Spectra of Adenine in Water.

—— , scan mode; - - - - , second derivative mode.

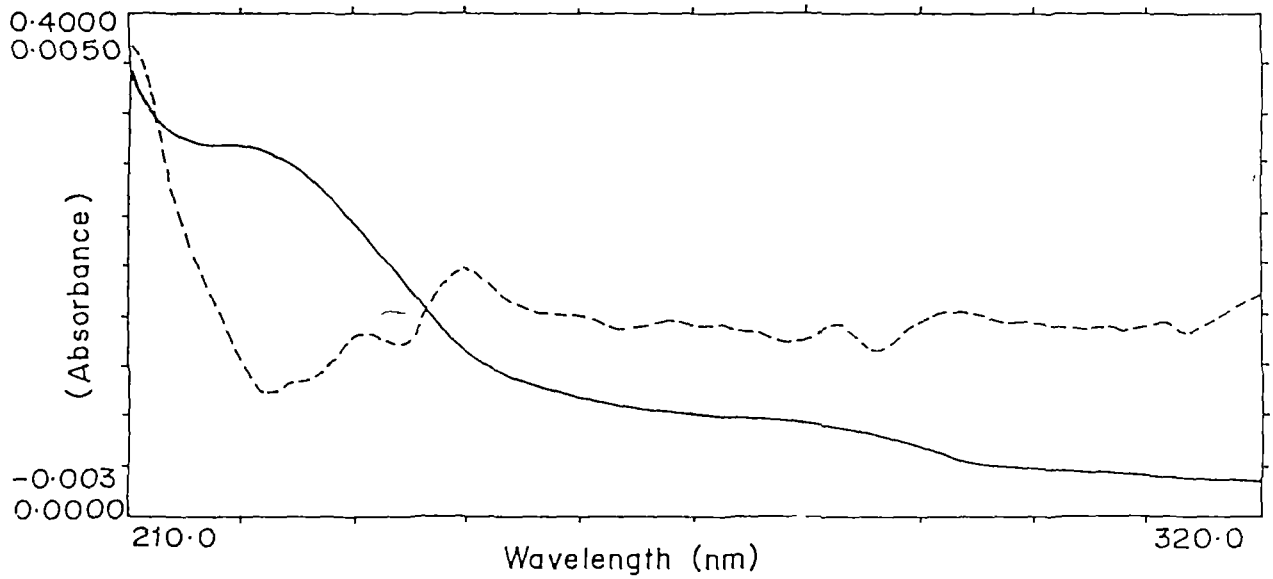


Fig. 4c. UV Spectra of Adenine in n-Hexane.

—— , scan mode ;- - - -, second derivative mode.

TABLE III. Band Positions of Adenosine in Solvents of Different Polarities (calculated from second derivative plot).

SOLVENT	BAND I	BAND II	BAND III	BAND IV	SPECIAL BAND
H_2O		261	265	278	
CH_3CN		256	266	277	
CH_3OH		260	267	276	
DIOXAN		260	268	279	
CH_2Cl_2				275	
n-HEX				277	226

TABLE IV. Band Positions of Adenine in Solvents of Different Polarities (calculated from second derivative plots).

SOLVENT	BAND I	BAND II	BAND III	BAND IV	SPECIAL BAND
H_2O	209	261	269	279(w)	
CH_3CN	210	261	269	279(w)	
CH_3OH		263	270	279(w)	
DIOXAN		259	270	279	
CH_2Cl_2			272	281	
n-HEX			274(w)	284	225

Note : Error limit is $\pm$ nm.

B. ASSOCIATION

The uv spectra of adenine in different solvents is shown in fig. 4a in the scan mode. The disappearance of the band at ca. 260 nm in dichloromethane is clearly observed and owes to the self-association. In n-hexane the dimeric band at ca. 225 nm was observed, fig. 4c

It deserves mention here, that shift of λ max's position with pH is ascribed to ionisation(Mason) as they have ionisable groups in them. The ionisable groups however have their pK values much lower than for the corresponding ionisation in the amino acids [11]. Because of these values there is no significant proportion of the bases in the charged form at pH 7. As all organic solvents used in the present investigation are at a pH ca 7, we rule out any possibility of ionisation.

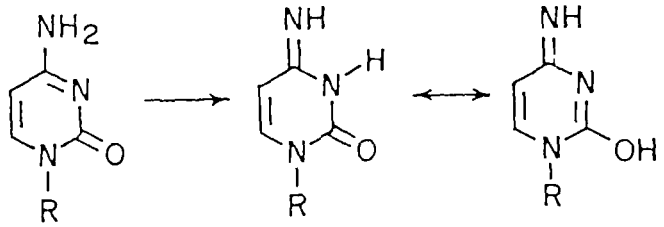
We, therefore, conclude that self association has definitely occured in non-polar solvents thus causing disappearance of the band due to monomeric molecules. In the absence of the hydrogen bond formation capability of the solvent, the molecules associate among themselves through hydrogen bonds.

CYTOSINE AND CYTIDINE:

A. CYTIDINE

The UV spectra of cytidine in solvents of different

polarities is shown in Fig.5a . The spectra are presented in the scan mode and band positions are calculated through their second derivative plots and given in Table V . A representative plot in methanol in scan mode along with its derivative mode is shown in fig. 5b. Scan mode shows only one well defined and highly intense band (band III) at ca. 275 nm with some indications for other bands, which were revealed through second derivative plot, band I at ca. 216 nm , band II at ca. 236 nm., another very weak ,band IV (shoulder) at ca. 284 nm . band I could be due to some high energy transitions eg. n--> sigma *. Band II as a pi-->pi* transition from chromophore NH2. As discussed in the case of guanine\adenine systems , the band at ca. 275 nm (very weak) has been assigned as due to imine tautomer (= NH) of the NH2 group, and a clear well defined band observed at ca. 265 nm was assigned due to carbonyl group.



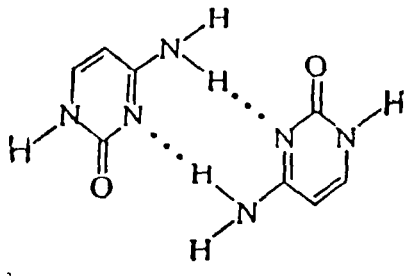
1 2 3

In this system we did not observe a band at ca. 265 nm, though the molecule contains a carbonyl chromophore. It seems quite obvious that the band due to carbonyl chromophore has red shifted to 275 nm. Therefore, the high

intensity at 275nm is in fact due to the overlapping of two bands as discussed above. This observation is further substantiated from the spectra in dichloromethane (from a substantially reduced intensity at 275 nm) , where the band due to carbonyl group has diminished because of dimerisation and band due to only imine chromophore is left. Band IV is due to enol tautomer.

B. ASSOCIATION

Fig.5c. shows the uv spectra in n-hexane in the scan mode and its second derivative plot. A band at ca. 225 nm is observed which has been assigned due to dimerisation, a consistent feature observed with all the nucleic acid bases studied so far. The structure of the possible dimer is as shown ;



b

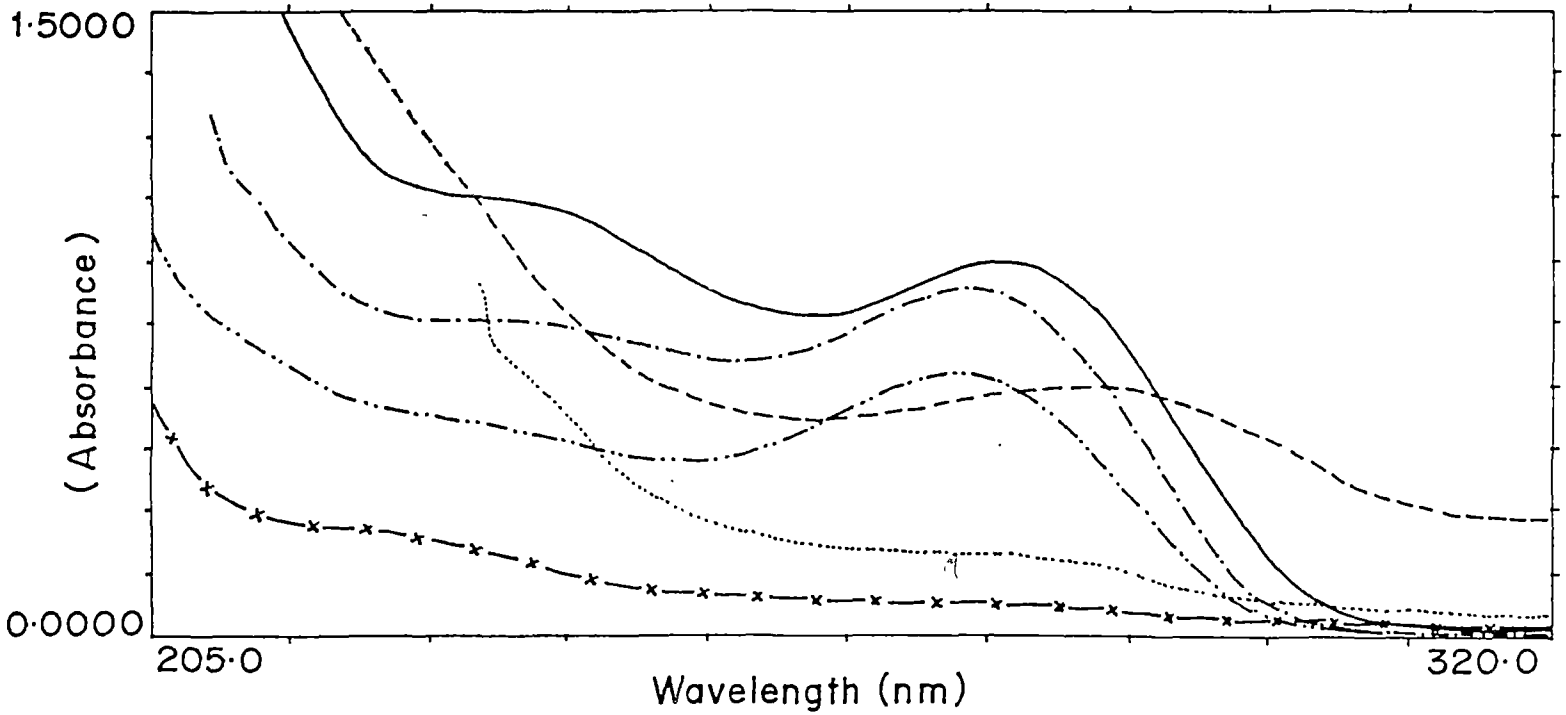


Fig. 5a. UV Spectra of Cytidine in solvents of different polarities. -·-·-·-, water; ——, methylcyanide;-·-, methanol; ·····, dichloromethane;----, 1,4,dioxan,x-x n-Hexane.

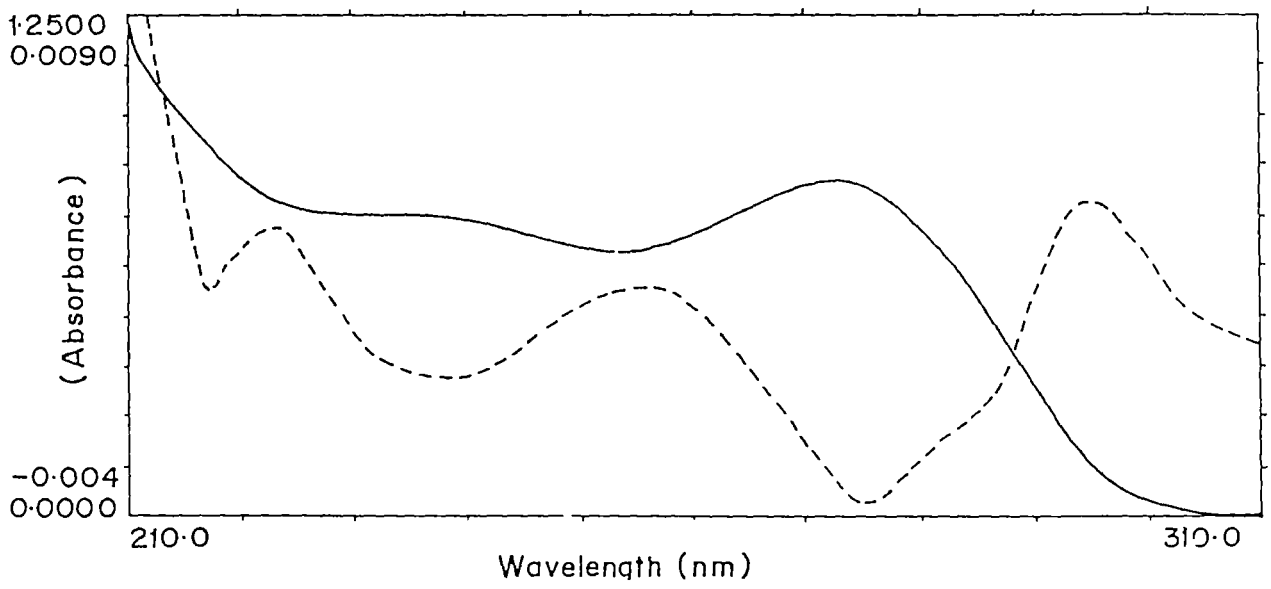


Fig. 5b. UV Spectra of Cytidine in Methanol.

—— , scan mode; - - - - - , second derivative mode.

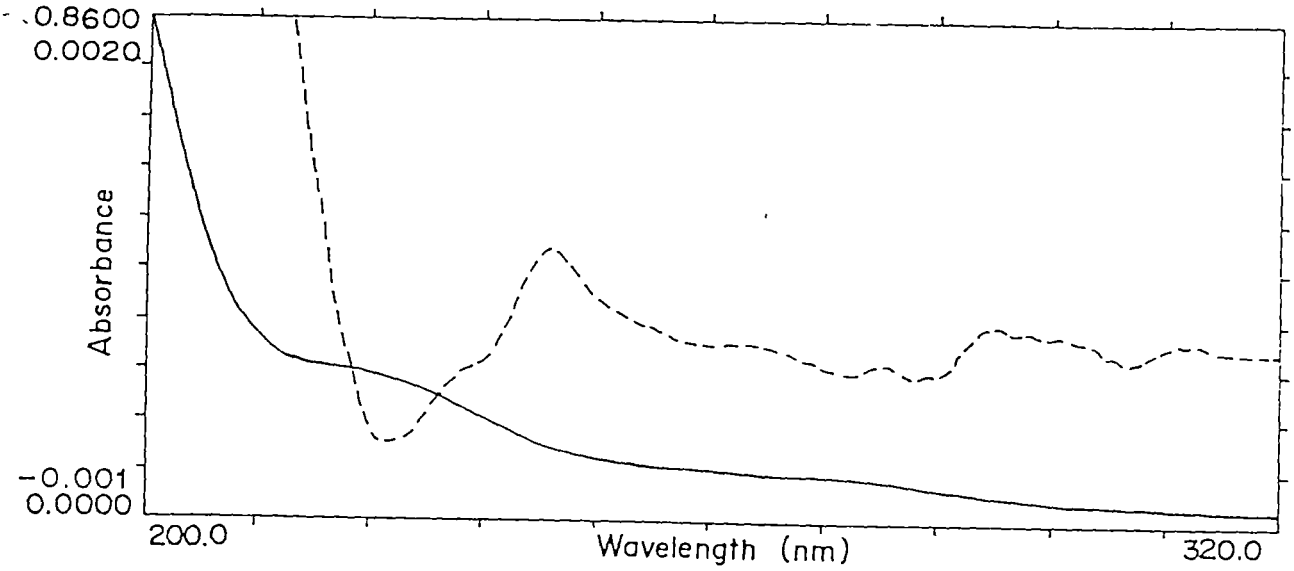


Fig. 5c. UV Spectra of Cytidine in n-Hexane.

—— , scan mode; - - - - - , second derivative mode.

CYTOSINE

The uv spectra of cytosine in solvents of different polarity in the scan mode is given in Fig.6a The positions of the bands have been determined from their respective derivative plots and are given in the table VI . The representative plot in methanol is given Fig.6b along with its second derivative plot. From the table it is apparent that four bands are observed. Band I ca. 215 nm , band II ca. 238 nm and band III ca. 270 nm and Band IV ca. 283 nm. Band I might be due to high energy transition whereas for Band II , 111 and 1V same assignments as made for cytidine holds good. In water band III is observed at ca. 269 nm where no self-association occurs . In dichloromethane and n-Hexane it is observed at ca. 275 nm (the band due to carbonyl group disappears due to self-association), and band only due to imine group stays back. This clearly implies that the band around 270 nm is actually a composite band formed by overlapping of two closely spaced bands due to carbonyl and imine group.

B. ASSOCIATION

Fig.6c shows the uv spectra in n-hexane in the scan mode along with its second derivative mode The spectral features are identical with cytidine, implying self-

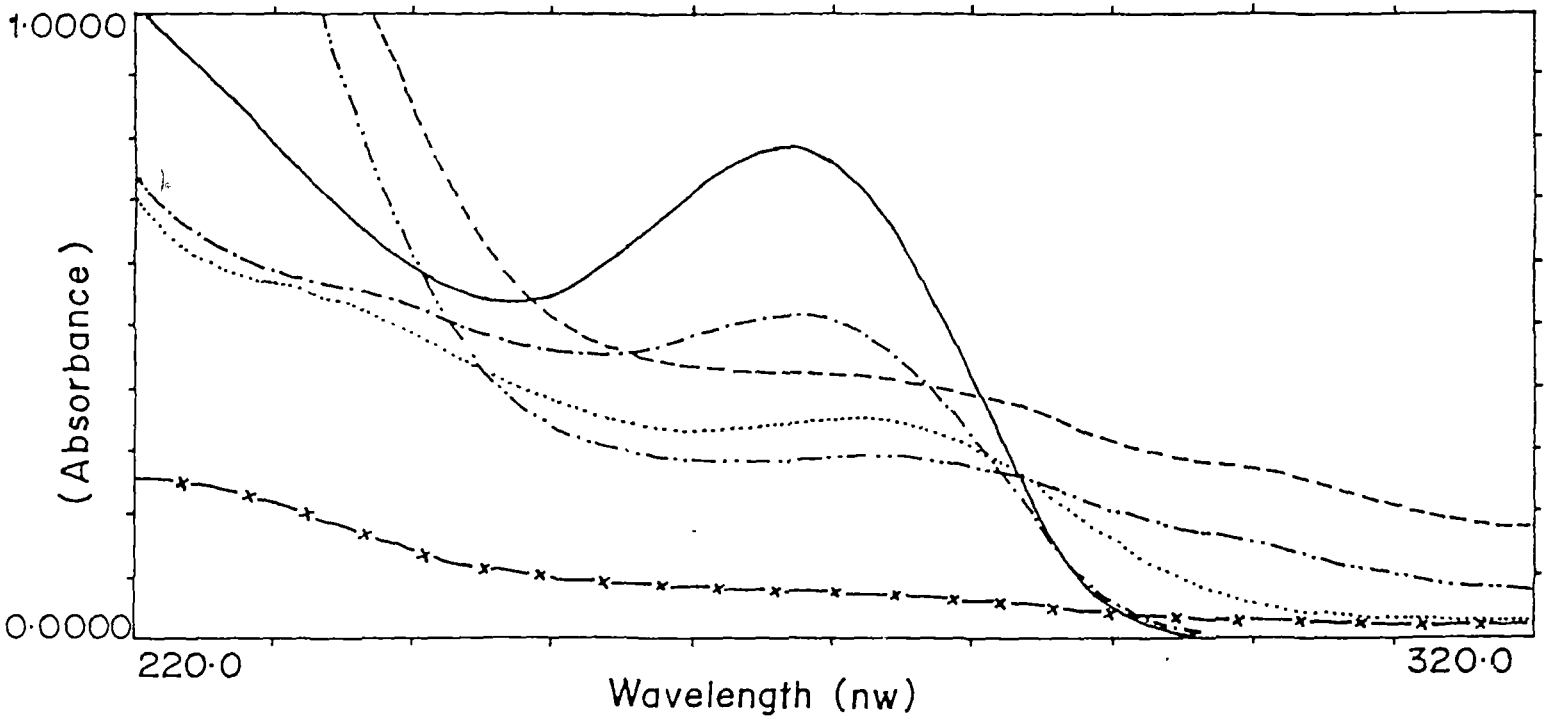


Fig. 6a. UV Spectra of Cytosine in solvents of different polarities. —— , water; . . . . , methylcyanide; -·-·-, methanol; —··— , dichloromethane; - - -, 1,4 dioxan; n-Hexane.

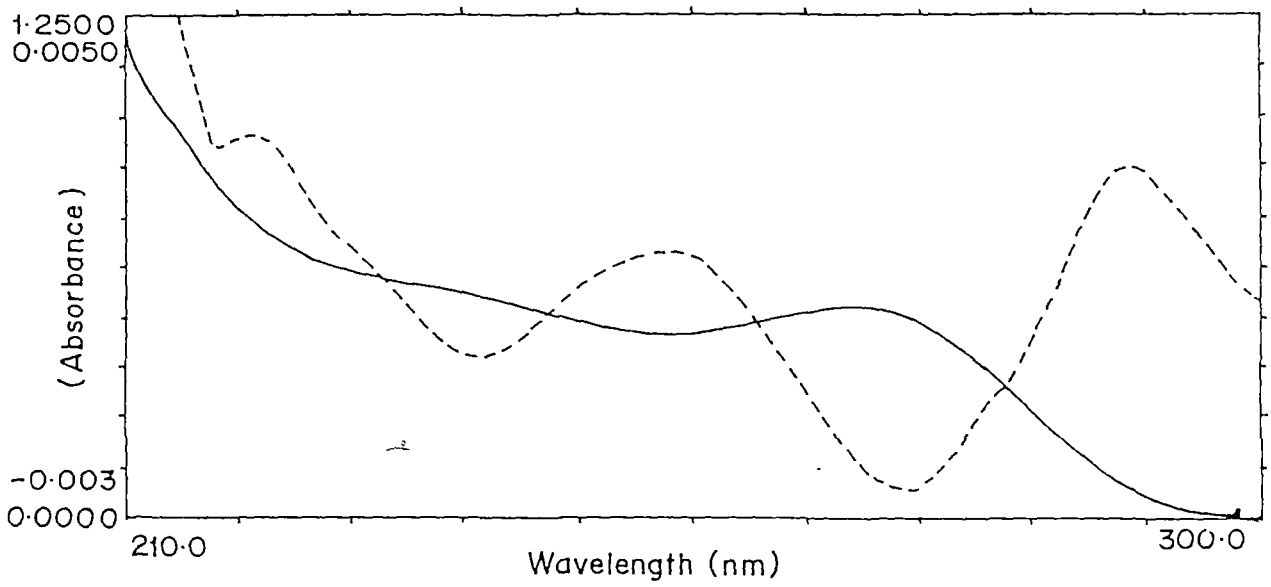


Fig. 6b. UV Spectra of Cytosine in methanol.

——— , scan mode; – – – – , second derivative mode.

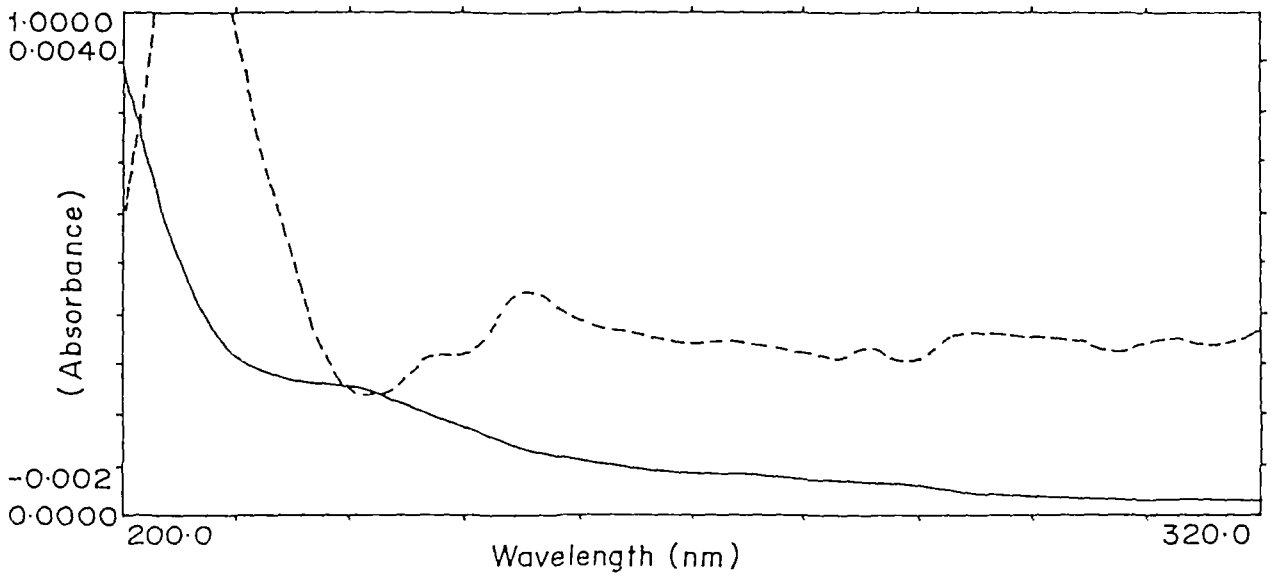


Fig. 6c. UV Spectra of Cytosine in n-Hexane.

——— , scan mode ; – – – –, second derivative mode.

TABLE V. Band Positions of Cytidine in Solvents of Different Polarities (calculated from second derivative plot).

SOLVENT	BAND I	BAND II	BAND III	BAND IV	SPECIAL BAND
H_2O	216	232	274	285(w)	
CH_3CN	216	236	275	286	
CH_3OH	216	238	278	284	
DIOXAN		231	273	284	
CH_2Cl_2		238	275	285	
n-HEX		236	274	283	225

TABLE VI. Band Positions of Cytosine in Solvents of Different Polarities (calculated from the second derivative plot).

SOLVENT	BAND I	BAND II	BAND III	BAND IV	SPECIAL BAND
H_2O	214	228	269	283	
CH_3CN	213	234	272	283	
CH_3OH	216	238	271	282	
DIOXAN		234	273	283	
CH_2Cl_2			275	284	
n-HEX		236	274	284	225

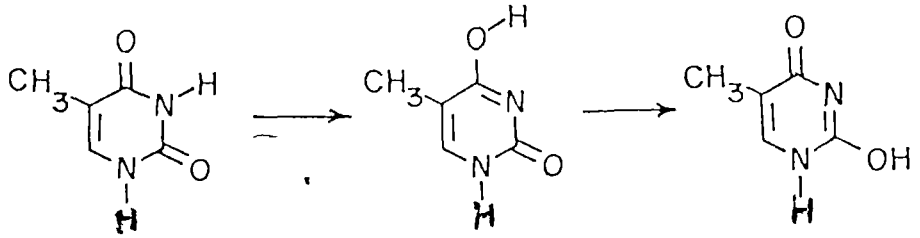
Note : Error limit is $\pm$ 1 nm.

association has occured. Since the bands responsible for monomers (in low intensity) are still present in dichloromethane and n-hexane it implies that in this system forces responsible for self-association are relatively weaker. This conclusion is in conformity with the reported information [12] that association with pyrimidine class of bases is weaker.

THYMINE AND THYMIDINE:

A. THYMIDINE

Fig.7a shows the UV spectra of thymidine in different solvents in the scan mode. In all two bands one at ca. 214 nm and other at ca. 268 nm is observed. The λ max values of the bands in different solvents are given in table VII . The molecule consists of carbonyl groups as the major chromophore and tautomerism occurs as shown.



The high intensity and position of the band points out that it is a pi--pi* transition. The band is well defined in all the solvents as respective derivative plots could not resolve into different bands in close proximity. Fig. 7b

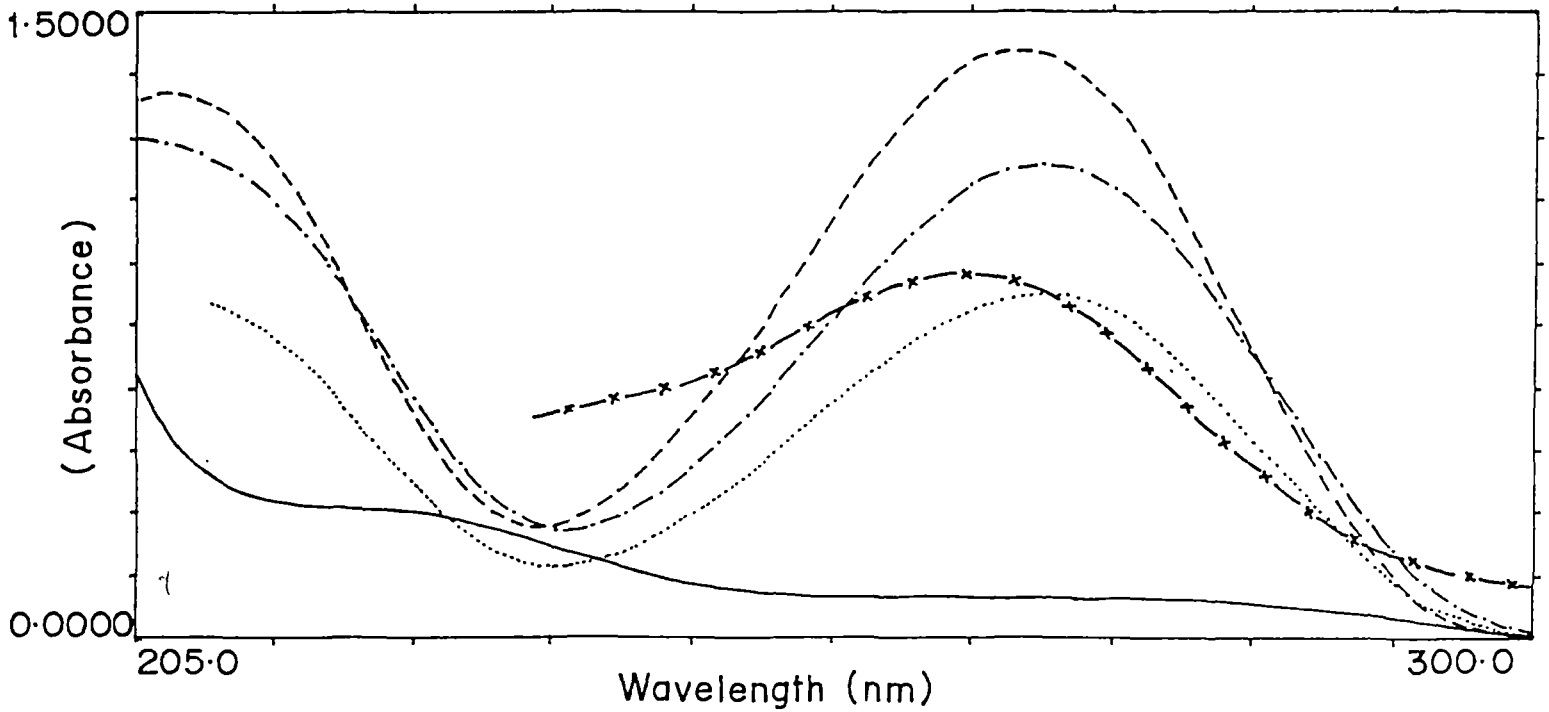


Fig. 7a. UV Spectra of Thymidine in solvents of different polarities. –·–·– ,water;– – –,methylcyanide;····, methanol; x–x–x–x, dichloromethane; , 1,4 dioxan; , n-Hexane.

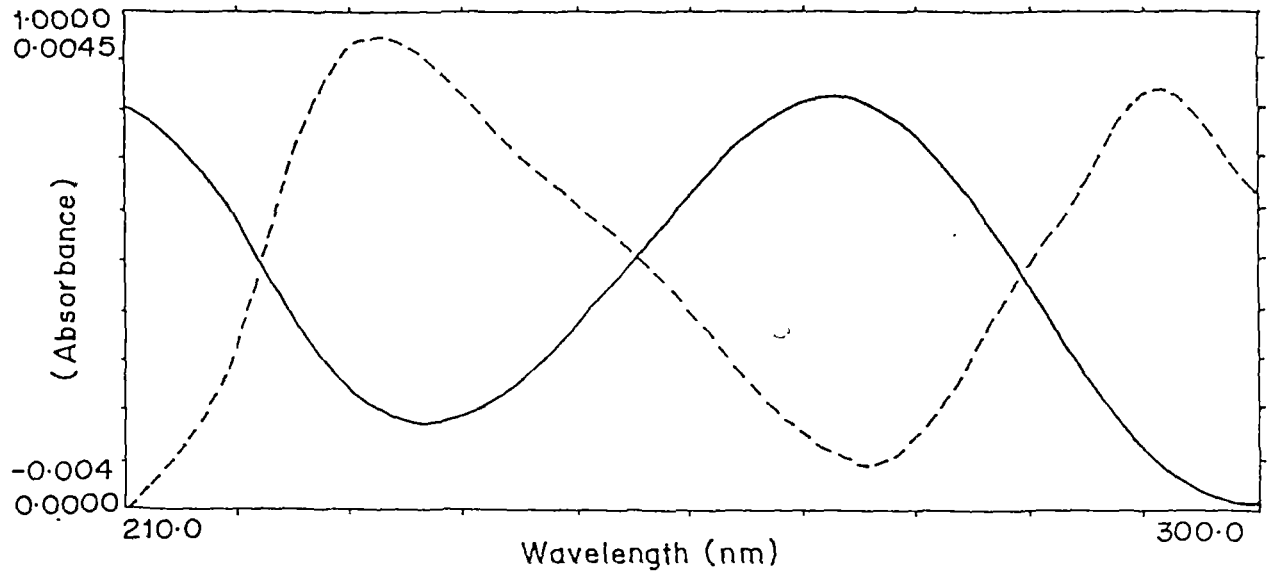


Fig. 7b. UV Spectra of Thymidine in Methanol.

—— , scan mode;---- , second derivative mode.

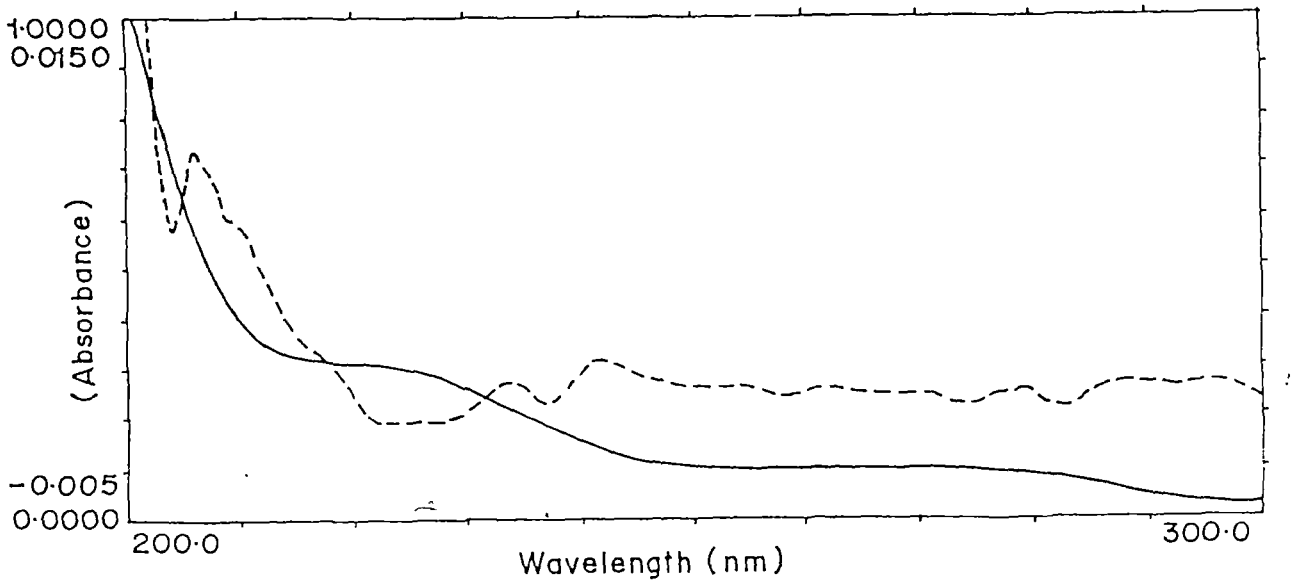


Fig. 7c. UV Spectra of Thymidine in n-Hexane.

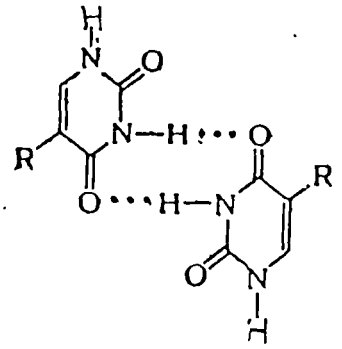
—— , scan mode; ---- , second derivative mode.

shows the UV spectra in methanol in scan and second derivative mode respectively. Inspite of our best efforts we could not find any band in the region of 280 nm (except in non-polar solvents like hexane) where we have unmistakably observed bands due to tautomeric forms even in polar and hydroxylic solvents for the bases discussed so far. This molecule contains two carbonyl groups and tautomerism is the characteristic property of carbonyl group provided the movement of proton is facile and the resulting tautomeric structures are stable. Here, NH proton is only one bond distance away from both of the carbonyl groups. It appears that the proton is not mobile, in other words thermodynamically the tautomeric structures are less favoured and requires stronger solvent interaction to bring about such a change. This molecule does not have amino group (NH2) in conjugation with carbonyl group and thus tautomerism is less facile. Hence absence of other bands corresponding to tautomeric structures in polar and hydroxylic solvents. Thus the most stable structure for thymine in the solution state would be the di-keto form.

B. ASSOCIATION

The fig.7c shows that in dichloromethane too ,the band is

well defined unlike the other bases. This remarkable observation seem to suggest that either no self-association has taken place or the extent of association is extremely weak which is in contrast to the preceding results. However, in n-hexane the solvent with the lowest polarity , the usual band at 225 nm due to dimerisation is clearly observed, according to structures shown below ;



along with a weak band at 283 nm which is due to the enolic form. Another band at 237 nm have been observed which we postulate as due to possible higher order associates for which the possibility exists because of the presence of two carbonyl and two NH groups. If one carbonyl and NH are involved in association with another thymidine molecule still one carbonyl and NH are available for association with a third molecule, and so on.

THYMINE

The uv spectra of thymine in different solvents in the scan

mode is given in Fig.8a and the band positions as calculated from their second derivative plots are given in table VIII . Here, too we observed two bands ; band I at ca. 214 nm and band II at ca. 265 nm, Fig 8b. The position and intensity of the second band shows that it is a pi--pi* transition. In dichloromethane, the band at 264 nm is still retained (implying weaker association) along with a band at 282 nm due to the enolic form. In n-hexane, too we observed the enolic band at 283 nm. The overall behaviour of the molecule is expected to be similar as observed with thymine and it was observed in actuality. The predominant structure even for thymine would be the di-keto structure.

B ASSOCIATION

We did not observe any self-association in dichloromethane for this molecule just as we did not observe with thymidine. However, in n-hexane the typical band at 225 nm was observed along with another band at ca 237 nm (fig 8c), the origin of which has been discussed for thymidine.

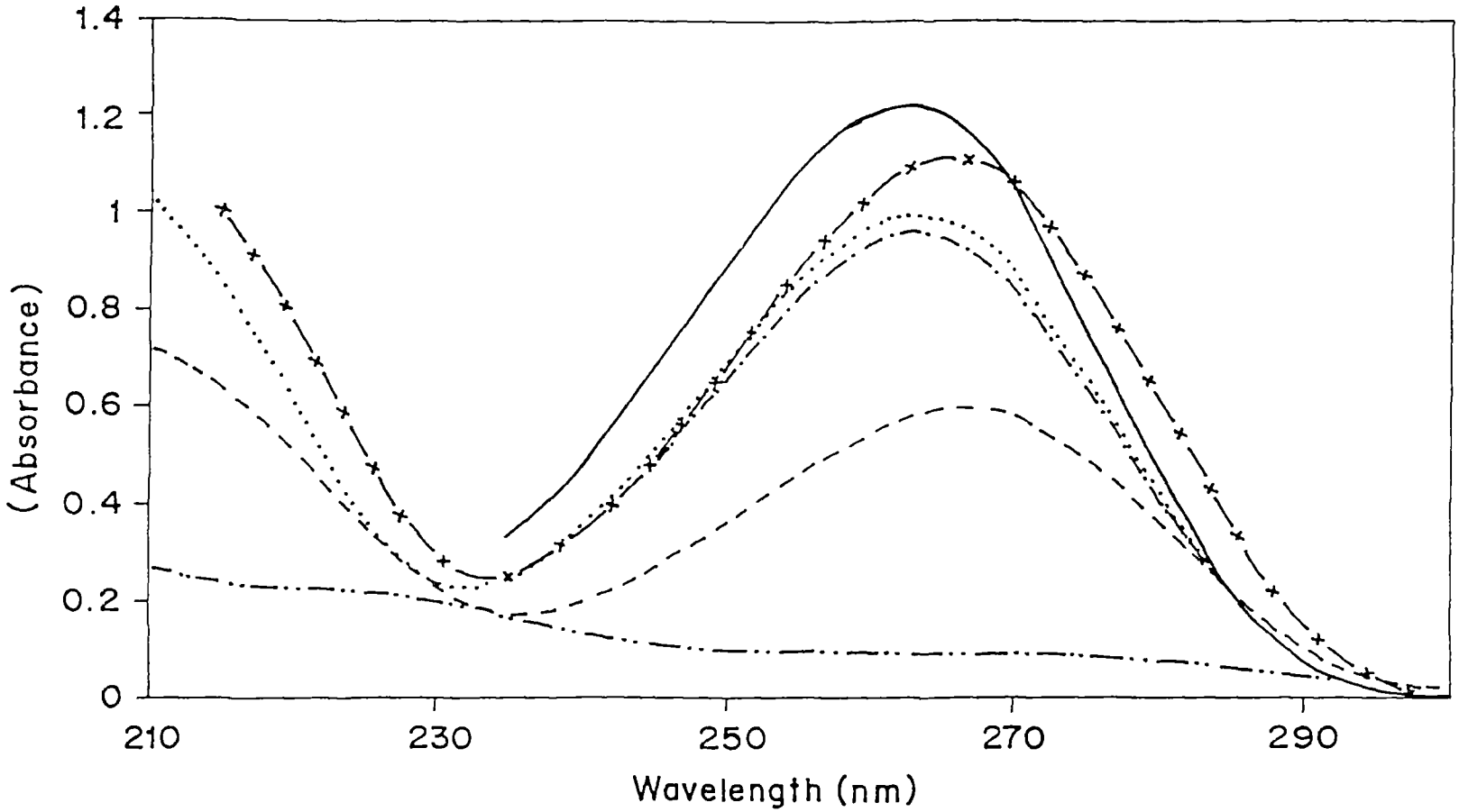


Fig. 8a. UV Spectra of Thymine in solvents of different polarities. ---, water; ....., methylcyanide; x-x-x-x, methanol ——, dichloromethane; -.-.-, 1,4 dioxan, -..-, n-Hexane.

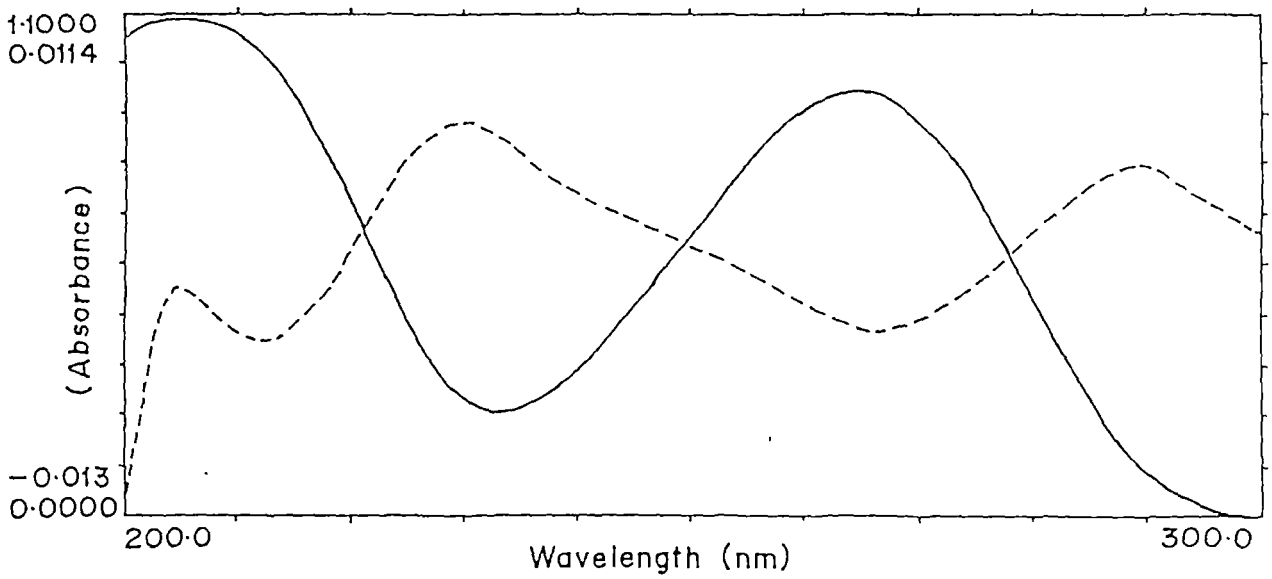


Fig. 8b. UV Spectra of Thymine in Water.

——— , scan mode; - - - - , second derivative mode.

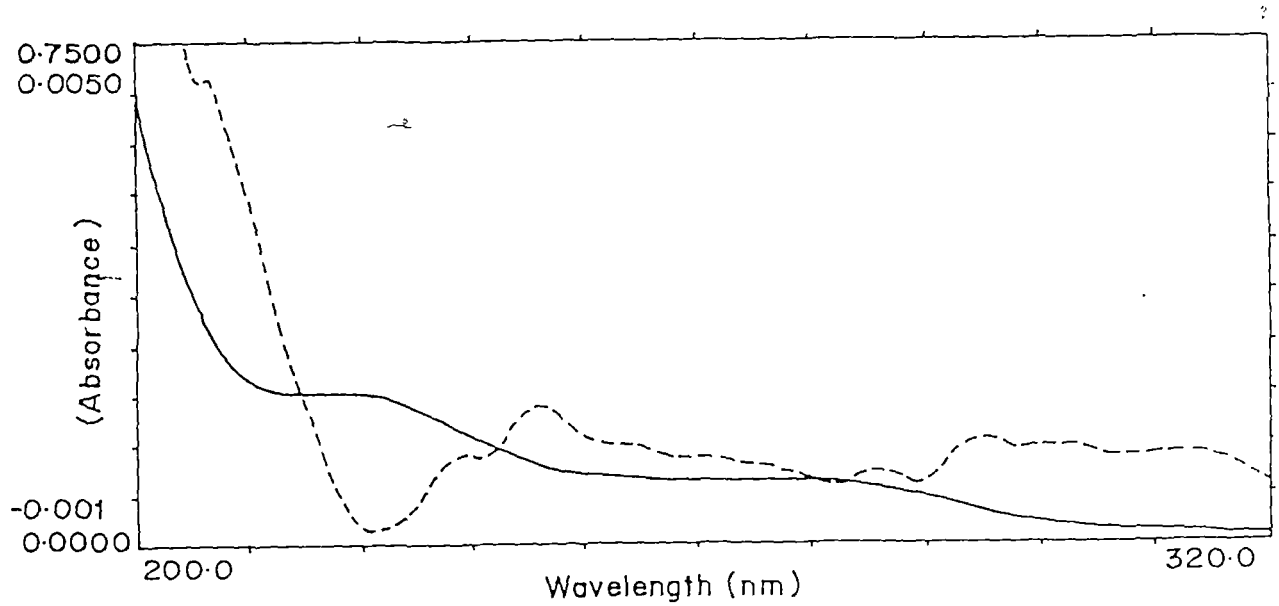


Fig. 8c. UV Spectra of Thymine in n-Hexane.

——— , scan mode; — — — , second derivative mode.

TABLE VII. Band Positions of Thymidine in Solvents of Different Polarities (calculated from the second derivative plots).

SOLVENTS	BAND I	BAND II	BAND III	BAND IV	SPECIAL BAND
H_2O	214		268		
CH_3CN	213		269		
CH_3OH	214		269		
DIOXAN			269		
CH_2Cl_2		237		283	
n-HEX		237		283	225

TABLE VIII. Band Positions of Thymine in Solvents of Different Polarities (calculated from the second derivative plots).

SOLVENT	BAND I	BAND II	BAND III	BAND IV	SPECIAL BAND
H_2O	214		265		
CH_3CN	214		265		
CH_3OH	214		265		
DIOXAN			265		
CH_2Cl_2		238	264	282	
n-HEX		237		283	225

Note : Error limit is $\pm$ 1 nm.

URIDINE

Fig. 9a shows the uv spectra of uridine in different solvents in their scan mode. Only two bands ; one at ca. 214 nm and the other at ca. 260 nm is observed. Their positions calculated from their second derivative plots are given in table IX . The band of interest is at ca. 260 nm. Fig.9c shows the spectra in methanol in the scan and second derivative mode respectively. The intensity and position of the band clearly suggests that it is pi--pi* transition from the carbonyl chromophore. In the case of water and acetonitrile, fig. 9c the band at ca. 260 nm splits up into two closely spaced bands ; one at 260 nm and the other at ca. 266 nm. We can not put forward any satisfactory explanation for this observation.

B. ASSOCIATION.

The uv spectra in dichloromethane is in contrast to that of thymidine , the band due to monomer at ca 260 nm diminishes significantly (while in thymidine it is unaffected) , though heterocyclic base units are structurally similar to both compounds. The difference lies in the substitution of

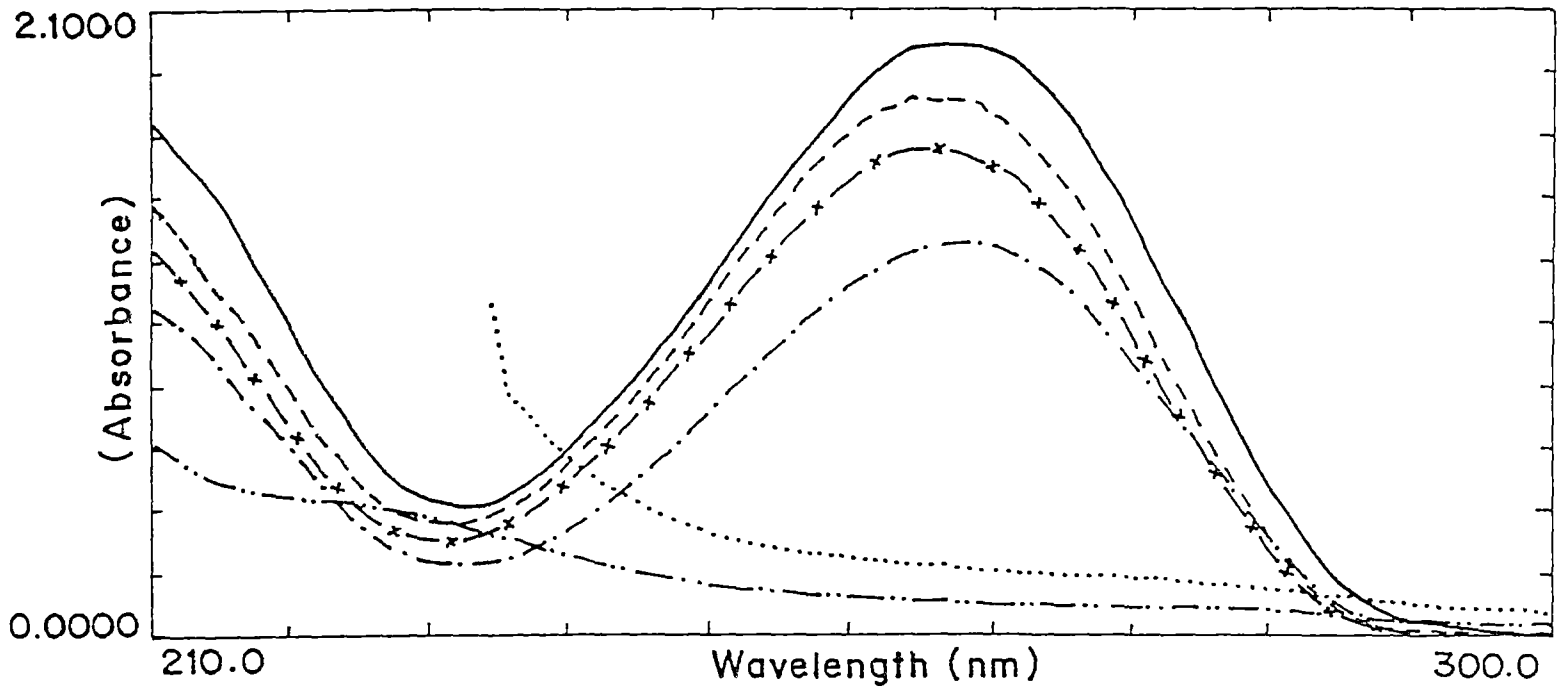


Fig. 9a. UV Spectra of Uridine in solvents of different polarities. —— , water; x—x—, methylcyanide; - - - , methanol; ....., dichloromethane; -.-.-, 1,4 dioxan; -•-•- , n-Hexane.

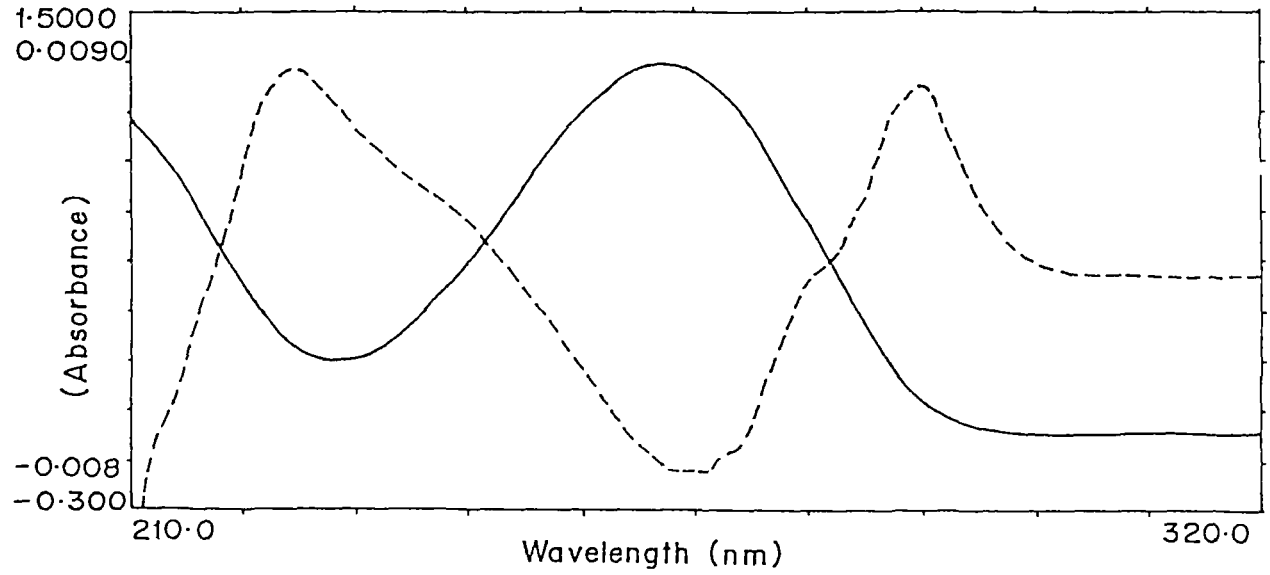


Fig. 9b. UV Spectra of Uridine in Methanol.

——— , scan mode; - - - - - , second derivative mode.

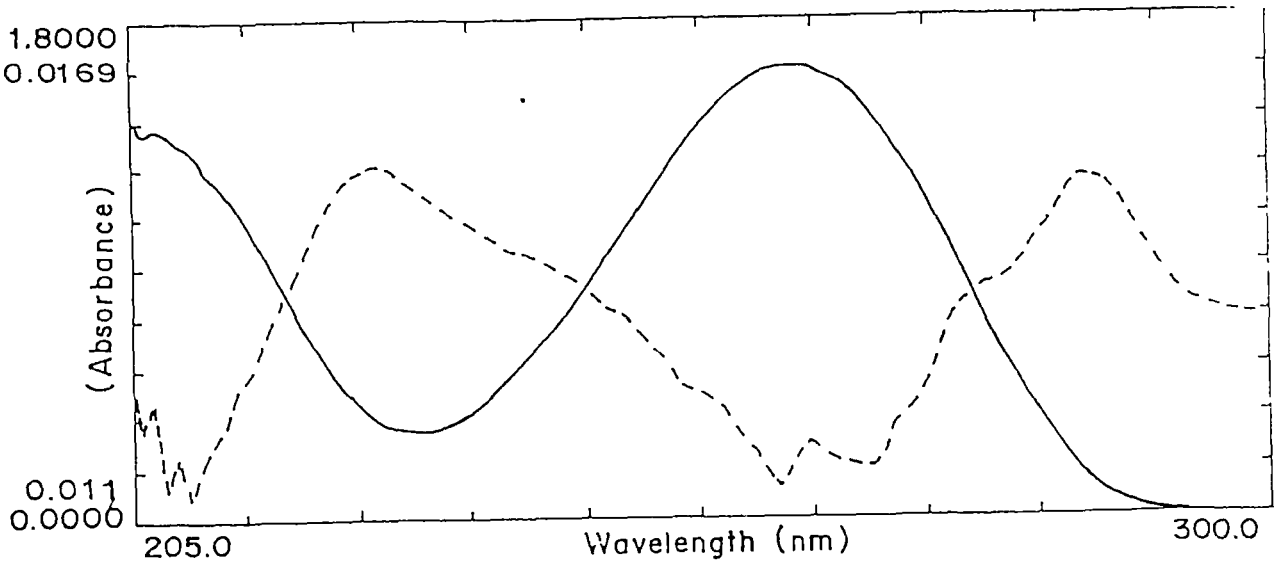


Fig. 9c. UV Spectra of Uridine in Acetonitrile

———, scan mode; - - - - -, second derivative mode.

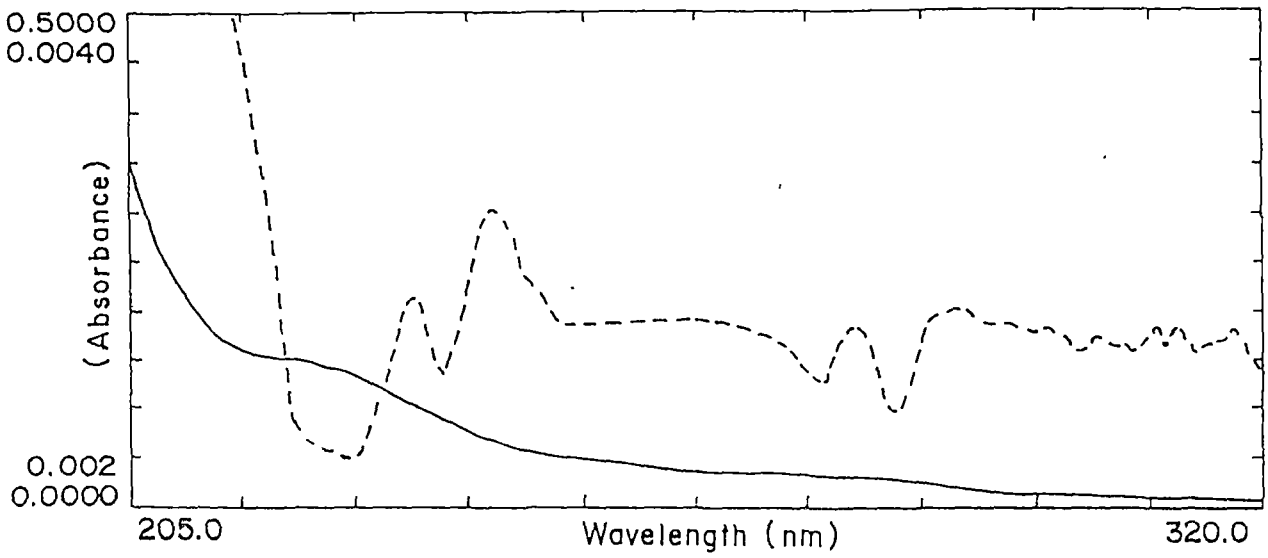
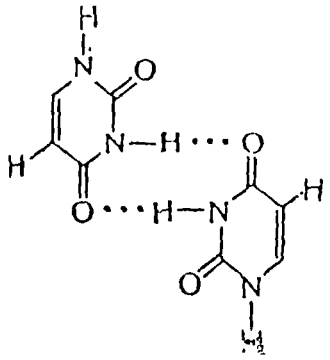


Fig. 9d. UV Spectra of Uridine in n-Hexane.

——— , scan mode; – – – – , second derivative mode.

different sugar moiety , and thymidine consists of a methyl group at C (5). In n-hexane, fig.9c, the spectral features with a band at ca. 225 nm are consistent with the observation and conclusions drawn so far. Another band observed at ca 238 nm owes same explanation as discussed for thymidine. The structure for the dimer is;



URACIL

Fig.10a shows the uv spectra of uracil in solvents of different polarity and the band positions are given in Table X . In all only two bands ; one at ca. 204 nm and other at ca. 260 nm are observed. The band of interest at 260 nm is a pi-->pi* transition. Again search in the region of 280 nm did not show any clear band , suggesting an extremely weak tautomerism. Fig 10 b and fig 10 c shows the spectra in methanol and n-hexane in the scan and second derivative mode respectively.

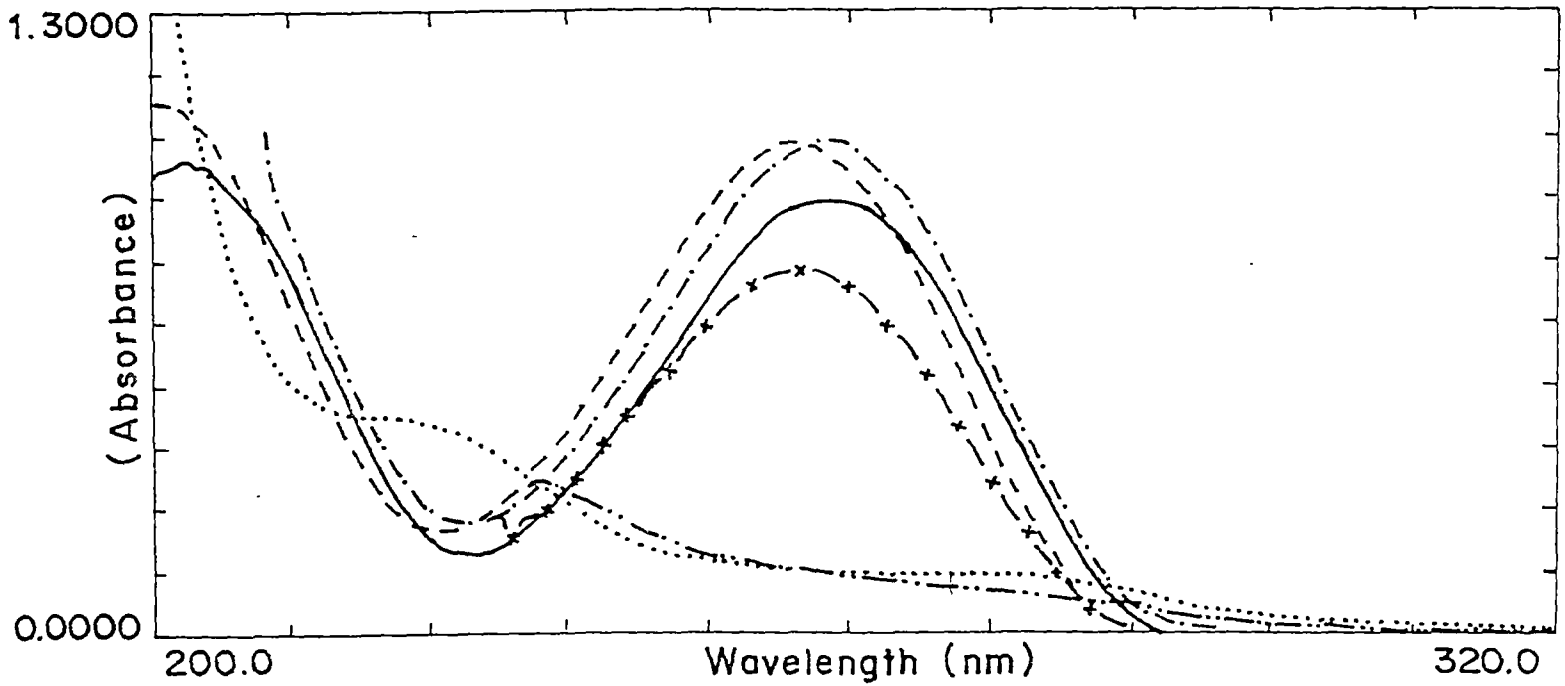


Fig. 10a. UV Spectra of Uracil in solvents of different polarities. —— , water; – – – –, methylcyanide; —·—·—, methanol —··—··—, dichloromethane; x–x–x, 1,4 dioxan; · · · ·, n-Hexane.

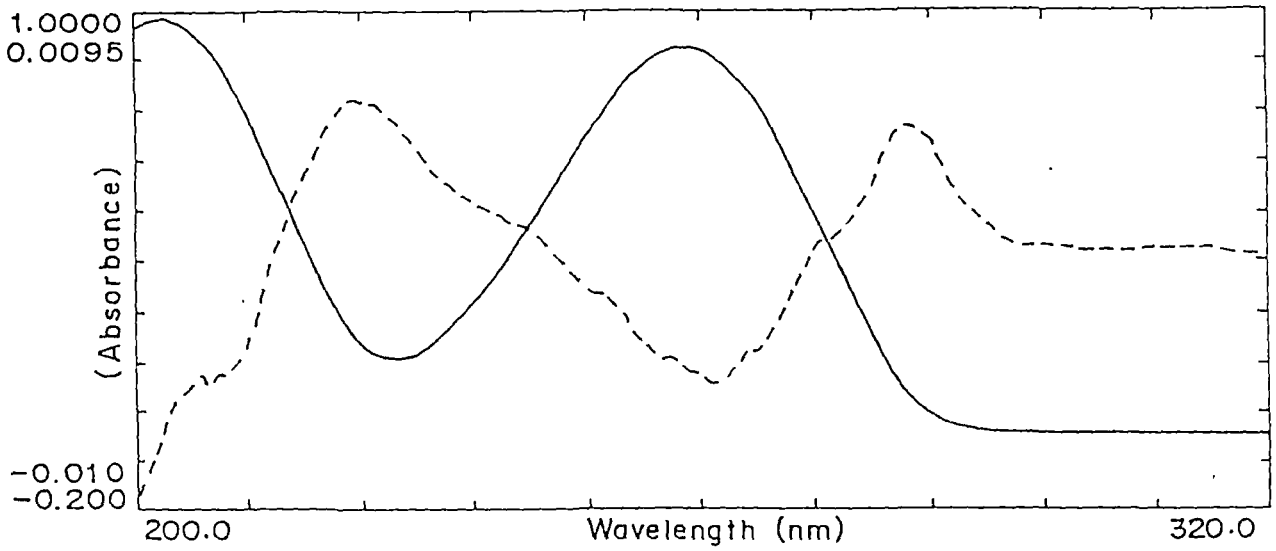


Fig. 10b. UV Spectra of Uracil in water

—— , scan mode; – – – – – , second derivative mode.

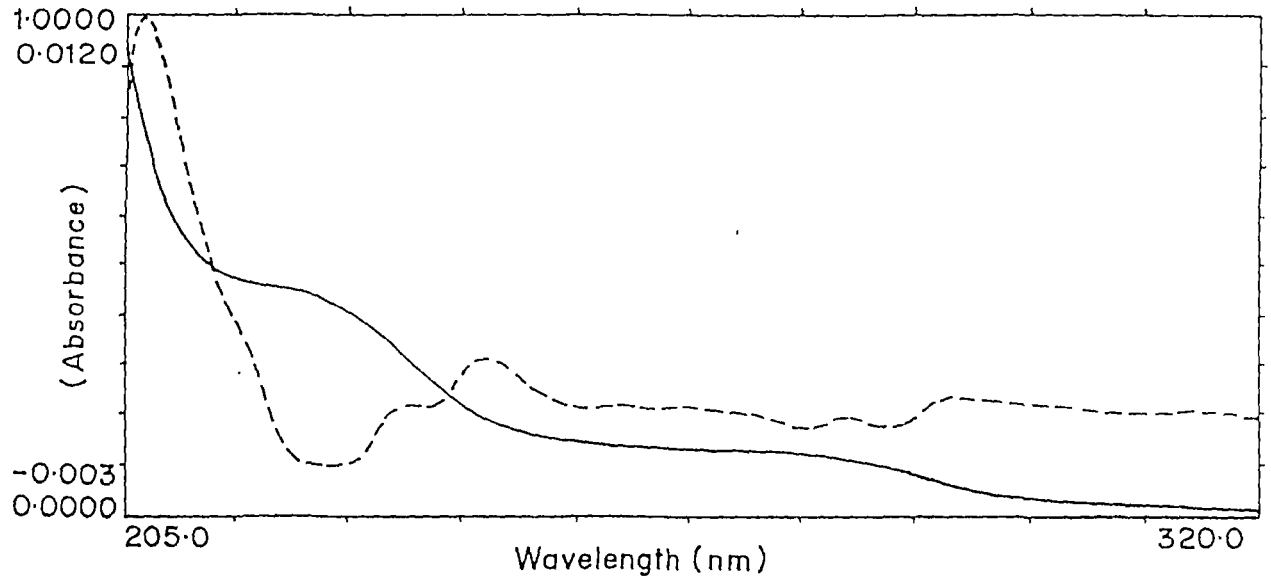


Fig. 10c. UV Spectra of Uracil in n-Hexane.

—— , scan mode; – – – – – , second derivative mode.

TABLE IX. Band Positions of Uridine in Solvents of Different Polarities (calculated from the second derivative plots).

SOLVENT	BAND I	BAND II	BAND III	SPECIAL BAND
H_2O	214	258		
CH_3CN		260		
CH_3OH		262		
DIOXAN		261		
CH_2Cl_2		261	283	
n-HEX		261	283	225

TABLE X. Band Positions of Uracil in Solvents of Different Polarities (calculated from the second derivative plots).

SOLVENT	BAND I	BAND II	SPECIAL BAND
H_2O	261		
CH_3CN	259		
CH_3OH	260		
DIOXAN	259		
CH_2Cl_2		283	
n-HEX	261(w)		225

Note : Error limit is $\pm$ 1 nm.

B. ASSOCIATION

Similar to the case of uridine, in uracil too we observed association in dichloromethane. Uracil being structurally similar to thymine, a similar behaviour was expected for both the bases. However the dimunition of the monomeric band in dichloromethane for uracil implies that the associative forces for this are stronger than for the case of thymine. In n-hexane , behaviour was similar as that of the other bases and the dimeric band observed at 225nm. The band at 237 nm owes to the same explanation as for thymine.

It was pointed out by by cavaliery etal [13] that the presence of either a 2-amino or 2-hydroxy group coincides with the appearance of a second maxima. In our experiments too, only in the case of guanine and cytosine both possessing a 2-amino or 2-hydroxy group did we observe two major absorption bands which could be resolved further..In the case of guanine as seen, self association is evidently stronger than for the other bases,immediately followed by adenine.In the case of the pyrimidine bases, self association is weaker as compared to the purine bases.Probably because of the absence of the imidazole ring there is a reduced electron density of the molecule.

BASE PAIRING :

As seen from the results of the preceding section self association of some of the bases occurs to a significant

extent in non-polar solvents viz dichloromethane and n - hexane. The use of these solvents to study base pairing was avoided. We also tried with hydroxylic and polar solvents viz alcohols but no significant association (base pairing) was observed probably some base units are engaged in hydrogen bonding with the solvent resulting in a very small concentration of the associates which may be below the detection limit. Therefore, the use of highly polar and hydroxylic solvents was avoided. We thus chose 1,4 Dioxan as the solvent as it has low polarity and relatively weaker hydrogen bonding ability, self association is also low as found from the results presented in the previous section. In 1,4 dioxan when we carried out this experiment,association between the naturally occuring pairs was observed. The two naturally occuring base pairs are ;

1. Thymine and Adenine
2. Guanine and Cytosine.

Pairing in the heterocylic acid bases was not studied because the nitrogen atom through which sugar is attached are free to form other hydrogen bonds and that might lead to structures very different from the particular hydrogen - bonded structure that are present in DNA. Therefore, pairing in the corresponding nucleosides were studied. For the first pair, the spectra of both the individual nucleosides and their mixtures in different conc. ratios are shown, fig. 11.

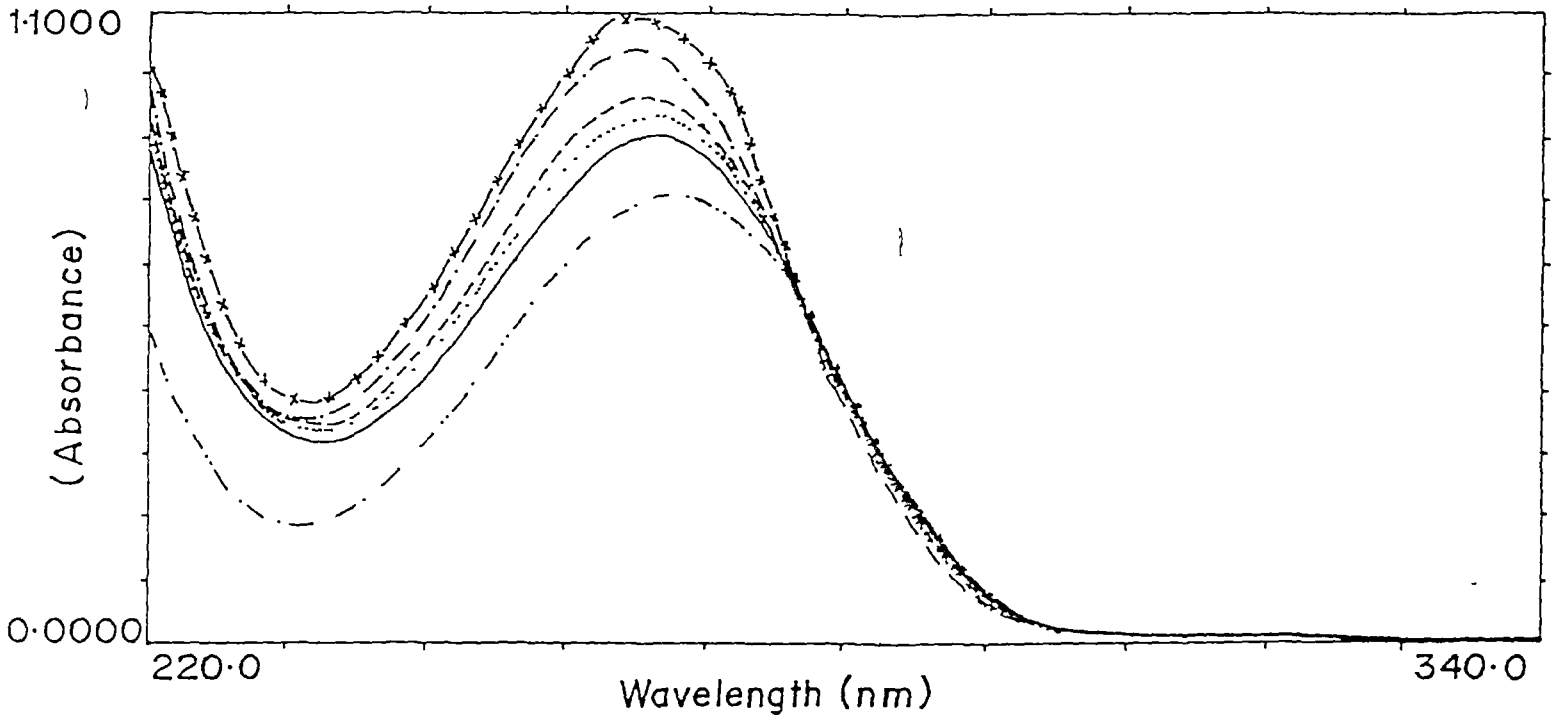


Fig. 11. UV Spectra of Adenosine and Thymidine in 1,4 Dioxan in different relative ratios of concentration.

—x—x—UV spectra of Adenosine , 7.5×10^{-5} M.

—··—··—UV spectra of Thymidine , 7.5×10^{-5} M.

UV spectra of Adenosine and Thymidine in—·—·—1:1 ratio,

---- 1:2 ratio, ... 1:3 ratio, ____ 1:4 ratio.

The spectra shows a very well defined isosbestic point. As the ratio of thymine to adenosine is increased , no shift in the position of the isosbestic point is observed i.e., for all ratios of thymidine to adenosine concentrations the spectra intersect through a fine point in the spectrum. The presence of a sharp isosbestic point is indicative of the presence of a single equilibrium between the free adenosine, thymine and the " complex " (an interaction products i.e., through H-bonds of the base pairs). This indicates that the stoichiometry of the complex between thymine and adenosine is probably 1 : 1 as has also been pointed out by others through different method using much higher concentration of the substrates.

However, for the other pair shown in Fig.12 no clear isosbestic point was observed. As the ratio of cytidine to guanosine was increased, a slight shift of the isosbestic point was evident. A band at ca 285 nm was also observed. This suggests that more than one equilibrium probably exists for this pair in this solvent, and as concentration of one is increased over the other, some other complex of a different stoichiometry may be formed. Hence for this pair too, the principal ratio of pairing is 1:1, other possibilities can not be ruled out because of higher no of H-bond forming sites available as compared to the first pair.

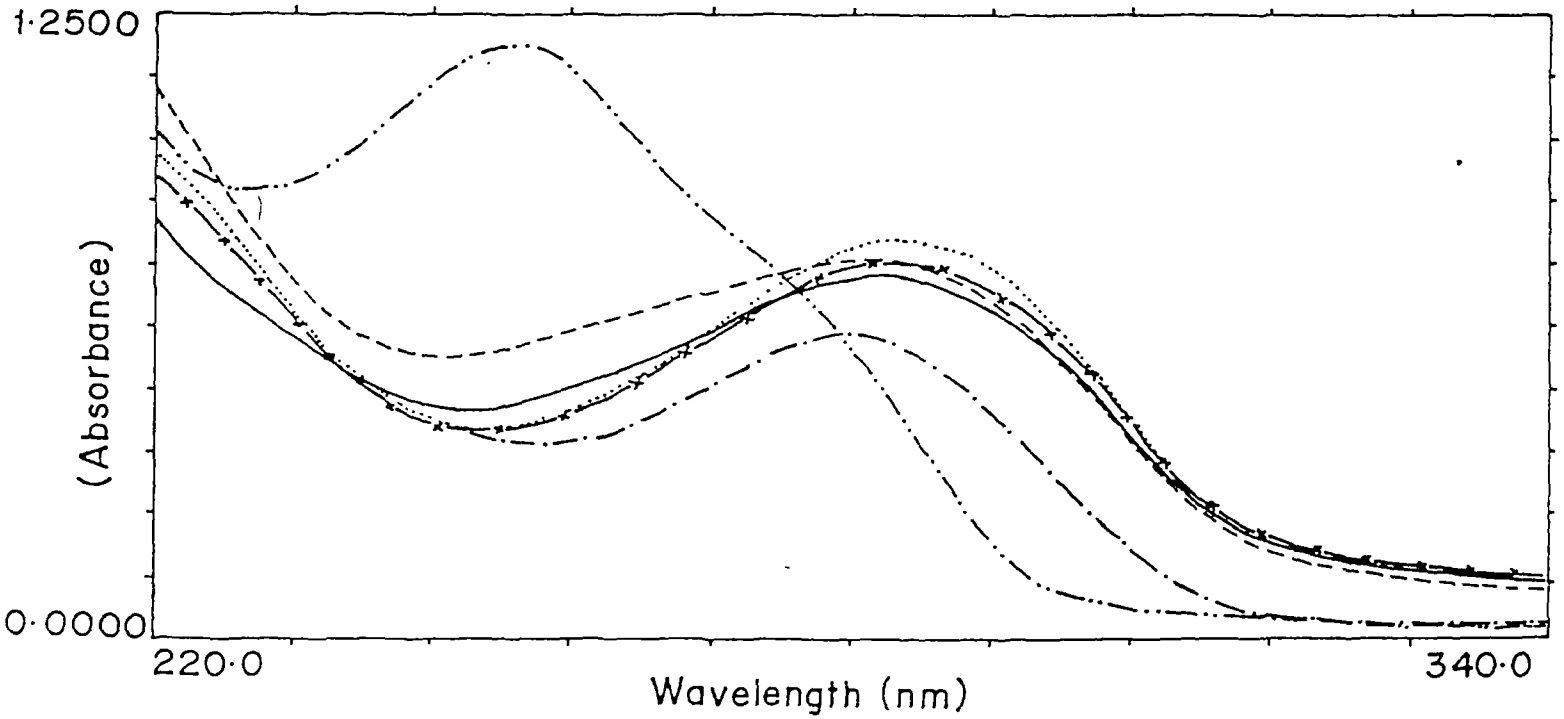


Fig. 12. UV Spectra of Guanosine and Cytosine in 1,4 Dioxan in different relative ratios of concentration.

—·—·— UV spectra of Guanosine , 8.0×10^{-5} M

—·—·— UV spectra of Cytosine , 8.0×10^{-5} M

UV spectra of Guanosine and Cytosine in ---- 1:1 ratio,

—·— 1:2 ratio, ····· 1:3 ratio, —×—×— 1:4 ratio.

CHARGE TRANSFER COMPLEXES

As pointed out in chapter 1, that electron transfer process can be viewed in terms of an outer sphere or an inner sphere mechanism. In the inner sphere mechanism the donor and the acceptor moieties maintain a substantial interaction in the transition state. This has been amply demonstrated by Kochi etal [14,15] who have emphasised the importance of the charge transfer complexes as precursors in electron transfer reactions. It was also pointed out by Taube that one of the methodologies of studying electron transfer processes is the study of the charge transfer complexes . As single electron transfer of the nucleic acid bases is the subject of study, of this project we attempted to study charge transfer formation by UV, if any , as a prelude to the study of single electron transfer by ESR , a versatile tool for detecting such a phenomenon. However, all results in this section are only qualitative in nature.

Proof for Charge transfer complexes.

As a rule charge transfer complexes are characterised by the appearance of a new band called " CT band " or hyperchromic effect caused by perturbation in the energy levels. This occurs when the electron from the HOMO orbital of donor molecule is transferred to the LUMO of the acceptor molecule. Such complexes are usually designated as strong

charge transfer complexes. However, numerous cases are known where electron is not effectively transferred from donor to acceptor orbital, but causes perturbation in the energy levels. This manifests as a net increase in the absorbance at λ max for donor or acceptor. Such interactions are weaker in nature and such complexes are called " contact charge transfer complexes ". In our system, there are three components ; nitrone as a spin trap , chloranil (tetra-chloro benzoquinone) as a powerful electron acceptor and the Nucleic acid bases as the target compounds under the investigation. Therefore, it became imperative to look into the possibility of charge transfer complexes between the constituents of the system.

The first step was to look into the posssibility of charge transfer complex formation between the spin trap and the Nucleic acid bases. As a representative, (i) spectra of guanine and nitrone, fig 13 and (ii) Spectra of uracil and nitrone fig. 14 in different proportions are shown . Clearly three isosbestic points at 216 nm, 230 nm , and 270 nm for the first and 268 for the second are observed. The isosbestic points are fairly sharp and do not shift with the change in the relative concentration of the constituents. Thus charge transfer complexes are formed between nitrone and Nucleic acid bases. Next, we looked into the same possibility with chloranil and nitrone and observed that

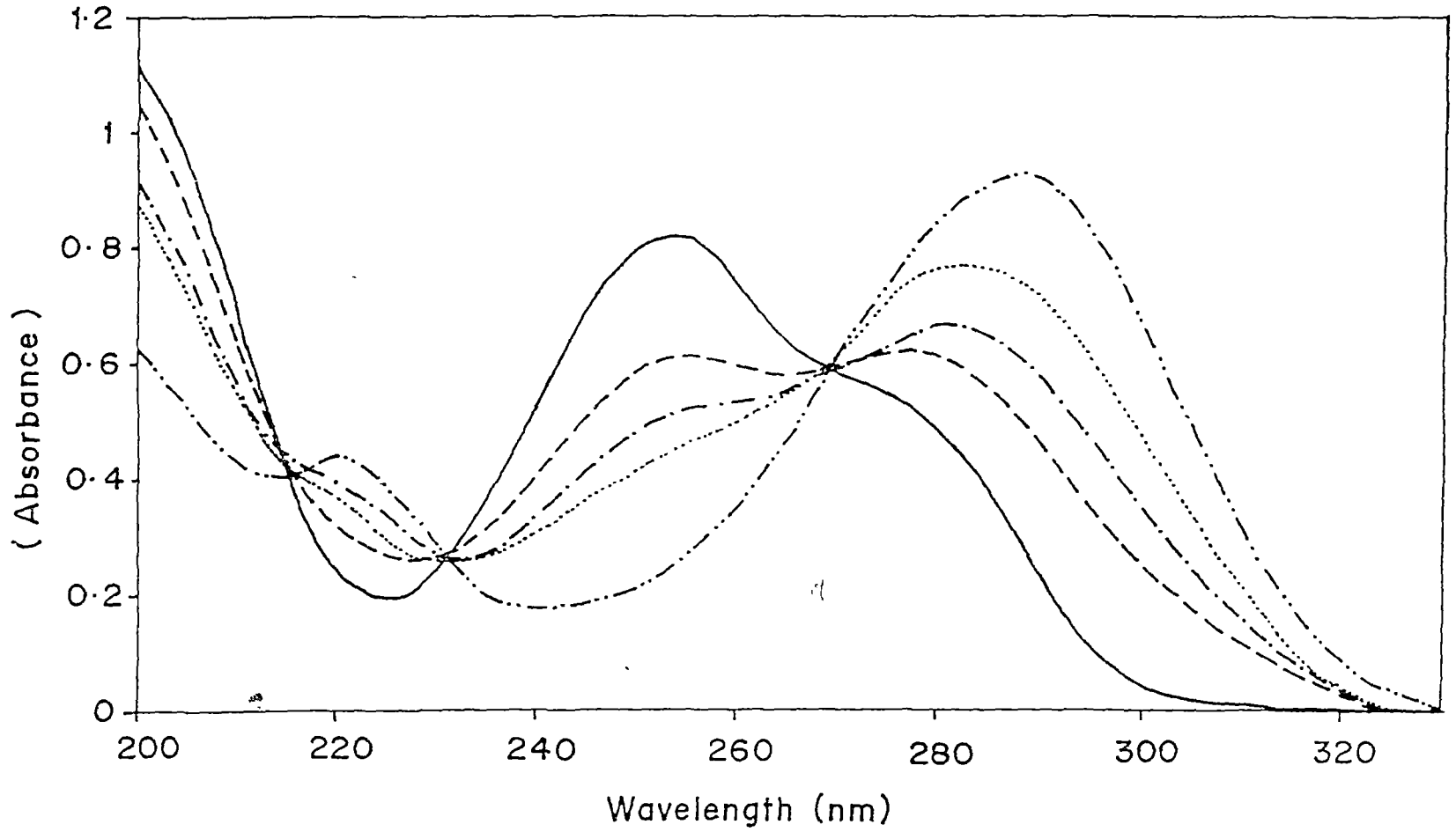


Fig. 13. UV Spectra of Guanosine and Nitrone in CH_3CN mixed in different proportions

— Guaonosine only (2.97×10^{-5}),—·—· Nitrone only(2.5×10^{-5})

—·—·— 1:1 (N:G), — — — 1:2(N:G), ... 1:3 (N:G)

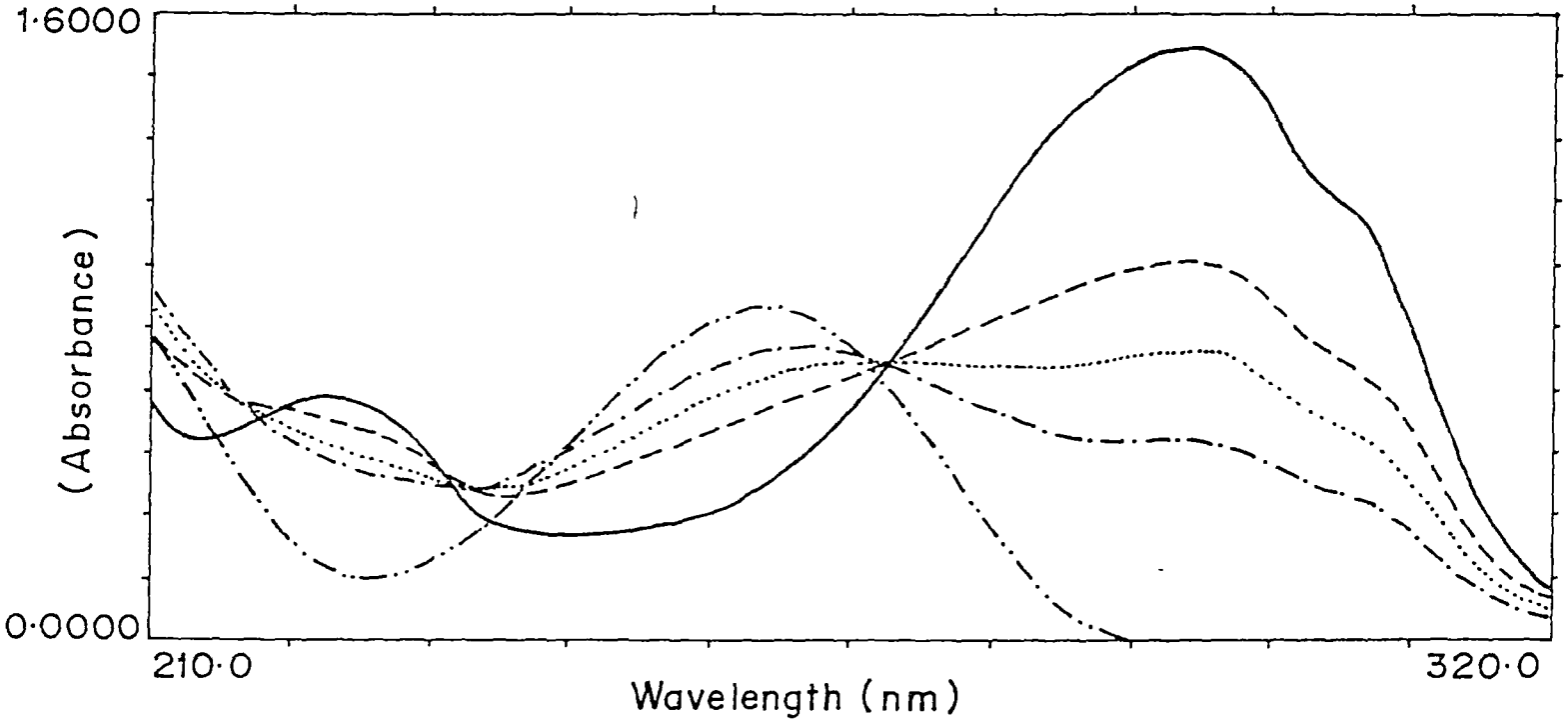


Fig. 14. UV Spectra of Uracil and Nitrone in CH_3CN mixed in different proportions.

—·—·—Uracil only(10.3×10^{-5}), ___ Nitrone only(10.3×10^{-5})

· · · · 1:1 (N:U), -·-·- 1:2 (N:U), - - - 2:1 (N:U)

charge transfer complex is formed in this case too. Then, the next step was to see the charge transfer formation between chloranil and Nucleic acid bases. As a representative, spectra of (i) adenine and chloranil, fig 15 (ii) thymine and chloranil, Fig.16 are shown . Fairly sharp and single Isosbestic point at 270 nm for the first system and 267 nm for the second system have been observed. This indicate that charge transfer complexes are formed in these systems too.

Then we studied the spectroscopic changes in the system where nitrone, chloranil and Nucleic acid bases were present. As a representative plot Fig. 17 the composite spectra of nitrone, chloranil and thymine in 1,4 dioxan is shown. The same solution was scanned over different period of time, even spectra were recorded after 24 hrs. Absolutely no change or any shift was observed as clearly visible from the spectra . This indicate that except simple complex formation, no material change has taken place.

Next, we studied the same system where oxygen was removed by bubbling nitrogen through the needle pierced through the self sealing septum. The cell was effectively sealed. Immediately spectra was recorded and it was identical to the aerated one. The scanning over the period showed growth in the some set of signals in the region of 230 to 270 nm which became quite intense after 24 hrs. Fig. 18 ,and the

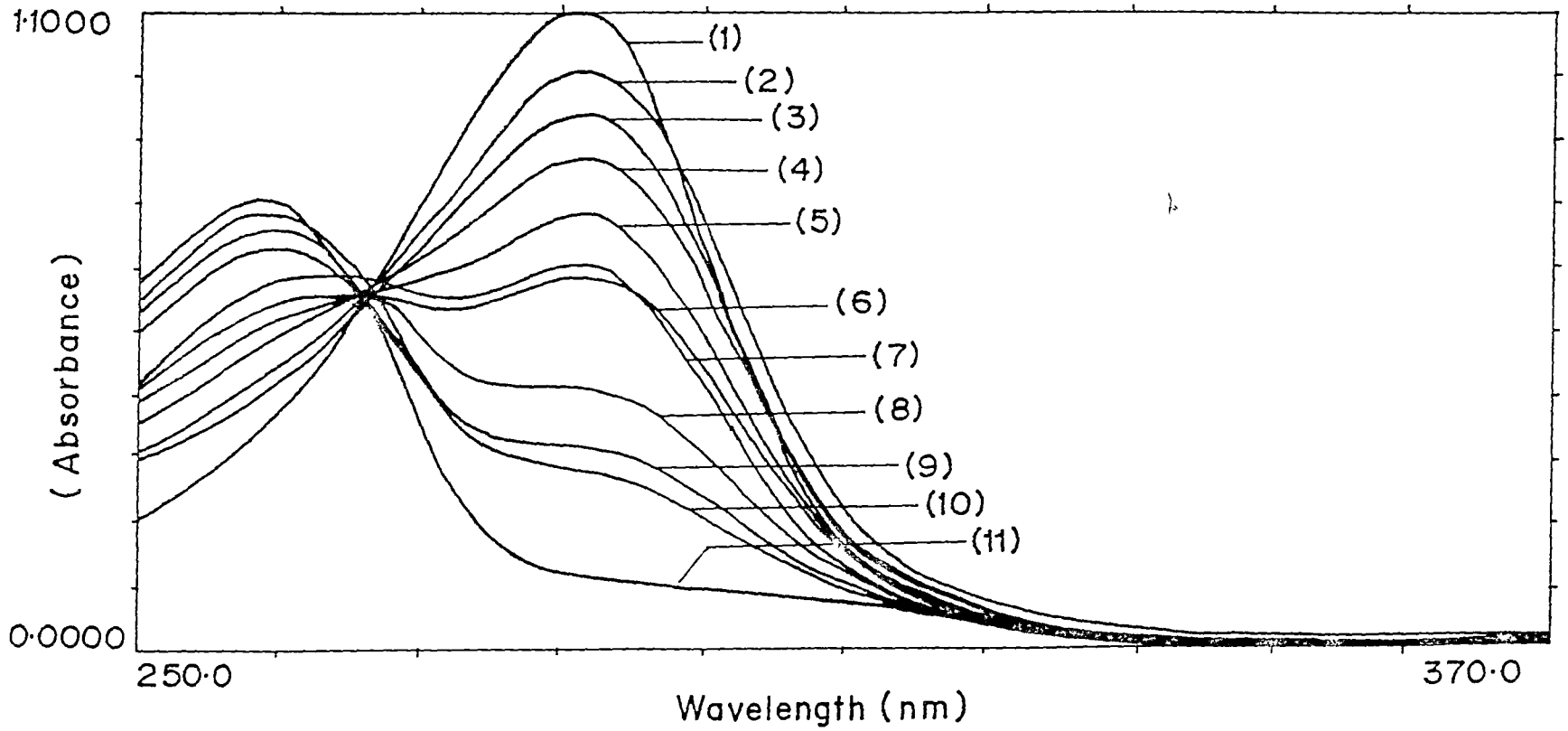


Fig. 15. UV Spectra of (1) Adenine (3.08×10^{-5}) and (11) Chloranil (3.08×10^{-5}) in CH_3OH mixed in different proportions.

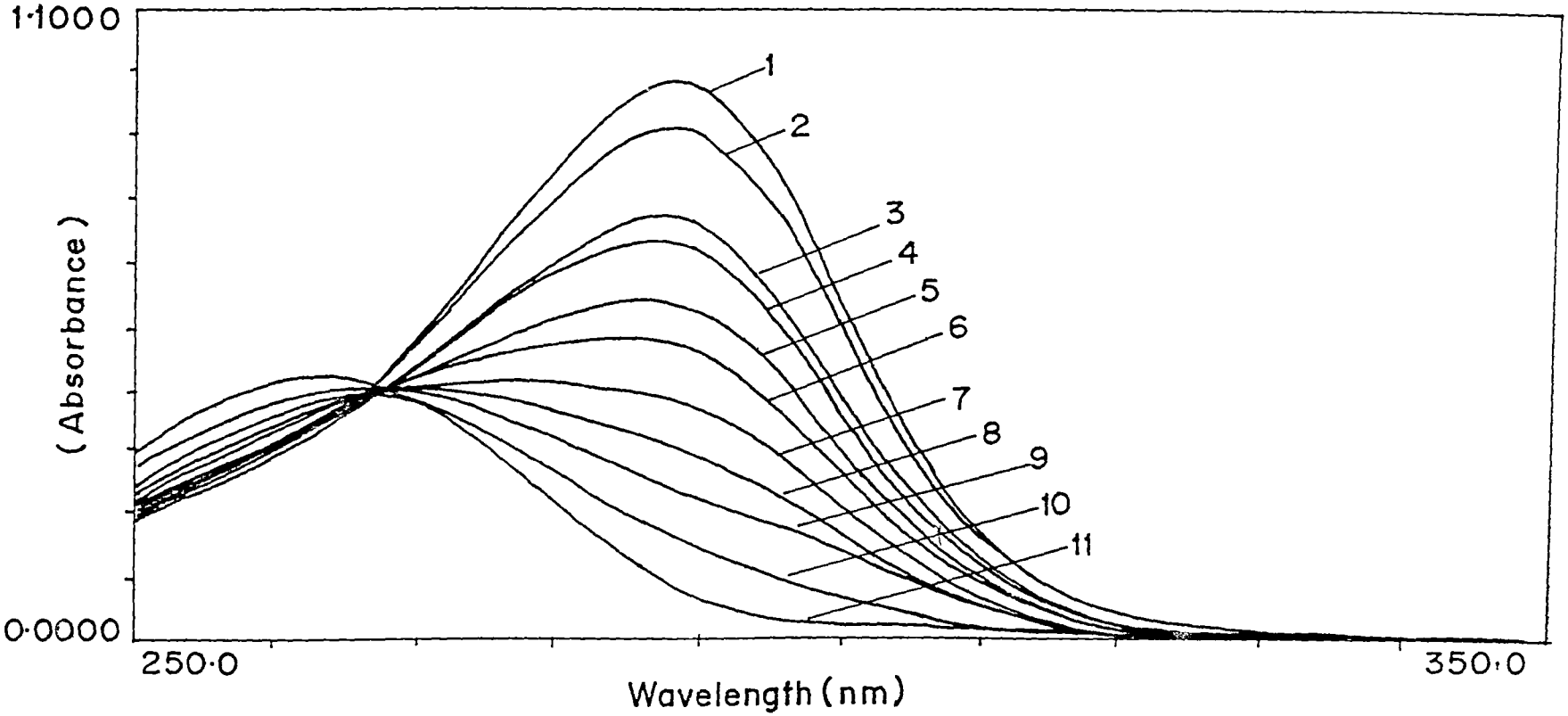


Fig. 16. UV Spectra of (1) Thymine (4.45×10^{-5}) and (11) Chloranil (4.45×10^{-5}) in CH_3OH mixed in different proportions.

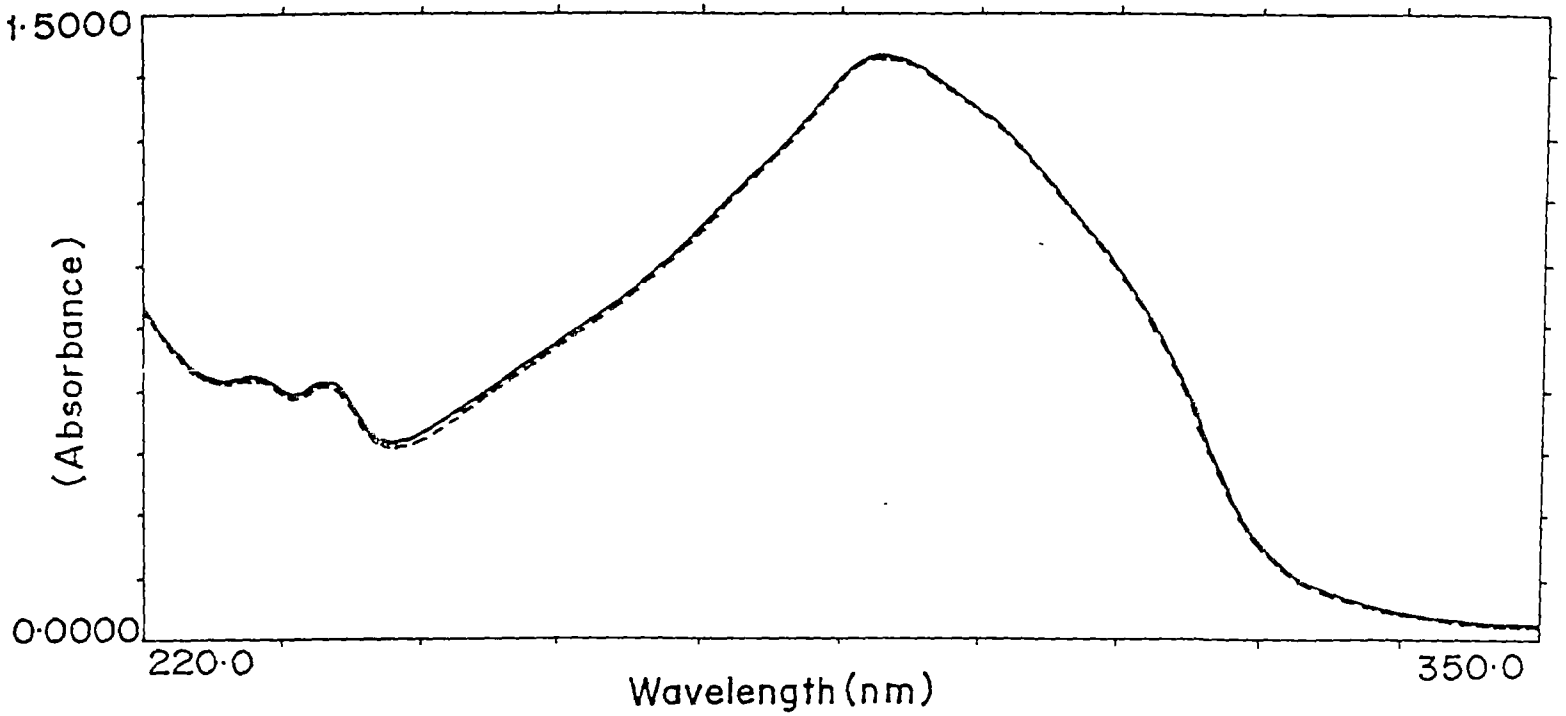


Fig. 17. UV Spectra of Nitrone, Thymine And Chloranil mixed in a 1:1:1 ratio.(conc of the substates 5.5×10^{-5}).

_____ Immediately on mixing, ... after 24 hrs.

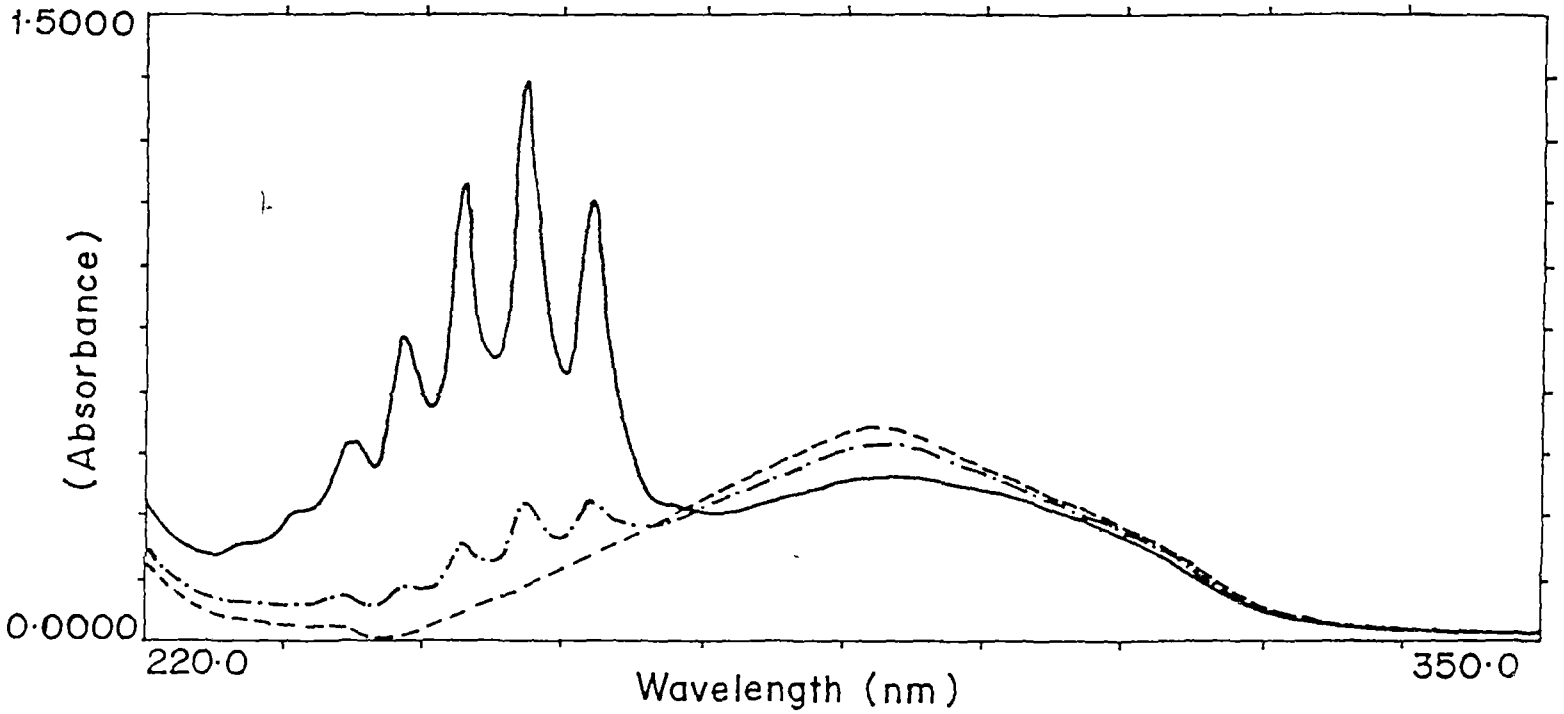


Fig. 18. UV Spectra of Nitrone, Thymine and Chloranil mixed in a 1:1:1 ratio under deareated condition.
-----Immediately on mixing,_._.after 4 hrs,—after 24 hrs.

significant decay in the signals due to nitrone and chloranil.This indicates that the constituents of the reaction mixture are consumed and new species are generated in the system. The appearance of such signals are usually indicative of the presence of an electronic transition accompanied by a vibrational transition, called "Vibronic Interactions" or it could be due to free radicals and/or some charged species. However , we could not make proper assignments.This experiment has clearly demonstrated that electron transfer has taken place and the decisive role of oxygen in the process of single electron transfer reactions. To the best of our knowledge this is one of the rare examples where such a crucial role of oxygen has been demonstrated. Fig 19 shows the UV spectra of an aereated and deaereated set after 24 hrs of mixing the solutions. Infact, this observation was confirmed even with the study for the similar system with ESR. When aerated solution of the above mentioned system was scanned, no ESR signals were observed, however, the moment solution was deaerated ESR signals started growing in intensity, which confirms the conclusions drawn from the uv results. On the role of oxygen we give following explanation ; Oxygen is well known to be paramagnetic in the ground state. One of its anti bonding orbital has only one electron, therefore, it will have a relatively stronger tendency to

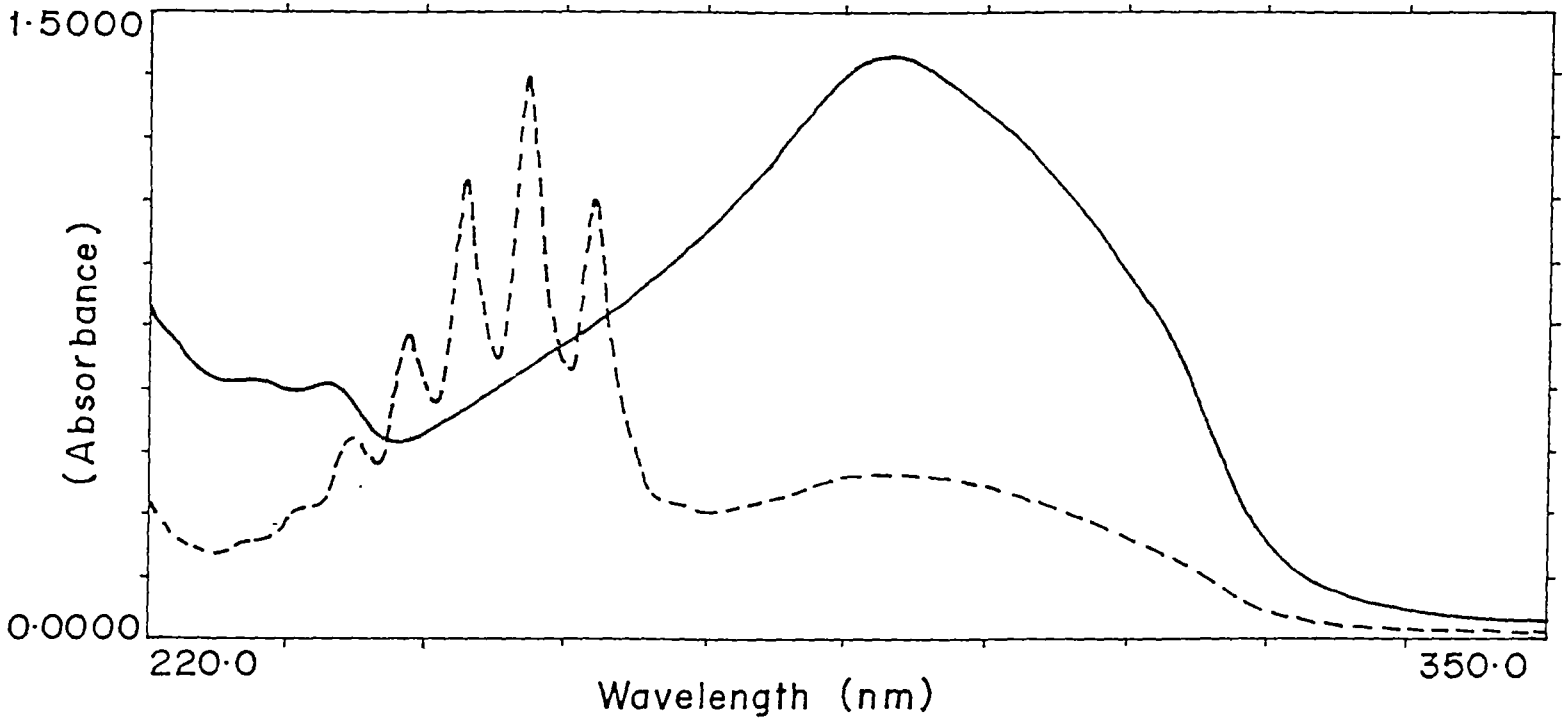


Fig. 19. UV Spectra of Nitrone, Thymine and Chloranil mixed in a 1:1:1 ratio (24 hrs after mixing the solution).

—— Aereated, - - - - - Deareated.

accept electron to acquire a state of lowest energy. This simply means that it will form a fairly strong charge transfer complex with the donor molecule in the system, which in a sense would remove donor from the system and hence electron transfer would be no more feasible. Since the concentration of oxygen is far more then the concentration of the substrate molecules, the probability of interaction of donor with oxygen would be certainly higher.

CONCLUSION

1. UV spectra of all the bases mentioned above have been successfully recorded in solvents of varying polarity.
2. The major transitions observed have been assigned as pi -pi*. No hyperchromic shifts were observed with the increase in the polarity of the solvent, contrary to general expectation. It appear that solvents, do not interact in any way , neither with pi orbital, nor with pi* orbital. It can thus be inferred, that these orbitals are stabilised through some other mechanism, which are thermodynamically more favourable.
3. The broad bands in the Scan mode were further split up through second derivative plots. These were assigned due to tautomers (keto - enol and/or amino - imine) existing in the system. The pi electrons responsible for

the transitions are now involved in tautomerism and are no longer available for stabilisation with the solvents. Thus no shift of band positions were observed. Only in cases where no tautomerism were observed (viz thymine) the normal hyperchromic shift could be observed.

4. Self association of the bases were observed in non polar solvents for the first time by UV spectroscopy. Association of the bases were confirmed either by the dimunition of the band of the monomeric species or by the appearance of a new band. In n-hexane the associative (dimeric) band could be observed for all the bases.

5. Through UV spectroscopy, we too have confirmed that self - association for purines is stronger then pyrimidine.

   The increasing order of association of the bases was found to be :

   Guanine > Adenine > Cytosine > Uracil > Thymine

   while the reported one is ;

   Guanine > Adenine > Thymine = Uracil > Cytosine.

6. Base pairing for the naturally existing pairs was confirmed by UV through the appearance of Isosbestic points with 1 : 1 stoichiometry.

7. Nitrone and nucleic acid bases when mixed in polar and hydrogen bonding solvents, isosbestic points were

observed, indicating a fairly strong CT formation. This further shows the feasibility of electron transfer.

8. Similarly isosbestic points were observed between chloranil and the nucleic acid bases, thus indicating the possibility of electron transfer. 9. When all the three components of the system ; nitrone, chloranil, and base were mixed, the composite spectra observed remains same over a period of time. Even the second derivative plots did not reveal the appearance or disappearance of any band. However, when the same system was deaerated, number of signals were observed. The intensity of signals increased with time and the composite band observed initially decreased . This indicate that some slow process has begun. This clearly demonstrates the critical role of oxygen in electron transfer processes for this system.

REFERENCES

(1) Single Electron Transfer Reactions, Lennart Eberson, Pg 83.

(2) Organic Chemistry Of NucLeic Acids-Part A , N.K.Kochetkov and E.I.Budovskii, Eds. Lord Todd and D.M.Brown.

(3) Pringsheim,P.; Flourescence and Phosphorescence, Wiley (interscience) New York(1959) pg 353-361.

(4) Pitha, J.; and Jones, R.N.;Can. J. of Chem., 44, 1045, (1966).

(5) Kyogoku,Y.;Rich, R.C., and Rich,A.;J.Amer.Chem. Soc, 89:3, 1967, P. 497.

6) Gellart,M., Lipsett, M.N.; and Davies,D.K.;Proc. Nat. Acad. Sci.USA, 48, 2013.

(7) Roehrig,G.H.; Oyler,N.A.;Adamowicz,L.; Chem.Phy. Letters. 225 (1994) p. 265.

(8) (a) Molecular Biology of Mutagens and Carcinogens, Ed. Singer,B.; Grunberger,D.; Plenum Press (1983) Plenum Publishing Co., New York, 1983, pg 23.

(b) Organic Chemistry of Nucleic Acids Part A N.K.Kochetkov and E.I. Budovskii, Eds. Lord Todd and D.M.Brown, p.147.

(9) Mason, S.F.; J.Chem.Soc, 1954, 2071.

(10) Stewart, R.F.; and Davidson,N.;J. Chem. Phys. 39 (2), 1963, p. 255.

(11) Yudkin, M.; and Offord,R.;Comprehensible Biochemistry, (Longman) 1973, p. 140.

(12) Organic Chemistry of Nucleic Acids- part A N.K.Kochetkov and E.I.Budovskii, Eds., Lord Todd and D.M.Brown p. 197.

(13) Cavaliery,L.F.; Bendich,A.; Tinker,J.F.,and Brown,G.B.; J.Amer.Chem.Soc, 70, 1948, p. 3875.

(14) Kochi,J.K.; Angew. Chem. Inter. Ed.English. 1988, 27, 1227

(15) Kochi,J.K.;Acta.Chem. Scand. 1990, 44, xxxx ?
85,1289 (1963)

CHAPTER-5

RESULTS & DISCUSSION
ESR
SPECTROSCOPY

SINGLE ELECTRON TRANSFER REACTION STUDIES BY ESR.

As discussed in the previous chapter, for an electron transfer to take place, two criteria must be met ;

First the Donor and acceptor must form some sort of complex, called Charge Transfer Complex. As postulated by J.K.Kochi etal " Charge transfer complexes are precursor to the single electron transfer reactions". It would be appropriate to add that the charge transfer complexes so formed must be strong enough to allow the transfer of an electron from HOMO of donor to LUMO of acceptor. The second most important is the energetic requirement ; the ionisation potential of the donor must be low and electron affinity of the acceptor must be high.

We did not use any radiation source to generate the radicals, instead we used only oxidising agents under very mild conditions. The results are thought to contribute to the better understanding of the mechanism of base radicals produced by " Chemical Oxidising " agents under normal or pathological metabolic processes.

Since the solubility of purine bases is lower then pyrimidine bases, for ESR studies satisfactory concentration of purine bases could not be attained, therefore, most of ESR work is confined to pyrimidine bases. As observed in

the uv spectroscopic studies, oxygen plays a very important role in charge transfer complex formation, therefore, all ESR experiments were carried out under degassed condition in a specially built cell as shown in the experimental section. Stock solutions of the order of 10 (-3) Mol. conc. of the bases, Nitrone, and Chloranil were used. Separately degassed solutions were mixed through the bridge tap and scanning was started immediately. Cell was left in the cavity of the spectrometer and continuous scans were recorded.

I. THYMINE / CHLORANIL / NITRONE SYSTEM

THYMINE / CHLORANIL / NITRONE SYSTEM IN 1,4 DIOXAN

(i) Thymine and Nitrone in 1,4 dioxan .

First blank experiments were done . 0.3 ml solution of Thymine and Nitrone each were degassed and mixed and the results are shown in Fig. 1a . Spectrum consists of a major triplet from primary nitrogen, each line splits up further into a doublet because of a secondary hydrogen, then each line of doublet splits further into a triplet in 1 : 2 : 1 ratio, suggesting the presence of two equivalent hydrogen in the tertiary position. The hyperfine splitting constants are aN = 14.5G aH_1=2.60G and aH_2=0.44G. The order and the magnitude of these constants unmistakably suggests that it is a nitroxyl type radical, the structure assigned is 7 of

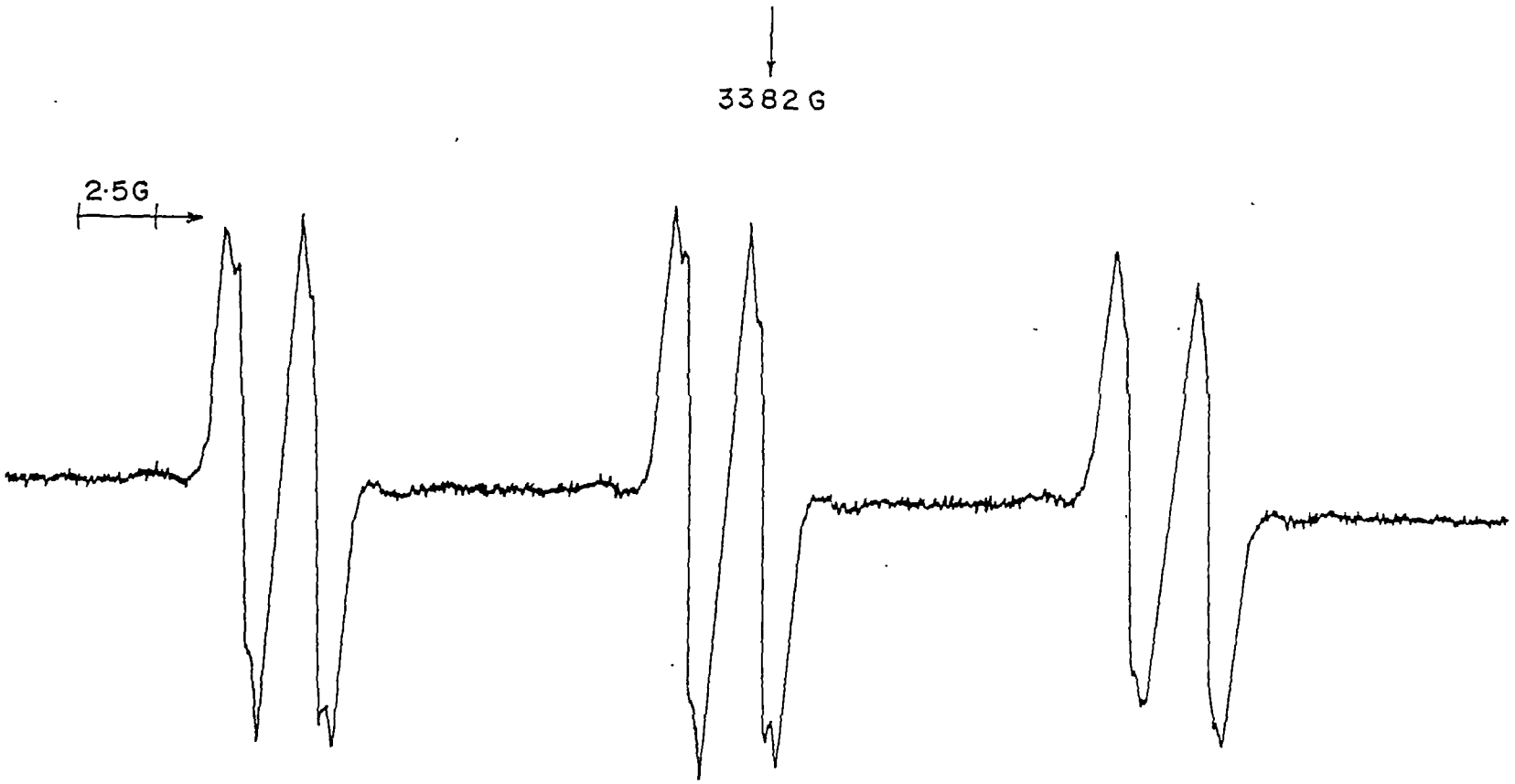


Fig.1a. ESR Spectra of the radical adduct 7 (scheme 1) obtained from Thymine with Nitrone in 1,4 dioxan.

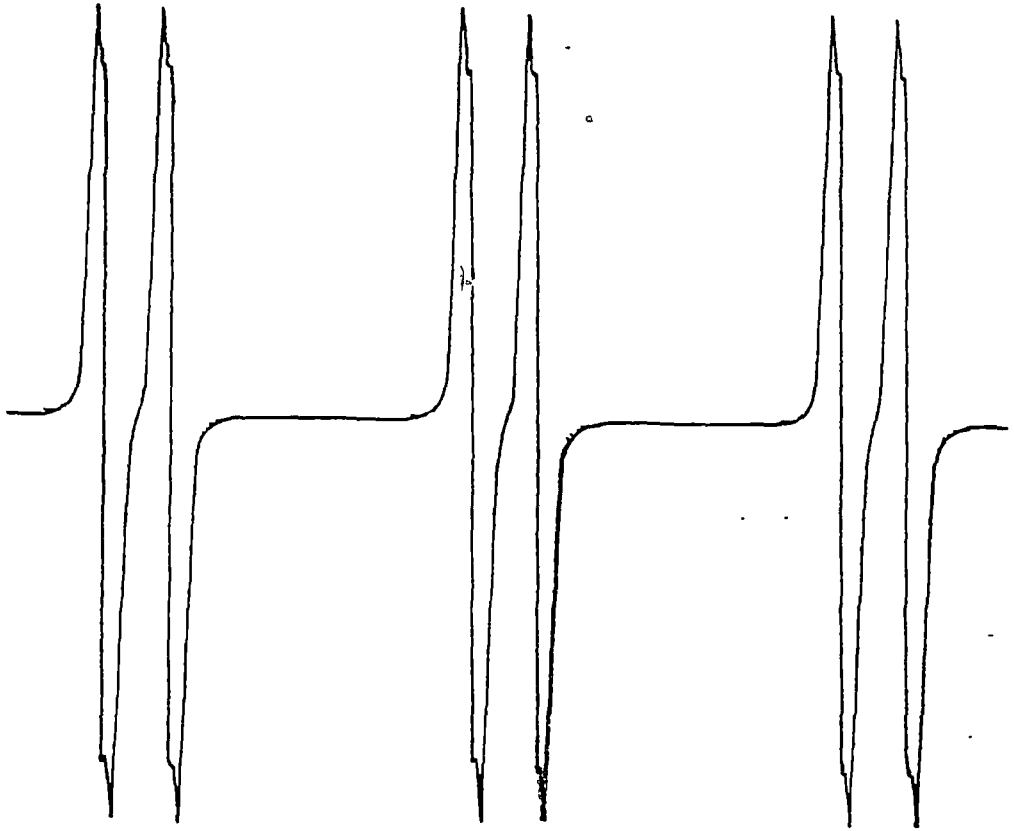
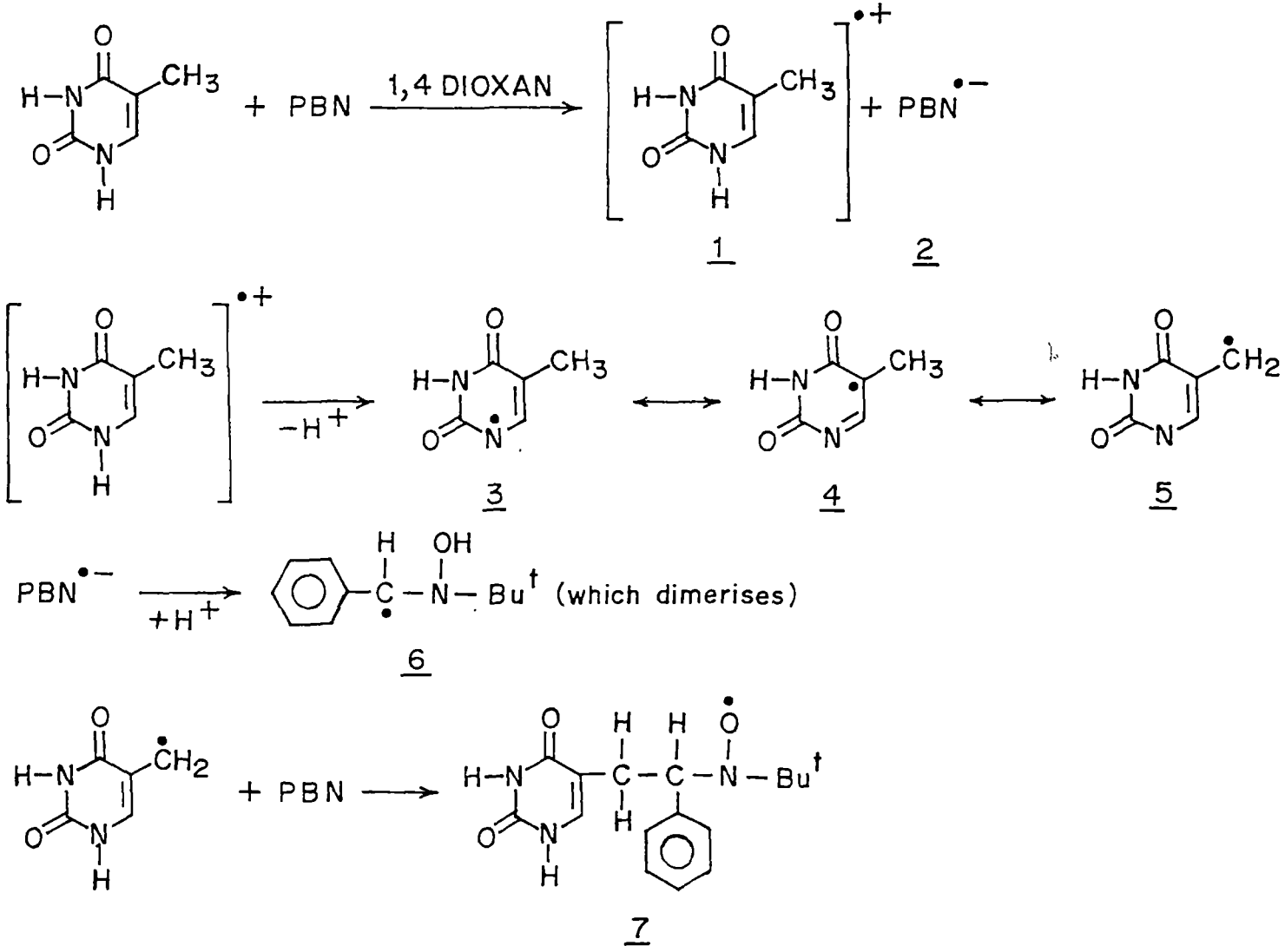


Fig.1b. Computer simulated spectra of 1a, using hyperfine parameters calculated from the experimental spectra and L.W = 0.43G.



Scheme – I

Scheme I.

MECHANISM

The redox potential of Thymine is 1.29 eV [1] and of Nitrone is 1.75 eV, therefore, in all probability the electron transfer from Thymine to Nitrone is thermodynamically feasible, resulting in the formation of respective cations and anions. We suggest the mechanism shown in scheme I.

The deprotonation of the thymine radical cation is reported to lead to the formation of the mesomeric structures (3), (4), (5). Our results indicate the trapping of the structure (5) by neutral PBN leading to the stable adduct (7). Such radical was also trapped by Gilbert etal [2]. The PBN anion generated undergoes protonation to form radical (6) which may dimerise. The spectra is very well simulated using the parameters, Fig. Ib calculated from the experimental spectra of the radical adduct (7).

(ii) Chloranil and Nitrone system.

Similarly, chloranil and nitrone were mixed under degassed condition. The spectra observed is shown in Fig. 2. Fig. 2 a shows only a singlet, which is a well known spectra of the chloranil anion, with a g value - 2.0042 (Rep. 2.0043) suggesting unambigiously that the electron has been transferred essentially from nitrone to chloranil (it is known to be powerful acceptor) . The oxidation pot. of nitrone is 1.47 eV and that of chloranil is 2.50 eV. Thus

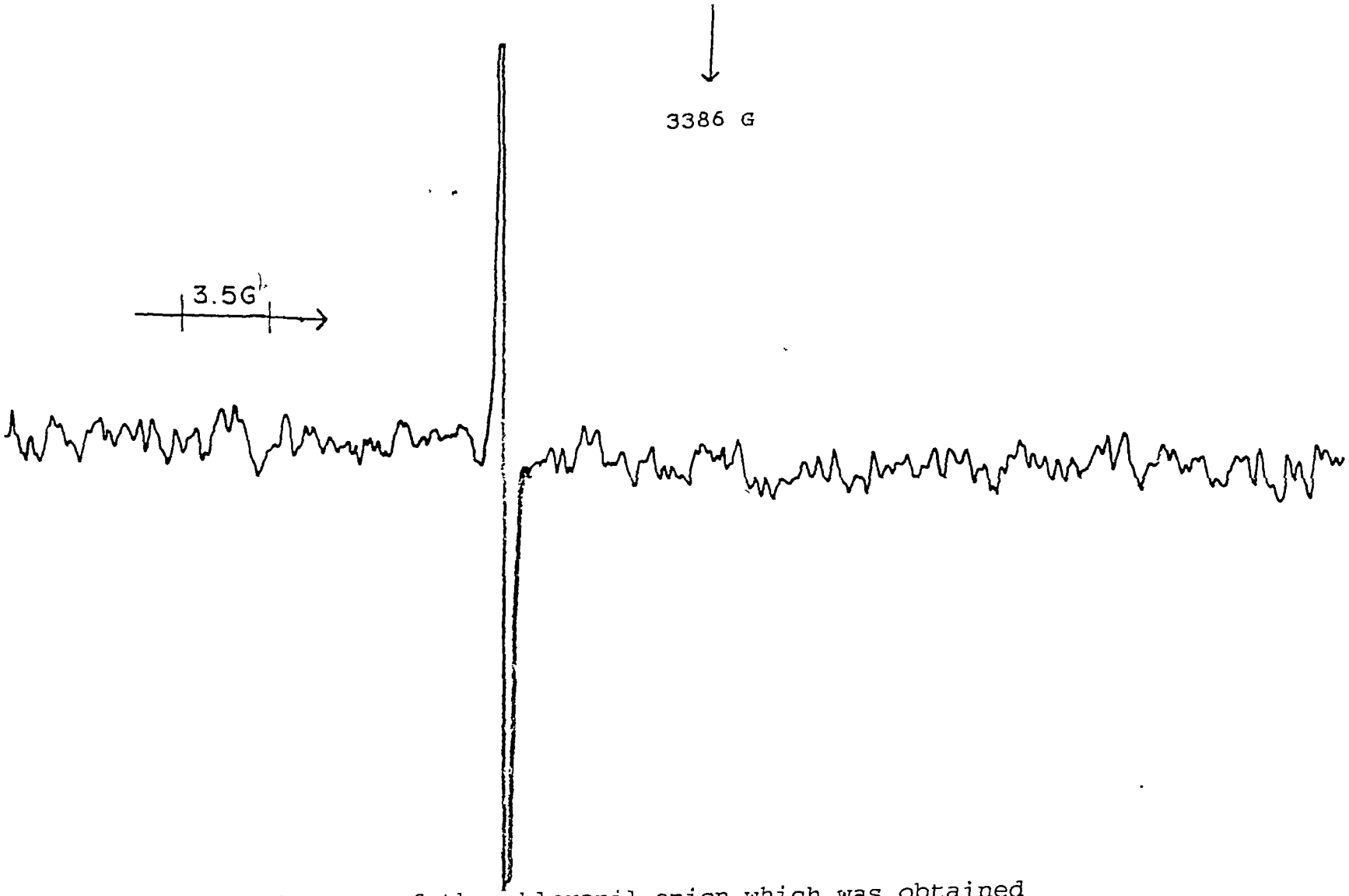


Fig.2. ESR Spectra of the chloranil anion which was obtained immediately from the reaction of nitrone with chloranil in 1,4 dioxan.

energetic consideration clearly points out the transfer of an electron from nitrone to chloranil. The anion is unstable and the spectra is followed by a doublet of a triplet, suggesting major splitting from primary nitrogen and secondary splitting from a β - hydrogen. The hyperfine splittings are a N = 13.75 G, a H = 1.88 G. This adduct is also unstable and spectra disappears in 30 mins. The order and magnitude of hyperfine splitting for adduct shown in Fig. 3a strongly suggests that hydroquinone adduct of PBN has been trapped. The parameter were used to simulate the spectra, Fig. 3b.

(iii) Thymine / chloranil system.

When degassed solutions of Thymine and Chloranil in 1,4 dioxan were mixed, immediately a singlet appeared. The radical is unstable. The g value of the radical is same as for Chloranil anion. It is clear that electron has been definitely transferred from Thymine to chloranil. Since , there is no spin trap in the system, we did not expect any other spin trapped adduct to be observed.

(iv) Thymine / Nitrone / Chloranil system.

Nitrone solution in one arm of the cell was degassed. In another arm chloranil (written as CA in Schemes) solution and thymine solutions were mixed and immediately freezed and degassed. Through bridge tap nitrone solution was added to the mixture and scanning started immediately. The spectra

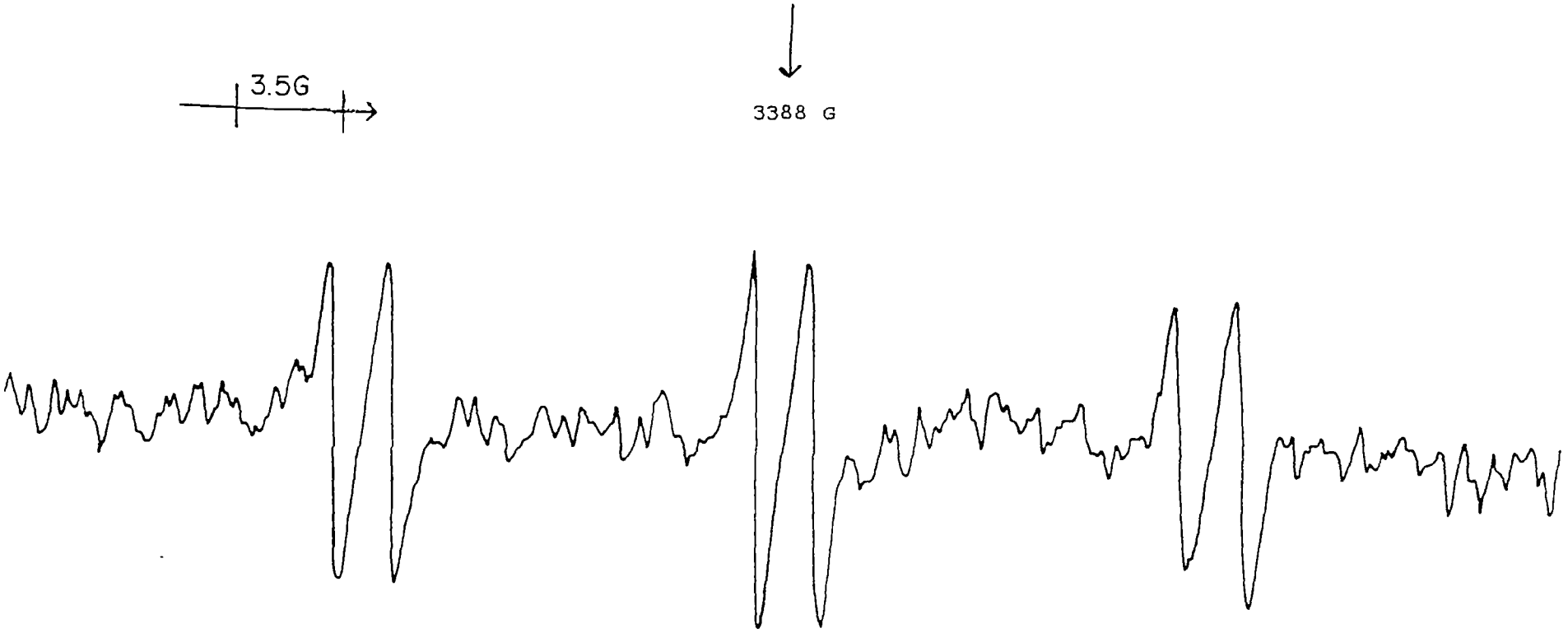


Fig.3a. ESR Spectra of the hydroquinone adduct of PBN in 1,4 dioxan.

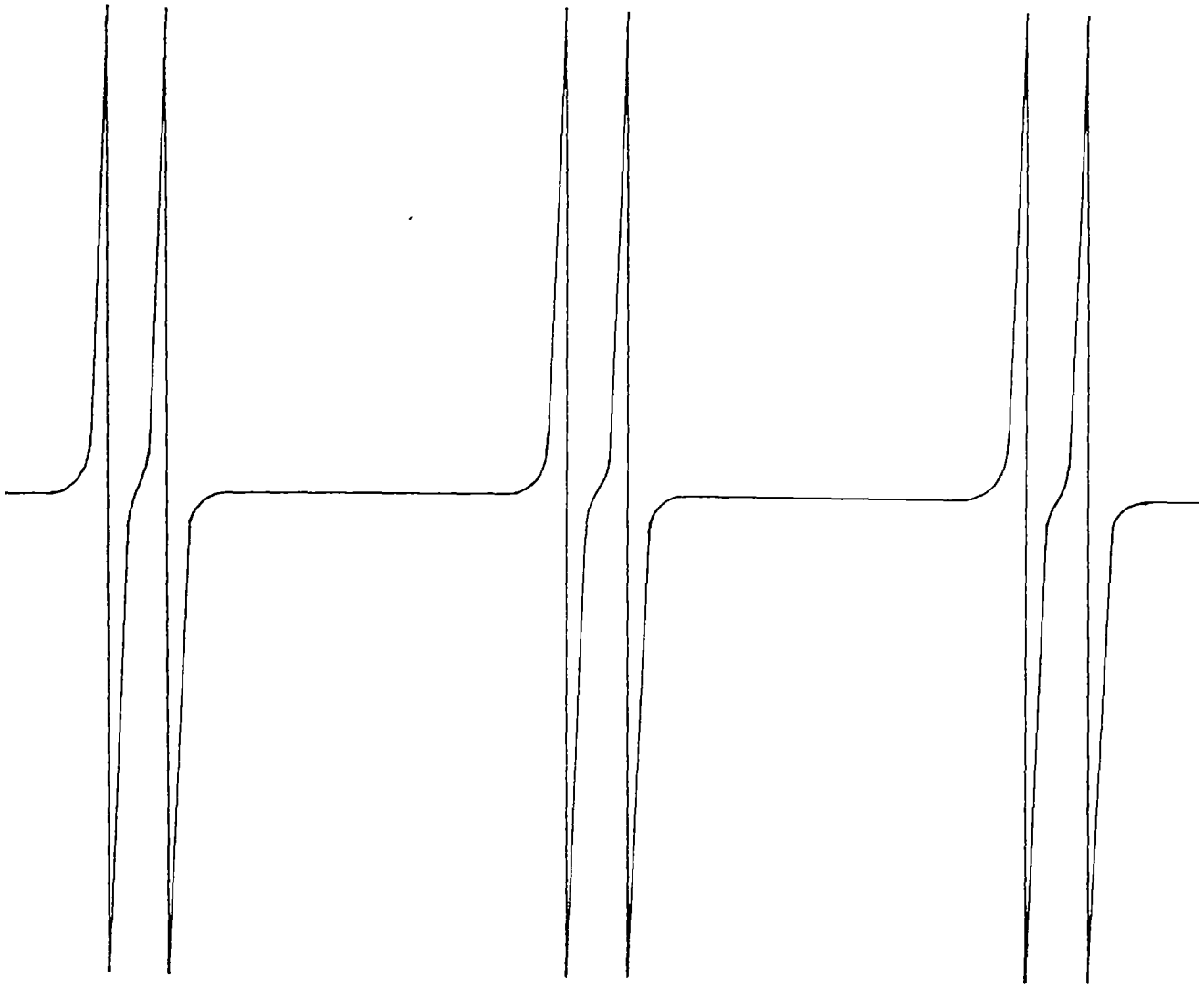
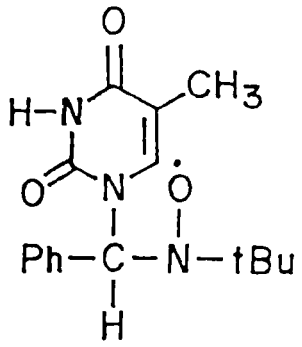


Fig.3b. Computer simulated spectra of 3a, using the hyperfine parameters calculated from the experimental spectra and L.W = 0.4G.

observed appears to be due to two species ; (i) a singlet due to chloranil anion, which is unstable and disappears after some time, (Fig. 4). (ii) some weak signals which grew over a time to a well resolved spectra, (Fig 5a). The spectra can be analysed as ; a major triplet with aN = 13.85 G due to a primary nitrogen, then each line again splits up into a triplet with a N = 3.0 G due to secondary nitrogen, then each line again splits up into a doublet with a H = 1.6 G. When one of the three component of the spectra was scanned over a small range of field at a very slow scan speed, further splitting of each line into a doublet was observed Fig. 5c with a hyperfine coupling of 0.4 G. This spectra is quite stable. The spectra can be postulated as due to the adduct shown below ;



MECHANISM

Mechanism leading to the formation of this stable adduct is shown in the scheme II . Chloranil being a powerful acceptor forces Thymine to donate an electron and thus chloranil

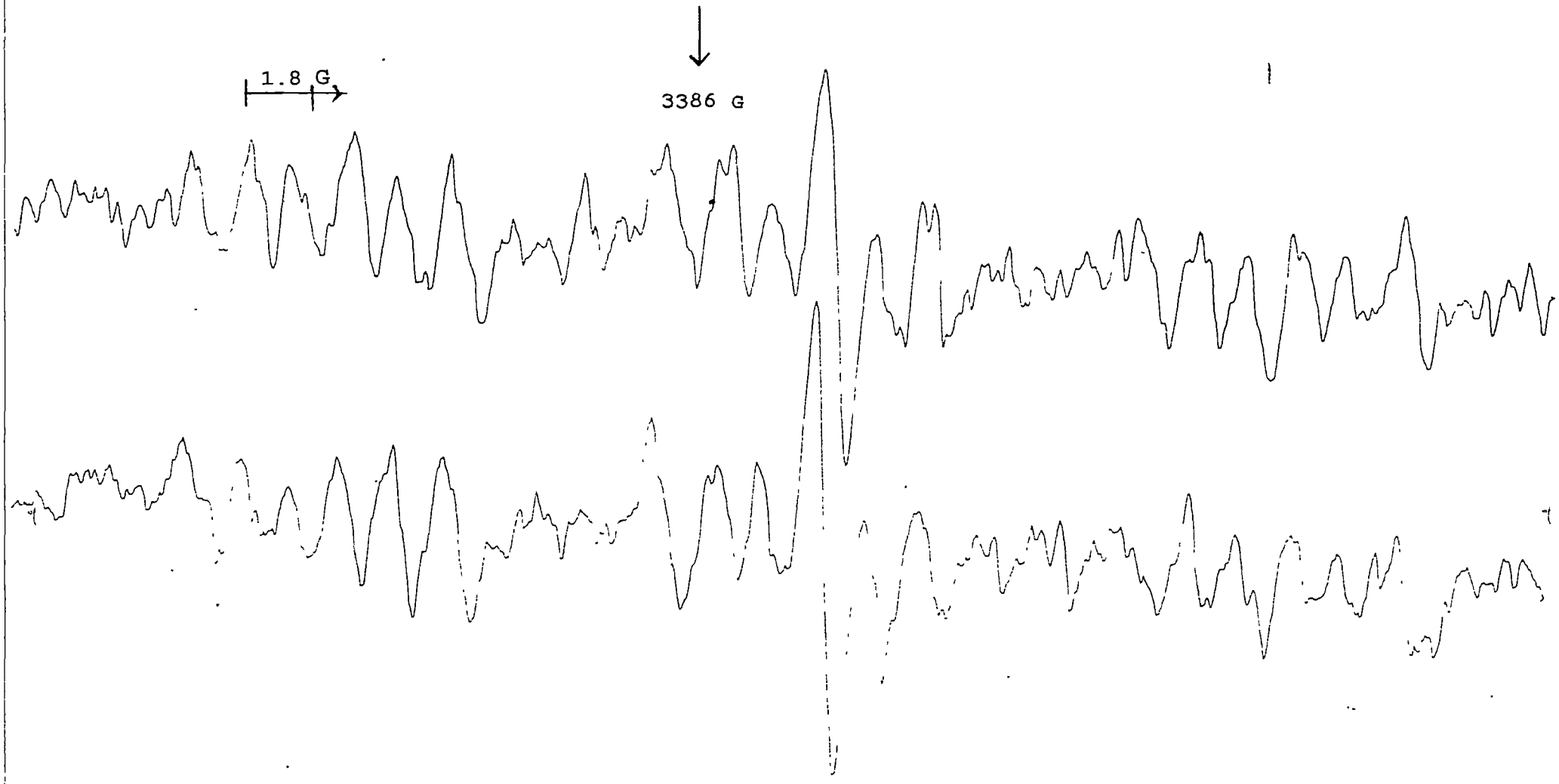


Fig.4 ESR Spectra observed immediately from the reaction of thymine, chloranil and nitrone in 1,4 dioxan.

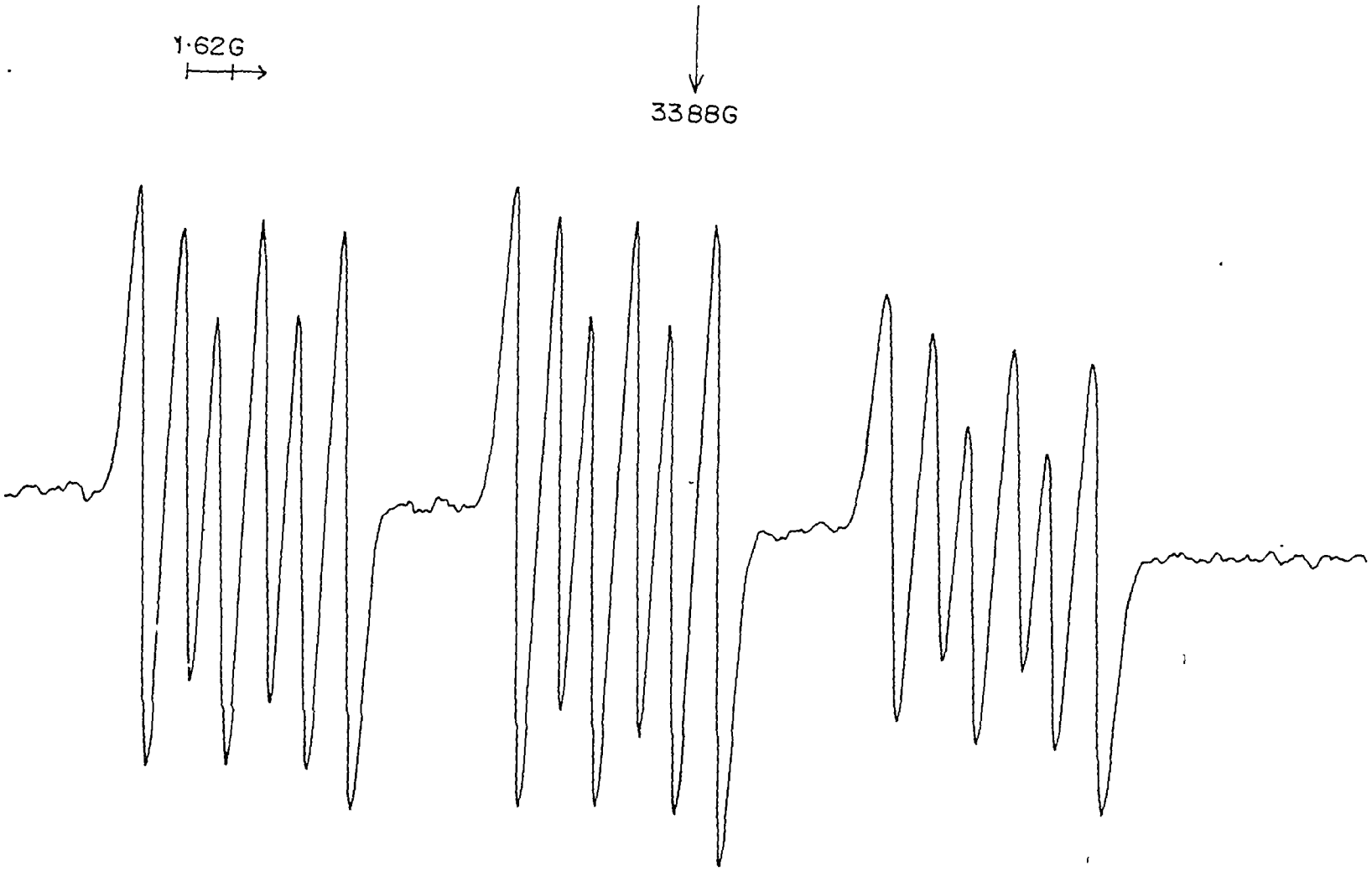


Fig.5a. ESR Spectra of the adduct 5 (scheme II) obtained from the reaction of thymine, chloranil and nitrone in 1,4 dioxan.

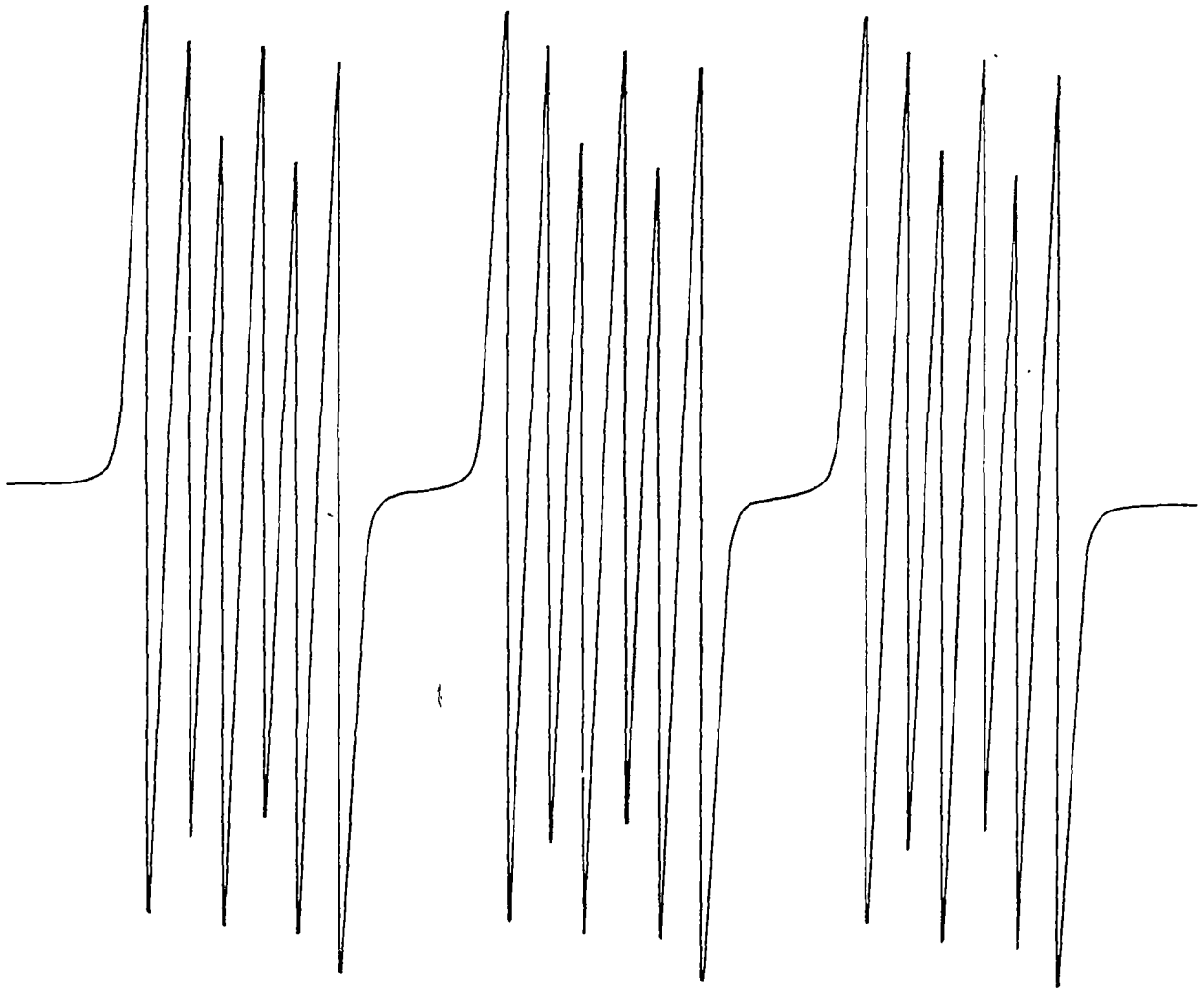


Fig.5b. Computer simulated spectra of 5a. using the hyperfine parameters calculated from the experimental spectra and L.W = 0.65 G.

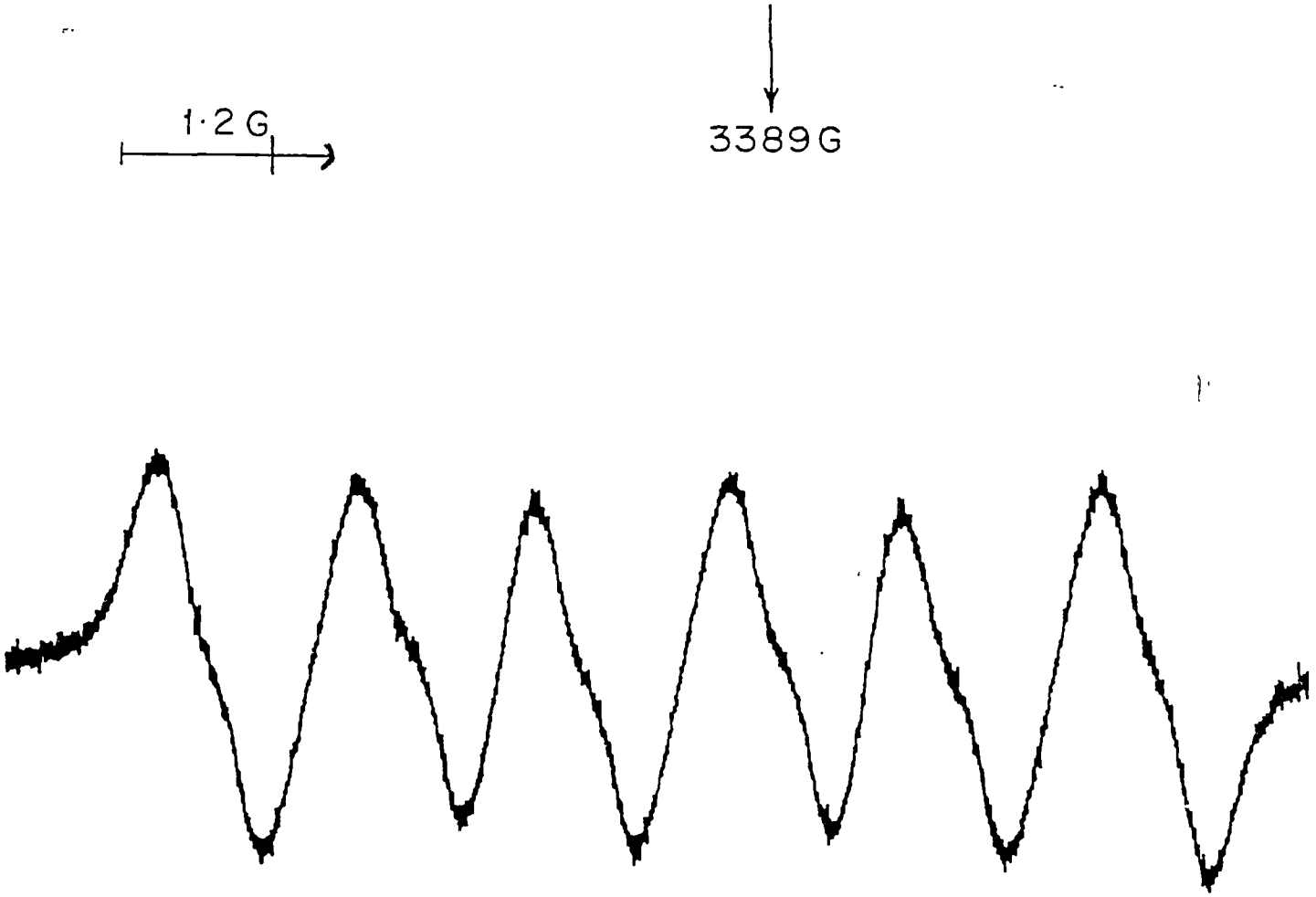
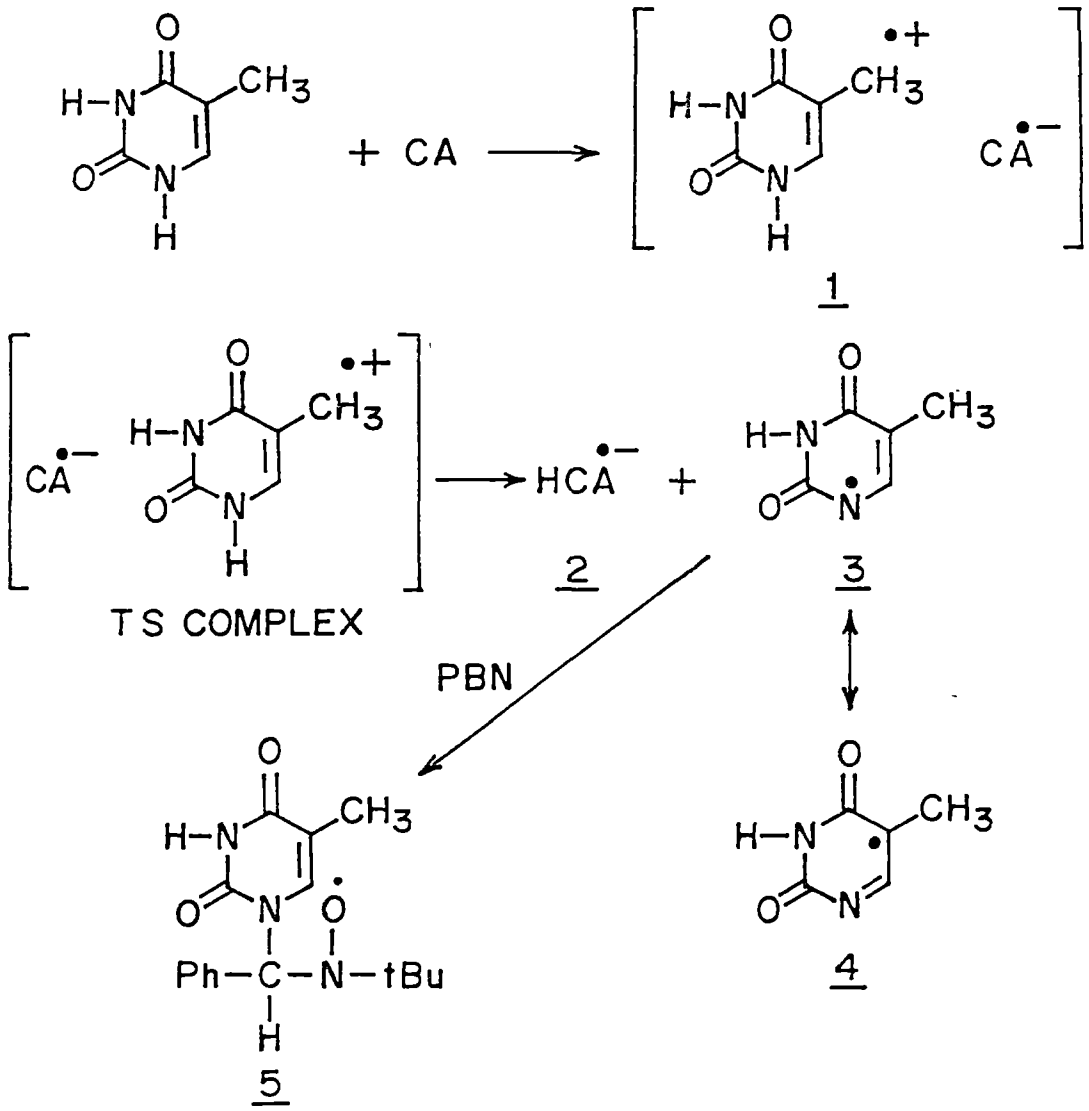


Fig.5c. ESR Spectra of one component of 5a run at a small field range and high scan time.



Scheme-II

anion ($CA^{\cdot -}$) and Thymine cation is generated. One may say that even nitrone may donate an electron to chloranil, because this has been demonstrated in a blank experiment. However, energetic consideration suggests that Thymine would donate electron preferentially as its redox potential (1.29 eV) is lower then that of nitrone (1.7 eV). The $CA^{\cdot -}$ is a transient specie and decays to $HCA^{\cdot}$ by the transfer of a proton of the labile cation radical [3]. The spectrum of the semiquinone radical ($HCA^{\cdot}$) decays over several seconds (to the spectral base line) and the spectrum left is that of the stable adduct. It is reported that thymine cation undergoes deprotonation at N (1) or N (3) to produce a nitrogen centered radical, which in turn would be trapped by nitrone. The splitting pattern suggests that in this case the deprotonation has occured from N (1), because aH2 splitting of 0.4 G would not occur through the trapping of N(3) centered radical. This observation is further substantiated by doing similar experiments with Thymidine (which shall be discussed later). The formulation of the transition state complex is based along the lines suggested by Kochi etal. The stability of such complexes depends very strongly on the nature of the solvents and would be very low in polar and hydroxylic solvents.

Since, $CA^{\cdot -}$ is a very reactive specie, it can abstract proton from the solvents molecules also. This was discounted by

doing an experiment, wherein degassed solutions of, hydrogen peroxide and nitrone in 1,4 dioxan were mixed and continuous scanning was done, and after some time a well resolved spectra developed, which was distinctively different from the one we described above, and that is identical to the reported one in which a proton has been abstracted from dioxan and the resulting radical was trapped by nitrone. We thus rule out the possibility of proton abstraction from the solvent and conclude that it has occured from thymine. Fig. 5b shows a well simulated spectra with the hyperfine splittings obtained from the experimental spectra.

It may be pointed out that in the experiment with thymine and nitrone , the spectra observed was assigned as due to deprotonation of the methyl group at C5, whereas, in this experiment $CA^{\bullet -}$ abstracts proton from N(1) exclusively. This difference is due to the fact that in the former case deprotonation occurs under its own forces of stabilisation, while in the latter case , proton abstraction occurs as a consequence of the transition state complex formed between $CA^{\bullet -}$ and thymine radical cation.

THYMINE / CHLORANIL / NITRONE SYSTEM IN ALCOHOLS

Alcohols being polar and hydroxylic can influence the electron transfer reaction pathway mainly in two ways: (i) they can solvate the charged species that may be formed and

thus can alter the course of the reaction (ii) and they can affect the stability of the transition state complex.

Before proceeding to do the actual experiments, blank experiments were done in this case too, and the results are basically similar in nature. With alcohols too, similar experimental procedure was followed as with dioxan. The spectra is shown in Fig. 6 and it consists of a singlet and a doublet of a triplet. The singlet is due to the chloranil anion formed by the transfer of an electron from thymine to chloranil. The chloranil anion formed here is extremely stable, indicating that some kind of stabilising forces are operative. As mentioned above, solvation with alcohol seems to be the stabilising force. This in fact would remove the anion from forming the TS Complex with Thymine cation as shown in scheme II. If this was the case, then the course of the reaction would be different from the one we discussed in 1,4 dioxan and indeed we observed it. The hyperfine parameters calculated for the doublet of triplet is very near to the $CH_3CH_2O^{\bullet}$ from ethanol and $CH_3O^{\bullet}$ from methanol, adduct of PBN [4].

Since, $CA^{\bullet-}$ is effectively removed through solvation, it is no longer available to influence the deprotonation of the Thymine cation. It is quite likely that thymine cation may follow the different pathway. The appearance of RO - PBN adduct means that hydrogen abstraction takes place from

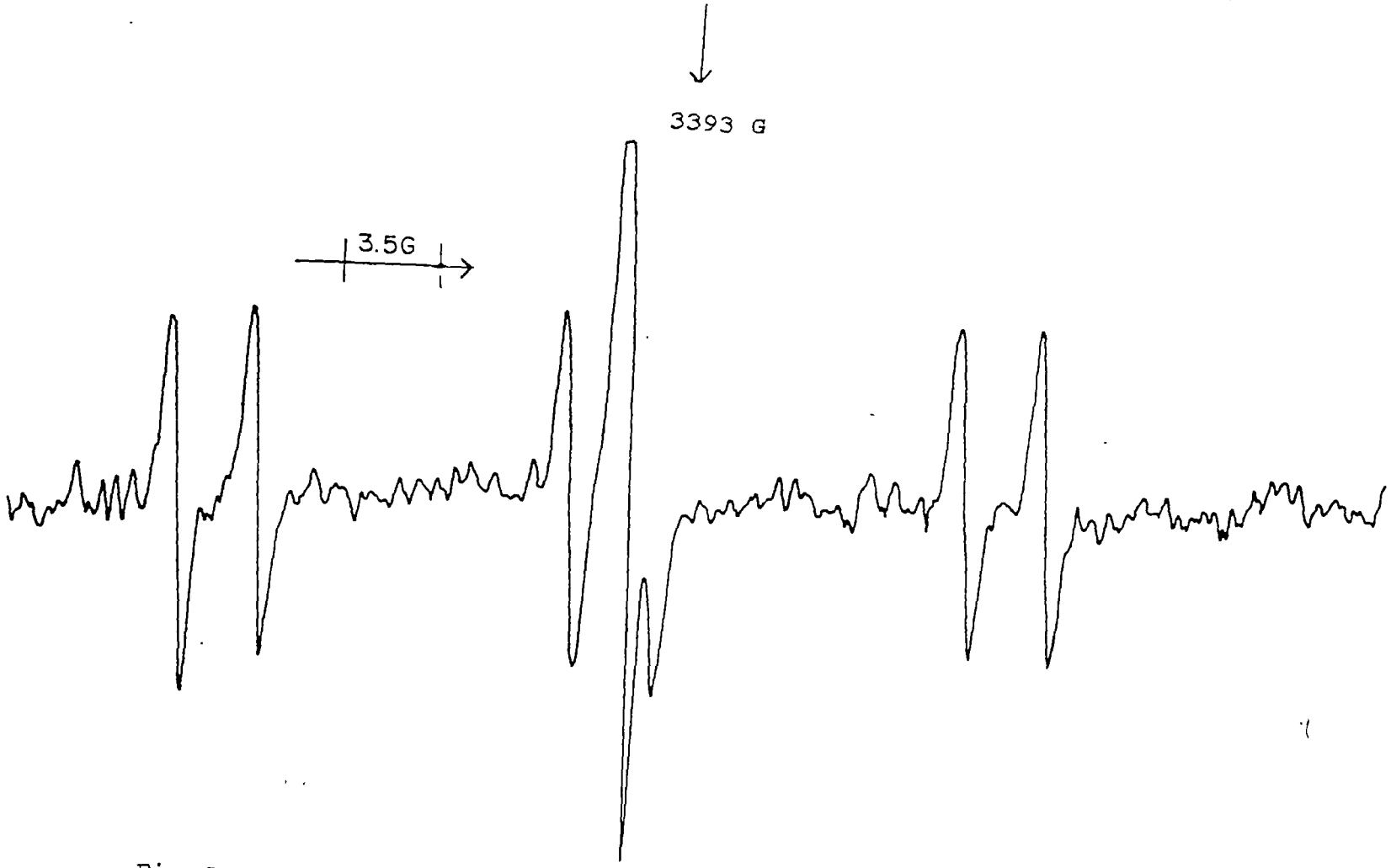


Fig.6. ESR Spectra obtained from the reaction of thymine, chloranil and nitrone in ethanol.

alcohol molecules. This is preferred over abstraction from Thymine cation because the solvated $CA^{\cdot-}$ has many surrounding alcohol molecules.

THYMINE / CHLORANIL / NITRONE SYSTEM IN ACETONITRILE

Methylcyanide is polar and very weakly hydrogen bonding and solvating agent. The objective of study in this solvent was to reduce the role of hydrogen bonding and solvation in the reaction pathway. In other words we wanted to explore the possibility of other pathways in the absence of strong hydrogen bonding but strong dielectric effect. Similarly , here too first blank experiments were performed .

(i) Nitrone \ Chloranil System.

Spectra observed consists of two species ; a singlet due to chloranil anion, and a doublet of a triplet. The hyperfine splittings calculated for the doublet of triplet (aN = 13.6G a H = 1.88 G) agrees very well with the hydroquinone adduct of PBN. After some time doublet of triplet disappears and only singlet is left behind. (Fig. 7)

(ii) Thymine \ Chloranil System.

Immediately a singlet appears, (Fig.8a) which is replaced with a doublet, (Fig. 8b). The hyperfine splitting of the doublet is 0.4 G, which appears to be due to semihydroquinone radical ($HCA^{\cdot}$), which decays out. No other signal was observed. This indicate that here too,

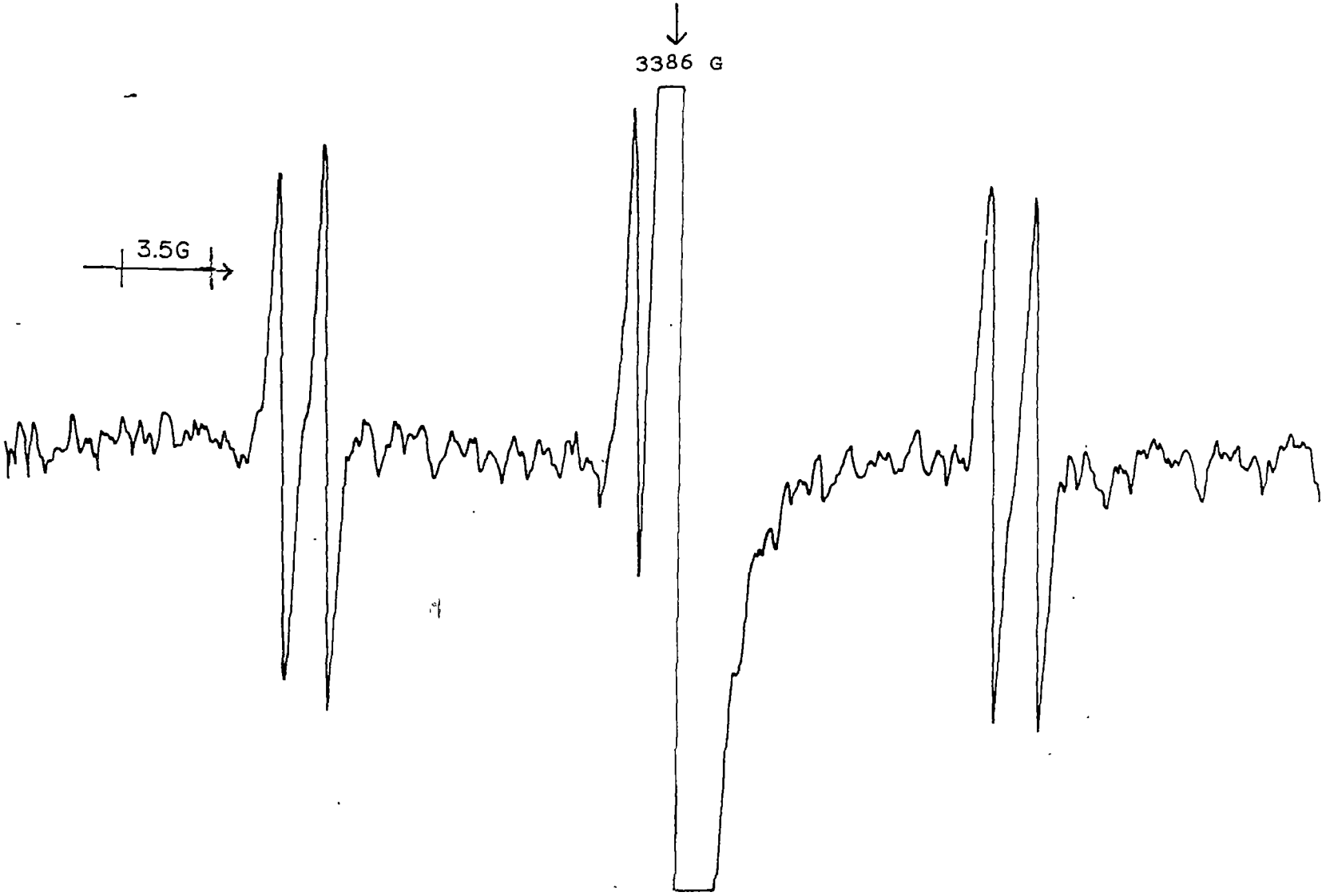


Fig.7 ESR Spectra obtained from the reaction of nitrone and chloranil in acetonitrile.

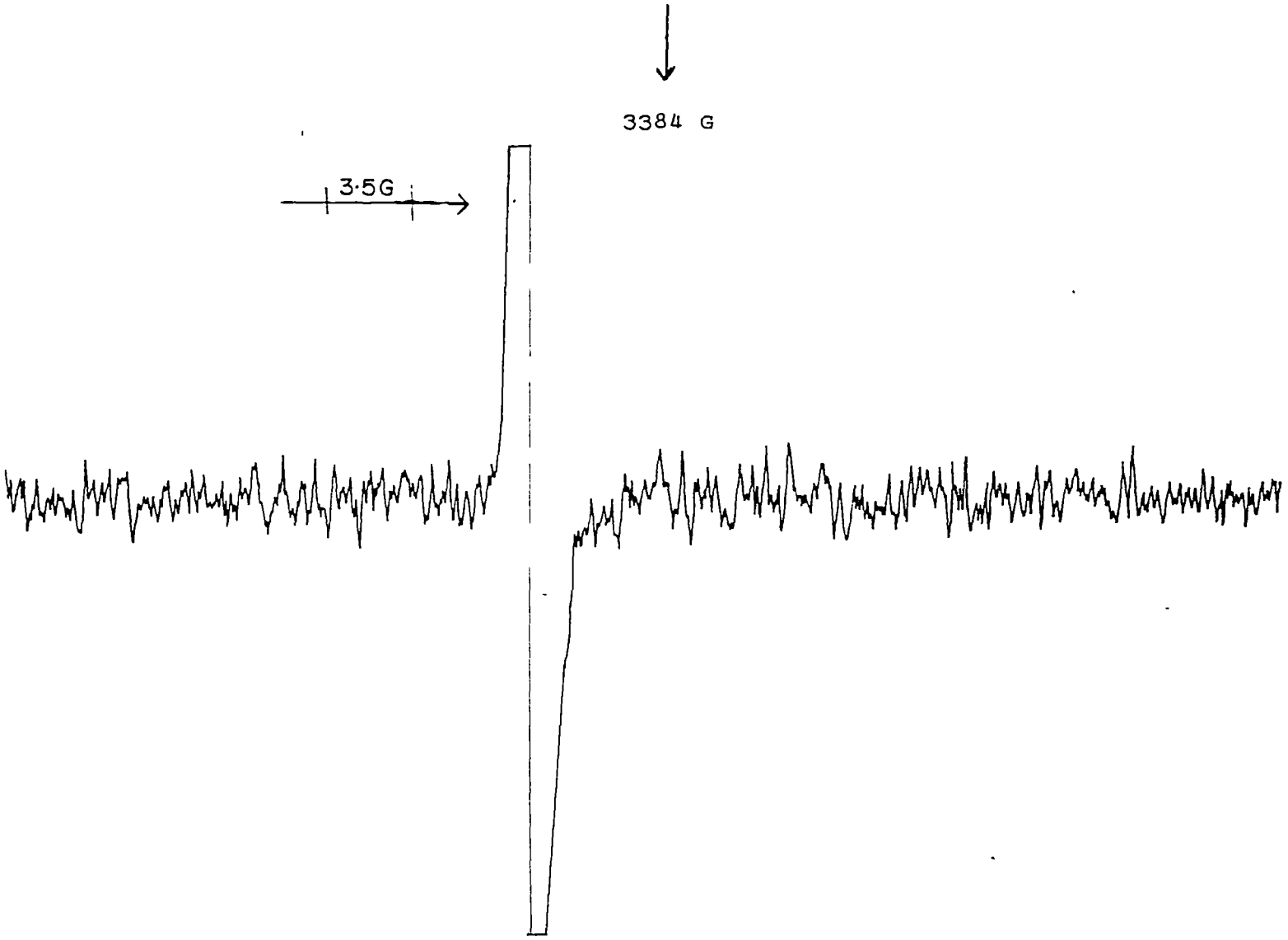


Fig.8a. ESR Spectra obtained initially from the reaction of thymine and chloranil in acetonitrile.

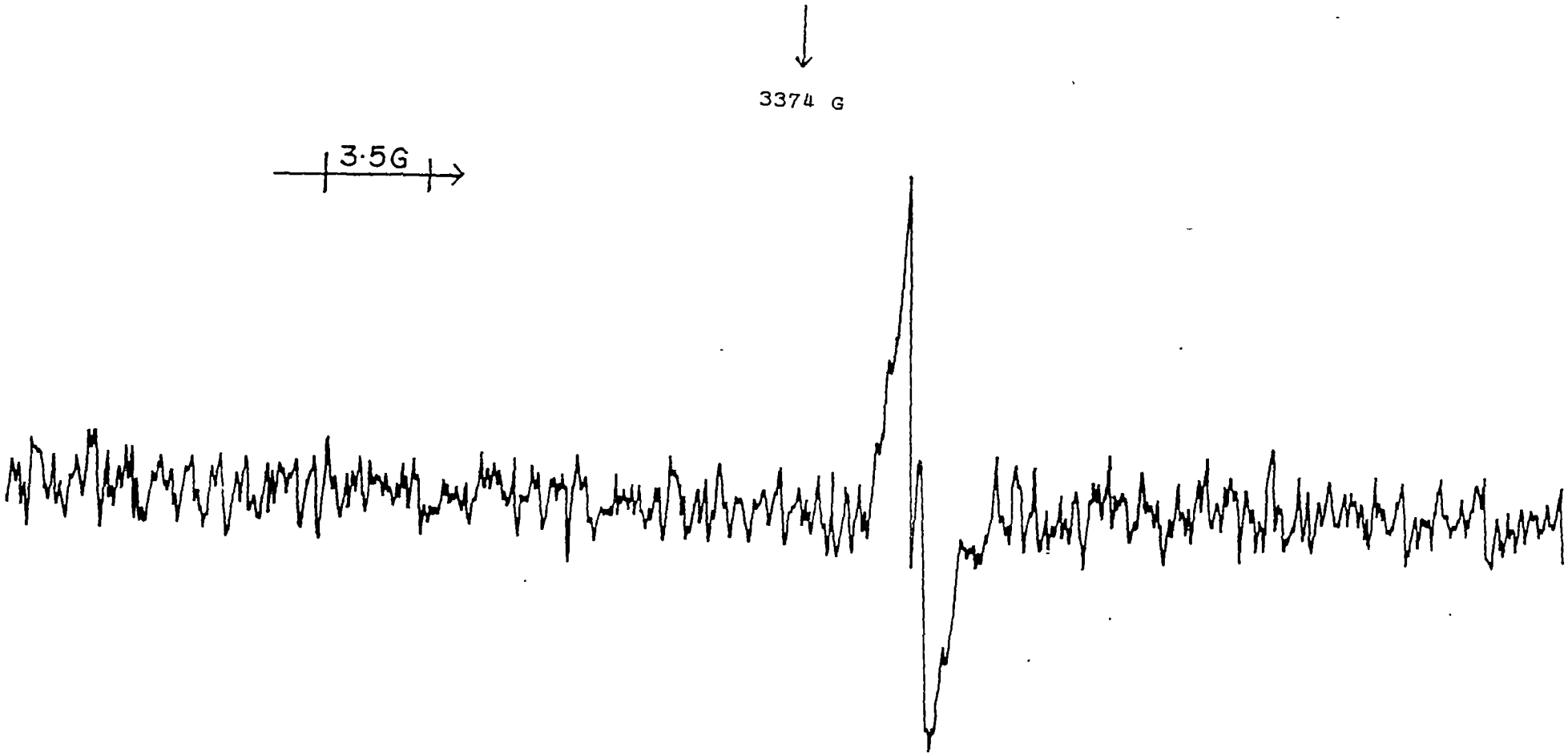


Fig. 8b. ESR Spectra, 8a recorded after a period of 1 hr.

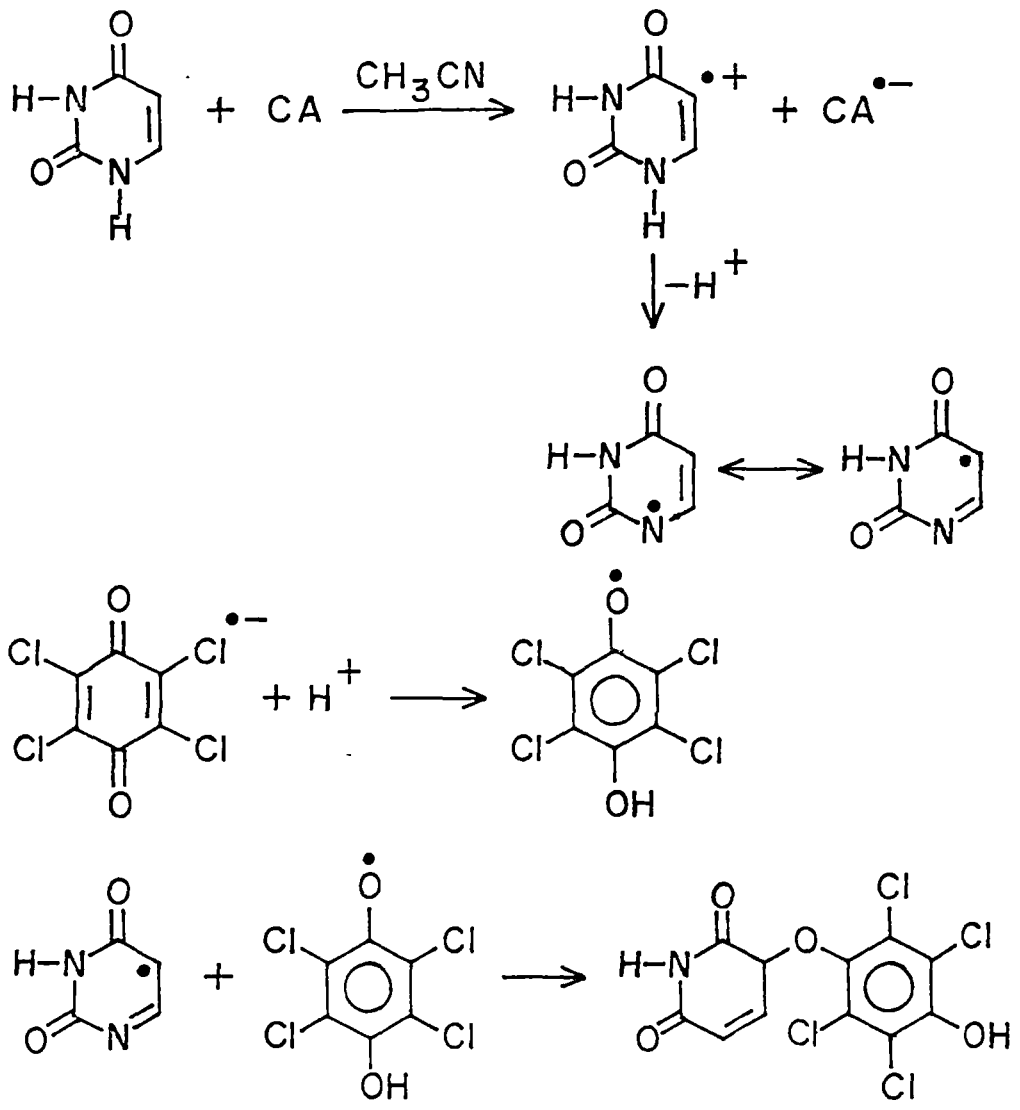
thymine has transferred an electron to Chloranil in the primary step. The replacement of singlet with a doublet strongly suggests that the abstraction of a proton by the $CA^{\cdot -}$ from the thymine cation has occured. This points out the possibility of the formation of a TS complex as shown in scheme II. In the absence of spin trap, no other specie could be observed.

(iii) Thymine \ Nitrone System

Degassed solutions of Thymine and Nitrone in acetonitrile were mixed and on immediate scanning no spectra was observed. After 2.5 hrs a poorly resolved spectra doublet of triplet with low signal to noise ratio was observed . The mere appearance of spectra indicate that electron transfer has taken place, but the reaction is so slow that it is very difficult to say that the spectra developed over 2.5 hrs. is due to only primary process. It is interesting to comment that in the case of 1, 4 dioxan, the electron transfer was fast enough and we could clearly identify and interpret spectra . This suggest strongly that the processes preceding to electron transfer are different from 1,4 dioxan.

(iv) Thymine \ Nitrone \ Chloranil System

The experiment was done exactly the way we did in dioxan. To our utter surprise we did not observe any spectra, neither immediately nor over a period of 1 -2 hrs. Though with blank



Scheme-III

experiments, spectra were observed indicating clearly the transfer of electron in the primary step. The absence of any spectra suggests that the radicals generated get involved in some other reaction processes. The $CA^{\cdot -}$ species that is generated is longer lived as indicated by the persistence of the spectrum in thymine and nitrone in blank experiment . Such a time span may be sufficient for the diffusive separation of the ion radical pair [3] to allow for a different reaction pathway, leading to the possibility of dimerisation reaction shown in scheme III. If our this inference is correct, then the additon of solvents like 1,4 dioxan should facilitate the formation of the adducts shown in scheme II, i.e it should lower down the reaction rate of dimerisation . Indeed when we added 10 % 1,4 dioxan to acetonitrile nothing was observed, then we added 40 % dioxan weak signals due to specie (vi in scheme II) were observed. Then we added 60 % dioxan, signals observed were higher in intensity and spectra similar to the one in dioxan was observed. This confirms our postulation that in acetonitrile , primary species formed follow different pathway.

II. URACIL / CHLORANIL / NITRONE SYSTEM

URACIL / CHLORANIL / NITRONE SYSTEM IN 1,4 DIOXAN

Uracil is structurally similar to Thymine except that at

C(5), there is no methyl group. In this system too, we proceeded in a similar way. First blank experiments were done and the results are described below ;

(i) Uracil \ Nitrone System

Degassed solutions of Uracil and Nitrone in 1,4 dioxan were mixed. Immediately observed spectra, Fig. 9 has very low signal to noise ratio, which improved over the time and doublet of triplet with aN = 14.12 G and aH= 2.1 G appeared. The redox potential of Uracil is 1.34 eV which is slightly higher than Thymine, which means that it would be weaker donor then Thymine. The reaction mechanism is shown in scheme IV. The absence of hyperfine splitting from proton at C5 suggests that the unpaired electron is delocalised as shown in structure 5 of scheme IV [5].

(ii) Uracil / Chloranil System

Degassed solution of thymine and chloranil in 1,4 dioxan when taken immediately produced a singlet. The radical is unstable as in the case of thymine and has the same g value. In the absence of the spin trap no other transient species could be observed.

(iii) Uracil / Nitrone / Chloranil System

Experimental procedure followed was the same as in the case of thymine. The spectra, initially consisted of a singlet due to chloranil anion which decays within a few minutes along with some weakly resolved signals, which however grew

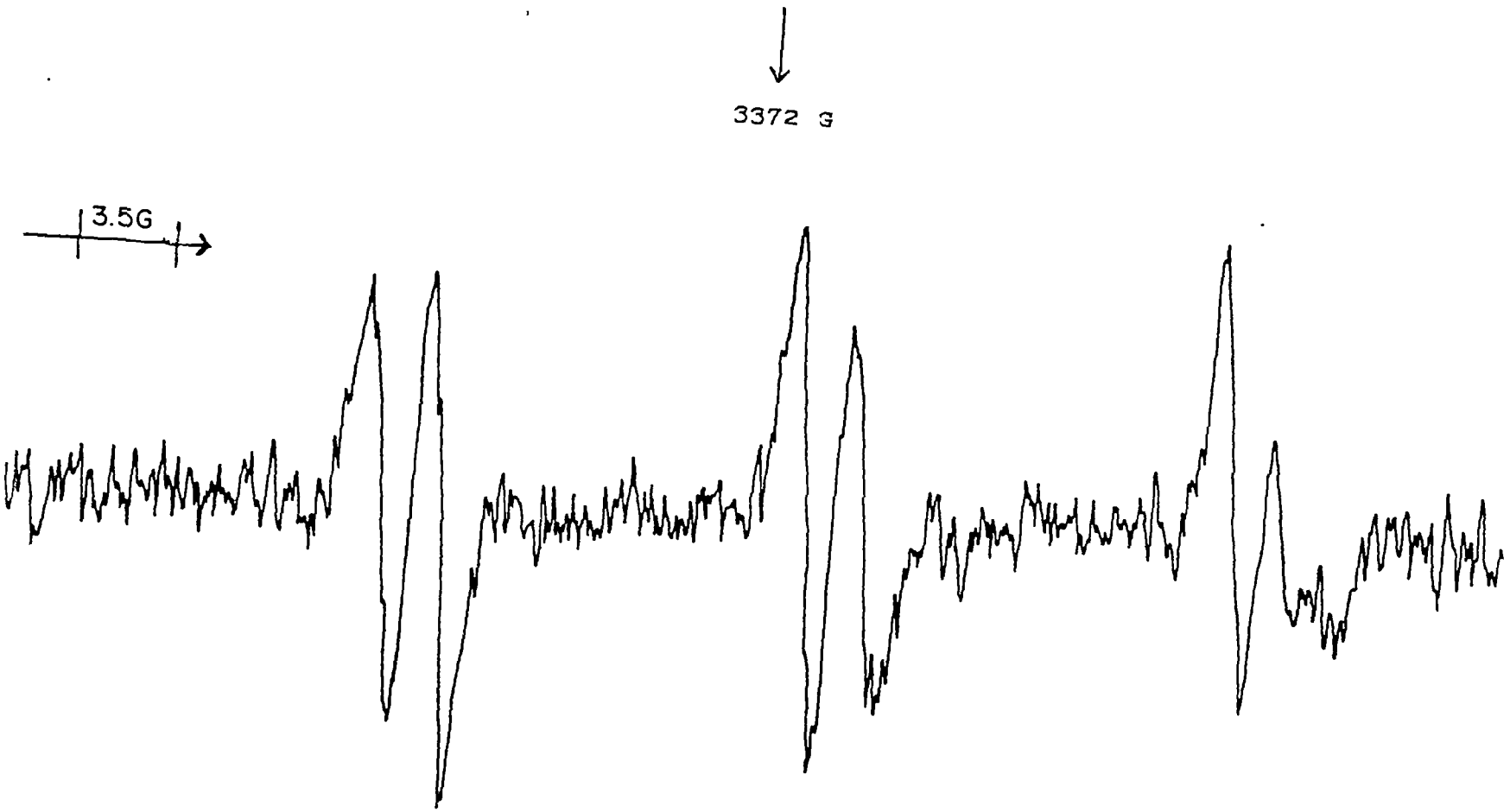
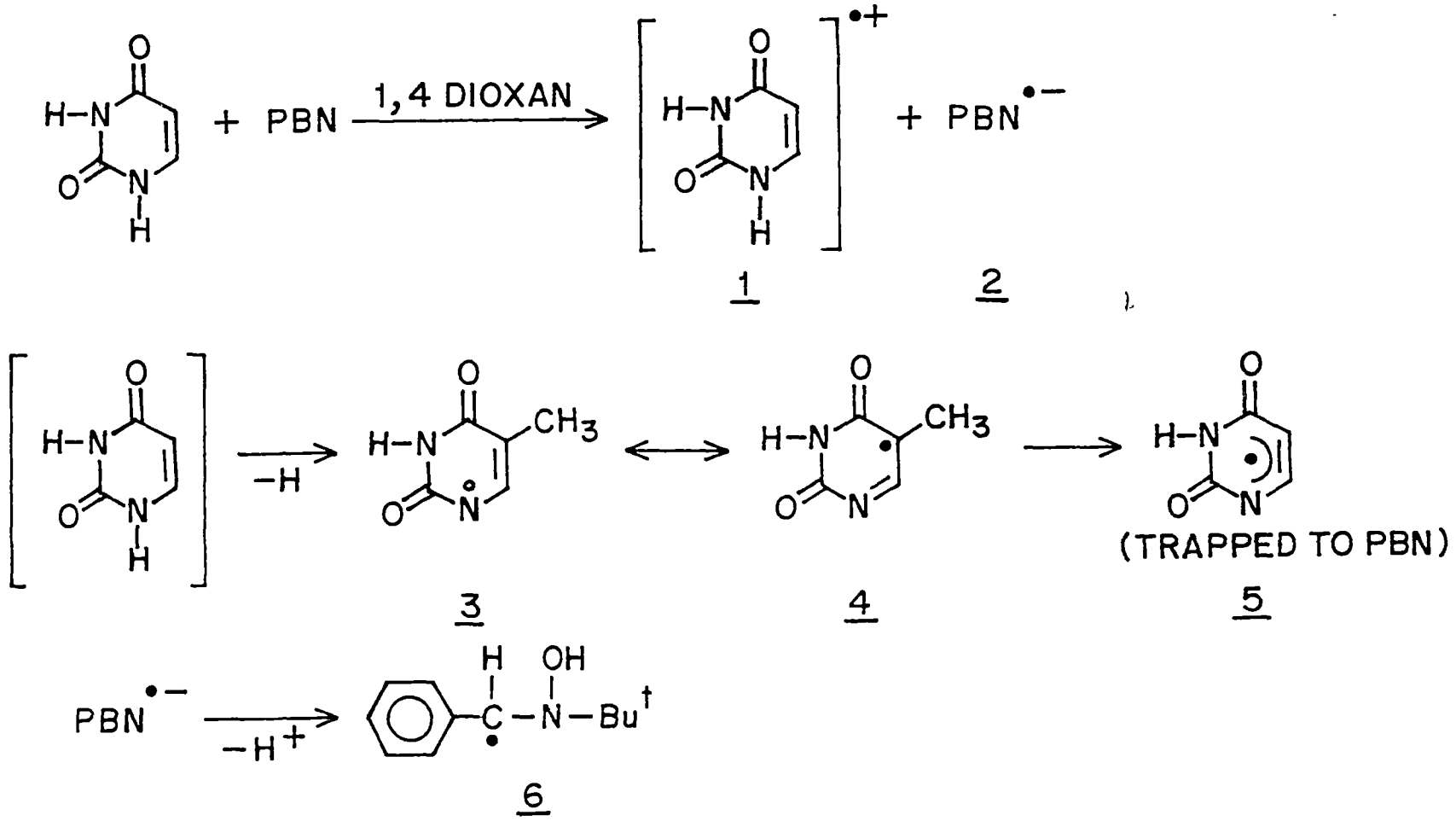
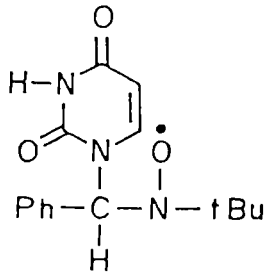


Fig. 9. ESR Spectra obtained from the reaction of Uracil and nitrone in 1,4 dioxan



Scheme-IV

in intensity over a period of time, (Fig. 10). The spectra can be analysed along similar lines as thymine , i.e it consisted of a major triplet with aN value 13.5G due to a primary nitrogen which further splits up into a triplet with aN value of 2.5g due to a secondary nitrogen which then further splits up into a doublet with aH value of 1.8G. There were further splittings as in the case of thymine caused due to a proton at C 6. The poor resolution due to C6 proton could be due to the absence of the methyl group at C5 in uracil which causes a reduced electron density at C6 as compared to thymine.C5. The spectra is attributed to the formation of the adduct.



MECHANISM :

The mechanism postulated is similar to that of thymine. Uracil donates an electron to chloranil generating $CA^{\cdot -}$ which abstracts a proton from the uracil radical cation and the subsequent addition of uracil radical to PBN to give the final adduct as shown above.

URACIL / CHLORANIL / NITRONE SYSTEM IN ALCHOHOL

As said earlier alcohols due to their high solvating power, influences the TS state formation. In the case of Uracil

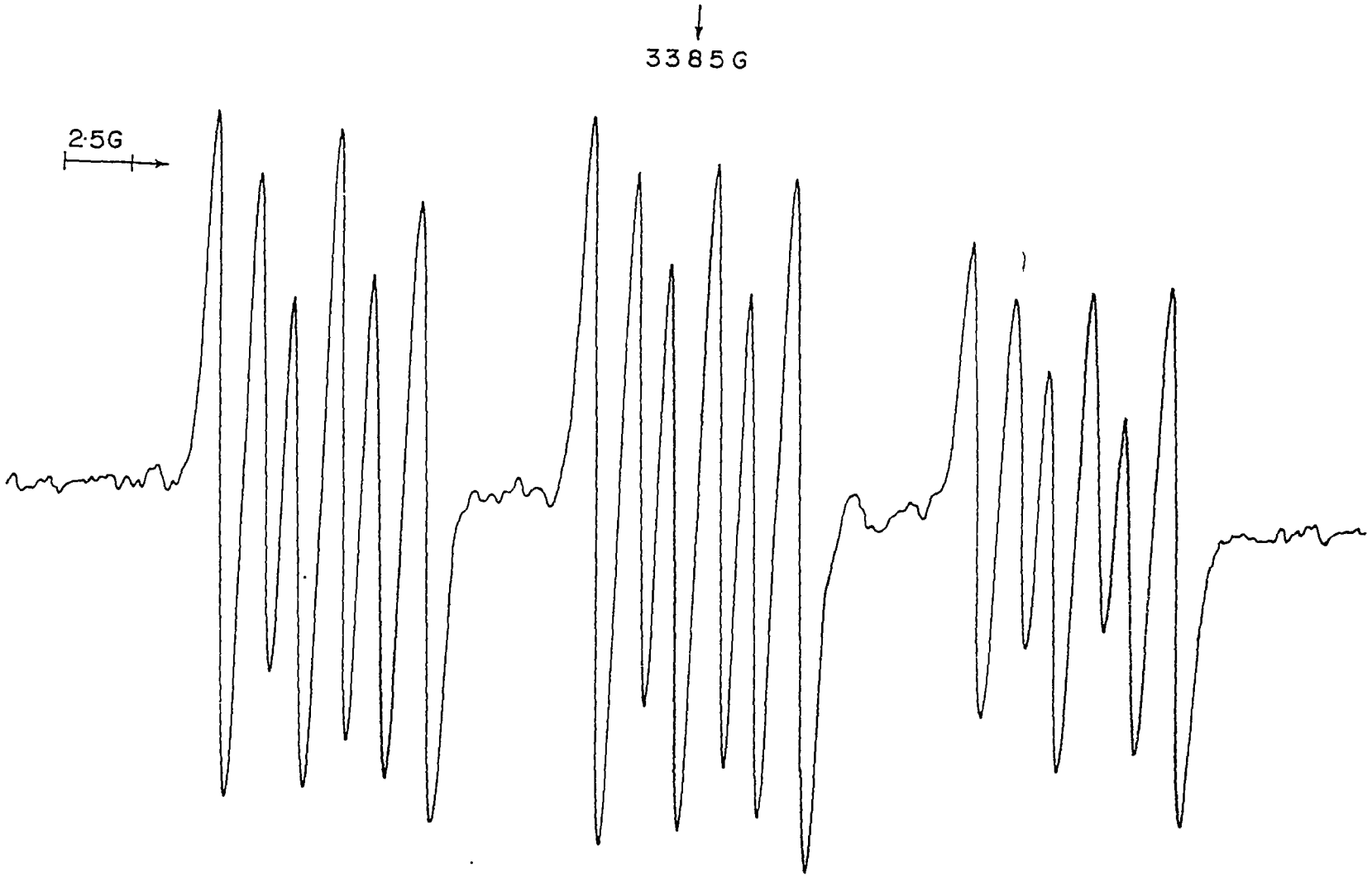


Fig.10. ESR Spectra of the reaction between uracil, chloranil and nitrone in 1,4 dioxan.

too, blank experiments were done and the results obtained were similar to that of thymine.

Uracil / Chloranil / Nitrone System

Degassed solution when mixed according to the procedure enumerated above, a singlet was initially observed due to formation of $CA^{\cdot -}$ which was stable. Another doublet of a triplet with aN = 14.4 G and aH = 2.8 G was also observed. As said in the case of thymine that $CA^{\cdot -}$ is stabilised through solvation with the alcohols molecules and hence abstraction of the proton occurs from the abundantly available alcohol molecules, and the spectra is exclusively of the RO-PBN adduct. The fate of the Uracil radical is however unknown in this process.

URACIL / CHLORANIL / NITRONE IN ACETONITRILE

The results of the blank experiments are similar to those of thymine

(i) Uracil / Chloranil System Initially a singlet is observed which is replaced by a doublet, again assigned to the semiquinone radical formed by transfer of electron from uracil to chloranil and subsequent deprotonation of Uracil cation.

(ii) Uracil / Nitrone System

In this experiment, only a doublet of a triplet which was poorly resolved appeared only after 3 hrs. with no signs of

any other signal. The result as in the case of thymine too indicate that electron transfer process is less facile in acetonitrile as compared to 1,4 dioxan.

(iii) Uracil / nitrone / chloranil system

Similar to the experiment with thymine initially no signals were observed. The $CA^{\cdot -}$ is sufficiently longer lived in acetonitrile leading to a fast dimerisation reaction as had been shown in scheme III. When attempts were made to slower down the reaction by addition of 1,4 dioxan, we obtained the similar results as we got from Thymine. This seems to suggest that although the primary processes are the same, the subsequent steps are different. The striking change over of the results in 1,4 dioxan and acetonitrile, derives from the strong solvent modulation of the ion-pair dynamics [6].

III. CYTOSINE \ CHLORANIL \ NITRONE SYSTEM.

CYTOSINE \ CHLORANIL \ NITRONE SYSTEM IN 1,4, DIOXAN

Cytosine also belongs to the pyrimidine class of bases. It differs from Thymine and Uracil in that a carbonyl group is replaced by an amino group. Similarly here too, blank experiments were done first and results are presented below

(i) Cytosine \ Nitrone System.

In this system, the results are identical to Uracil system, (Fig. 11a), a doublet of triplet with aN = 14.5 G , aH = 2.5

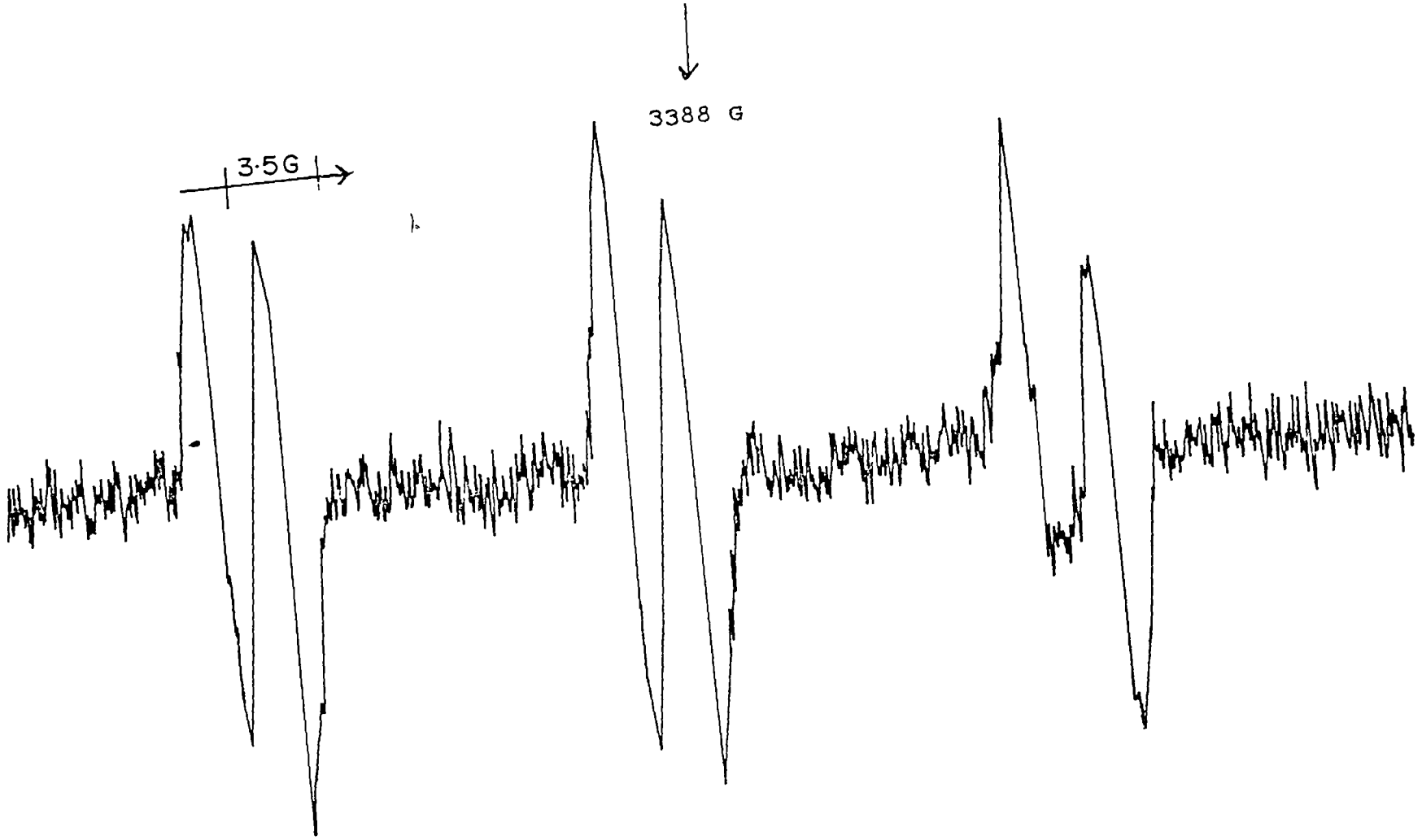


Fig.11a. ESR Spectra of the reaction between cytosine, and nitrone in 1,4 dioxan.

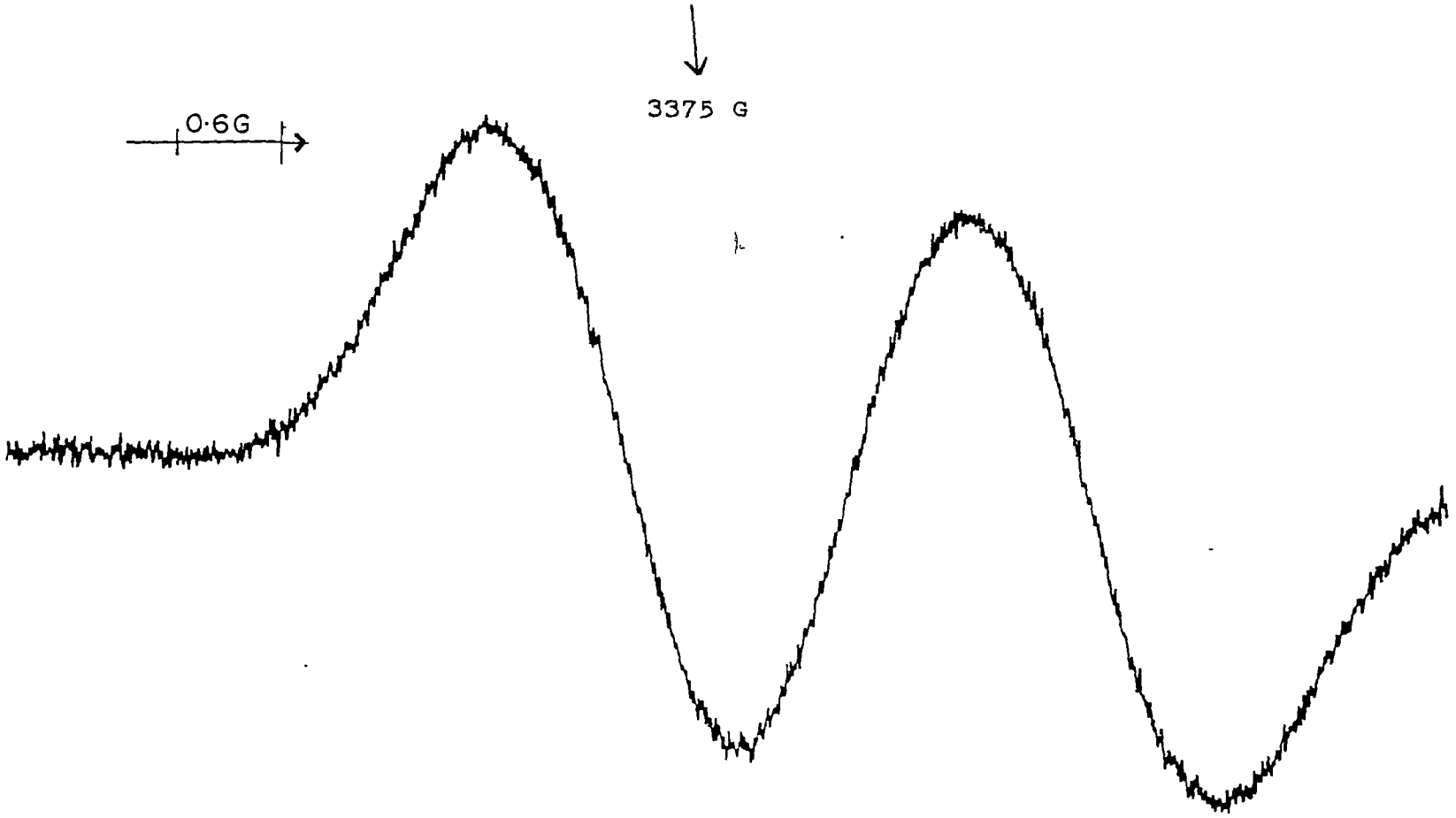
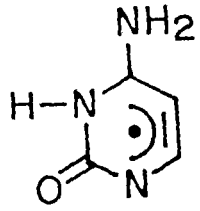


Fig.11b. ESR Spectra of one component of 11a run at a small field range and a high scan time.

G. The structure of the radical trapped is given below ;



MECHANISM

Fig. 11b shows one spectra scanned over a small range of field at a low scan speed The mechanism for the formation of the radical shown above is similar to the one discussed for Uracil.

(ii) Cytosine \ Chloranil System

A singlet due to chloranil anion was observed, a consistent feature with the pyrimidine bases.

(iii) Cytosine \ Chloranil \ Nitrone in 1,4 dioxan.

When we mixed the degassed solutions as we did with other pyrimidine bases, the spectra developed immediately is doublet of triplet with aN = 13.40 G and aH = 1.56 G, Fig. 12a. This spectra was identified as due to hydrogen abstracted adduct of 1, 4 dioxan with PBN. Inspite of our repeated efforts, we did not see a singlet due to chloranil anion ($CA^{\cdot-}$). It seems that $CA^{\cdot-}$ formed reacts very fast with the cation formed from Cytosine or with 1,4 dioxan.

At a later stage the doublet of triplet is replaced with a

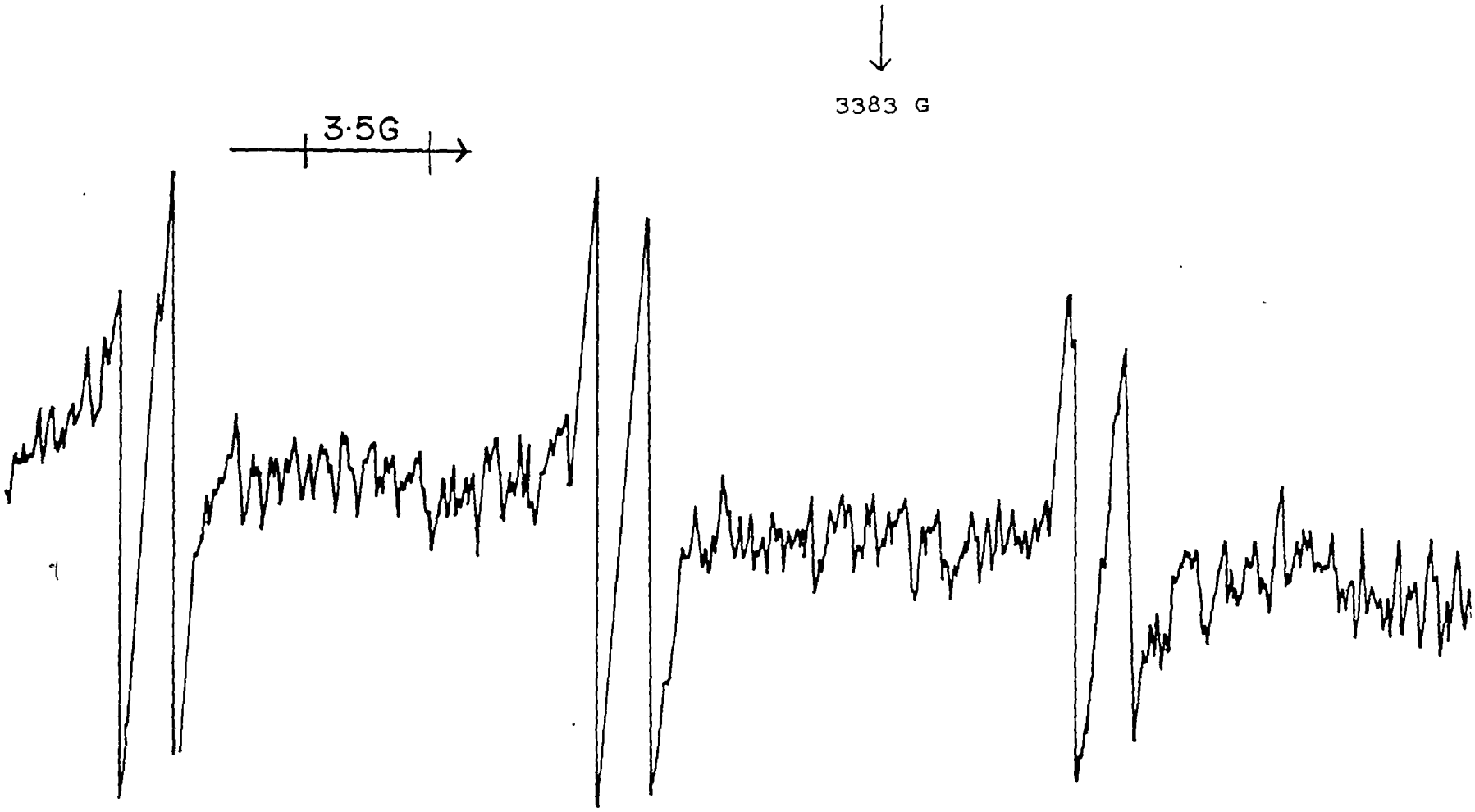


Fig.12a. ESR Spectra obtained initially from the reaction of cytosine ,chloranil and nitrone in 1,4 dioxan.

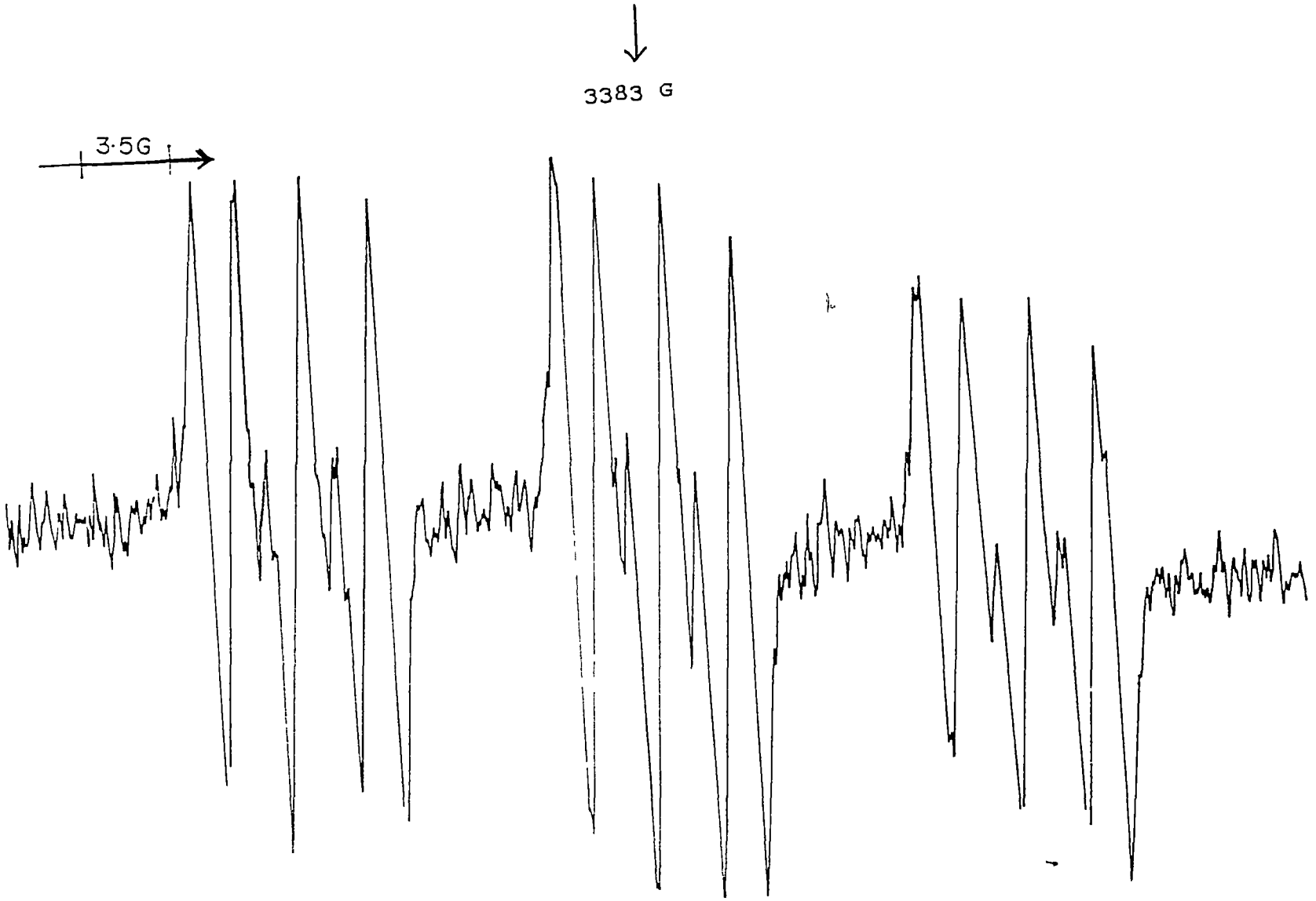


Fig.12b. ESR Spectra , 12a obtained after a period of 20 mins.

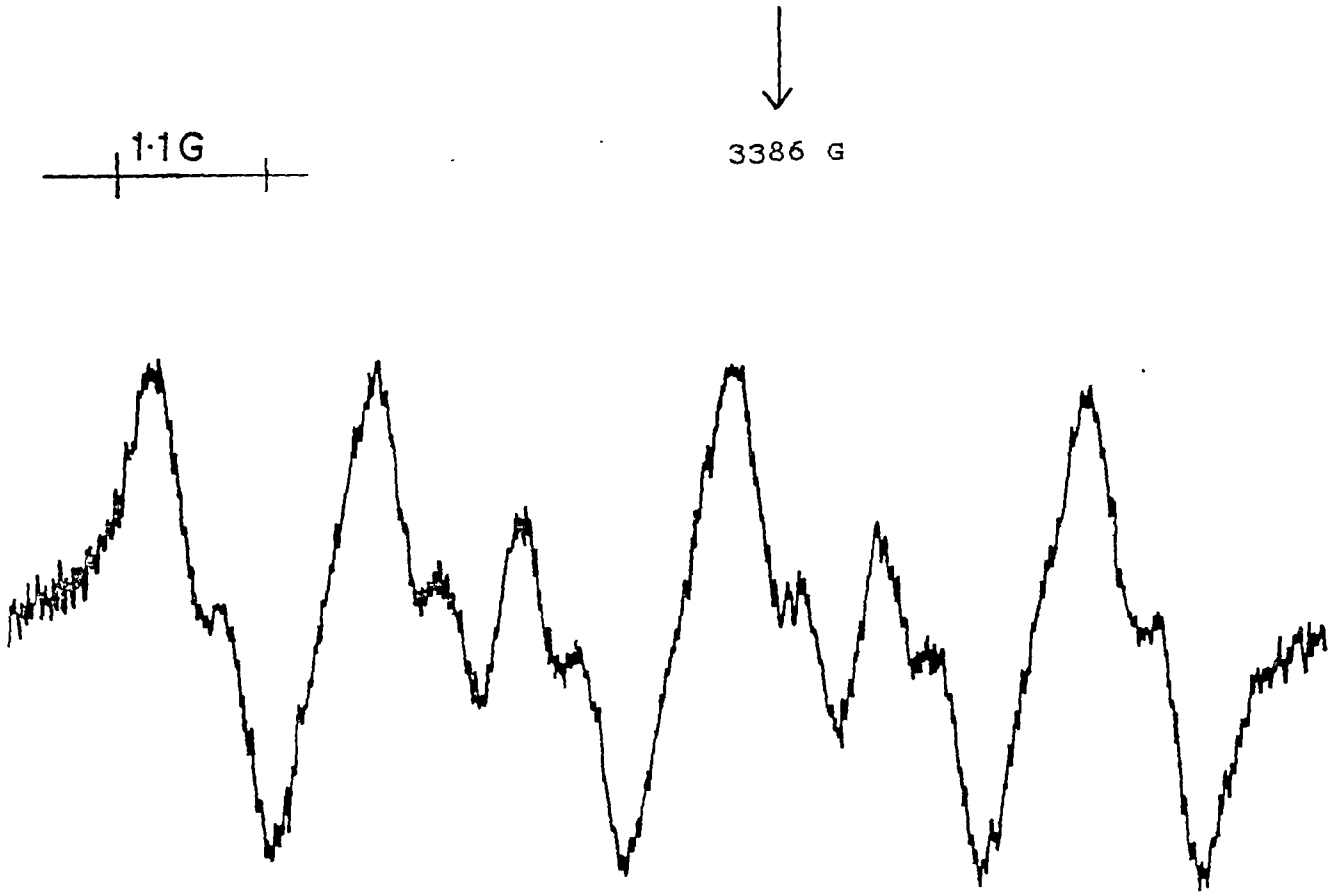
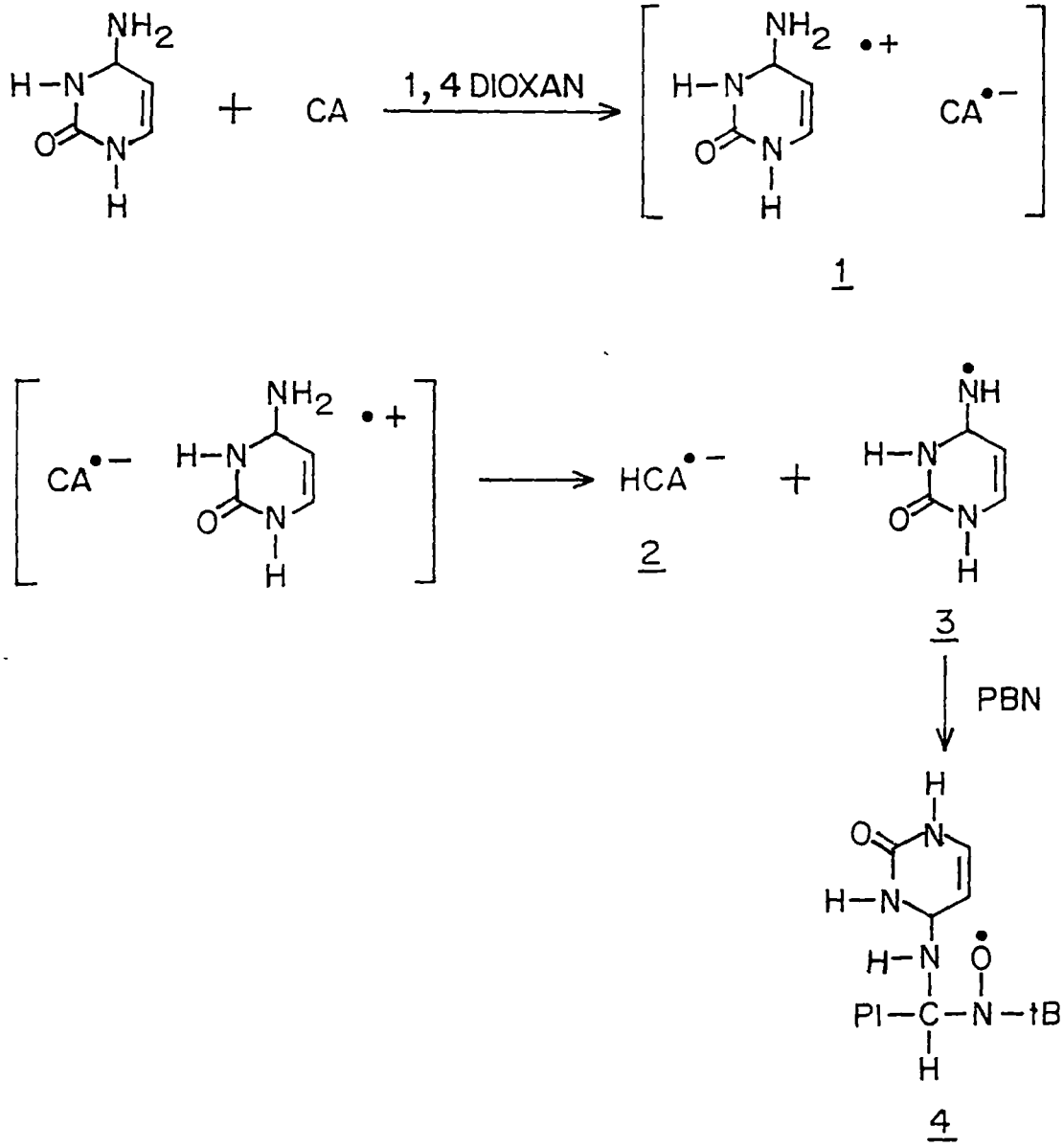


Fig. 12c. ESR Spectra of one component of 12b run at a small field range and a high scan time.


NH2
H–N
O
N
H
+
CA
1, 4 DIOXAN
NH2 •+
CA•–
1
CA•–
NH2 •+
HCA•–
2
NH
3
PBN
H
O
N
H–N
H–N
O
Pl–C–N–tBu
H
4

Scheme – V

spectra identical to the adduct 5 (scheme II). The hyperfine parameters are aN = 13.75 G , aN2 = 2.5 G, aH1 = 1.8 G, Fig. 12b. When one of the components of a triplet was scanned over a small range of field at a very low scan speed, further splitting of each line into a doublet with a hyperfine splitting of aH= 0.7 G, Fig. 12c was observed. On the surface the spectra can be interpreted on similar lines as for adduct 5 in scheme II. But, to our surprise identical spectra was observed when we replaced Cytosine with Cytidine (N (1) is substituted with sugar molecule). This points out clearly that in Cytosine deprotonation does not occur from N (1) unlike Thymine or Uracil ,but from amino group at C4. The reaction scheme V is shown. To our knowledge our this result is in contrast to that of Steenken etal, who has shown deprotonation from the ring nitrogen and the delocalisation of unpaired electron [7].

CYTOSINE \ CHLORANIL \ NITRONE SYSTEM IN ALCOHOL.

In alcohols the blank experiments were similar to those of Thymine and uracil.

Cytosine \ Chloranil \ Nitrone System

The spectra due to this was dominated by a doublet of a triplet with splittings of aN = 14.4 G and aH =2.8 G, which results basically from the abstraction of proton from alcohols to PBN. The rate of addition of the RO· radicals

being fast we could not observe any other signals.

CYTOSINE \ CHLORANIL \ NITRONE IN ACETONITRILE

Here too the blank experiments were similar to that of Thymine and Uracil.

Cytosine \ Chloranil \ Nitrone

In pure acetonitrile no spectra was initially observed. However in acetonitrile dioxan mixtures, a singlet was initially observed which soon disappeared with the appearence of signals as in the case of pure dioxan and hence can be assigned as due to the same radicals.

IV. ADENINE / CHLORANIL / NITRONE SYSTEM

Adenine belongs to the Purine class of bases. It consists of an additional group imidazole, which increases electron density on the purine ring. Spectroscopic studies have indicated that purine bases undergo stronger association in non-polar solvents as compared to pyrimidine bases. So far we have observed that the deprotonation of the pyrimidine bases is a common process and fairly fast. In Purine bases , the probability of electron delocalisation is relatively stronger, therefore, the stability of cationic species formed may be higher, which in turn may follow different pathways.

ADENINE \ NITRONE \ CHLORANIL IN 1,4 DIOXAN

(i) Adenine \ Nitrone System.

The spectra developed immediately on mixing degassed solutions, consists of a doublet of triplet, with aN = 13.88 G and aH = 2.50 G, Fig. 13. Adenine has red. pot. of 1.32 eV. It will therefore, transfer an electron to Nitrone. The cation generated can undergo either deprotonation or hydration (anion addition) [8]. In this case probably the deprotonation of the cation is relatively a slower process as compared to that of the pyrimidine bases. This relatively longer life time is probably responsible for a different pathway. We suggests the following mechanism, scheme VI ; The probability of C6-OH has been shown to be slightly more [9].

(ii) Adenine \ Chloranil System

In this blank experiment we initially observed a singlet along with some very weak signals , which decayed out quickly. The singlet is replaced with a weak doublet due to hydroquinone radical ($HCA^{\cdot-}$).

(iii) Adenine \ Nitrone \ Chloranil System

On mixing degassed solutions, immediately no spectra was observed. However, after some time spectra observed could be analysed due to two species A and B, Fig. 14. The hyperfine parameters for A are ; aN = 13.75 G , aN2 = 2.80 G, aH = 1.70 G. For B ; aN = 14.00 G, aN2 = 4.10 G and aH = 1.70 G.

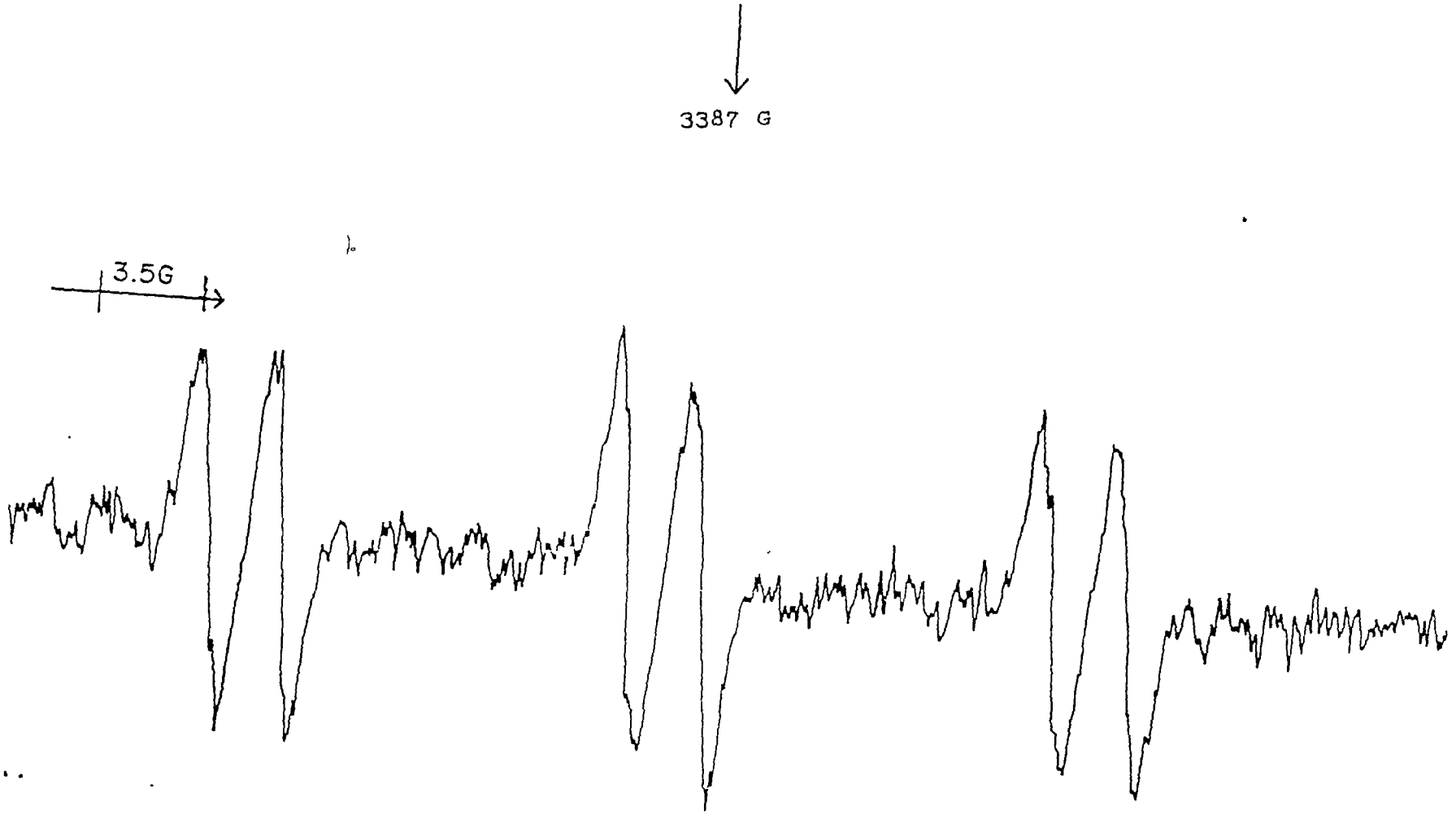
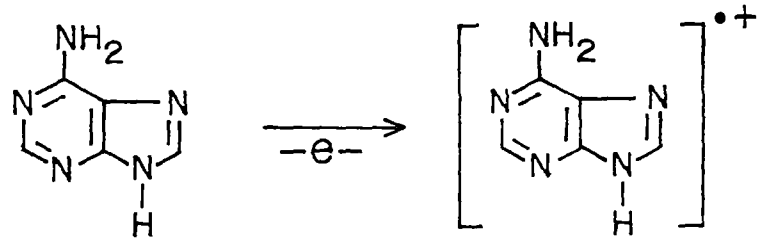
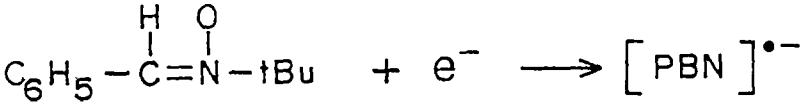


Fig. 13. ESR spectra obtained from the reaction of adenine and nitrone in 1,4 dioxan.

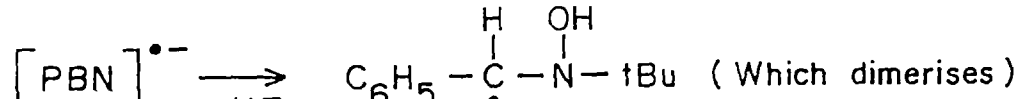


1

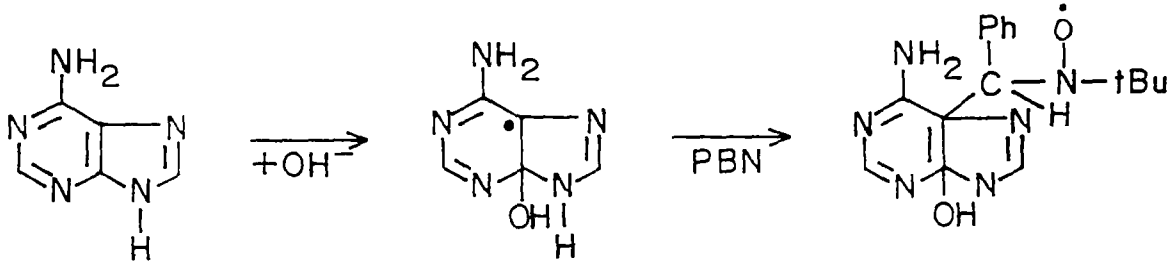


2

$$H_2O \rightarrow H^+ + OH^-$$



3



4 5

Scheme – VI

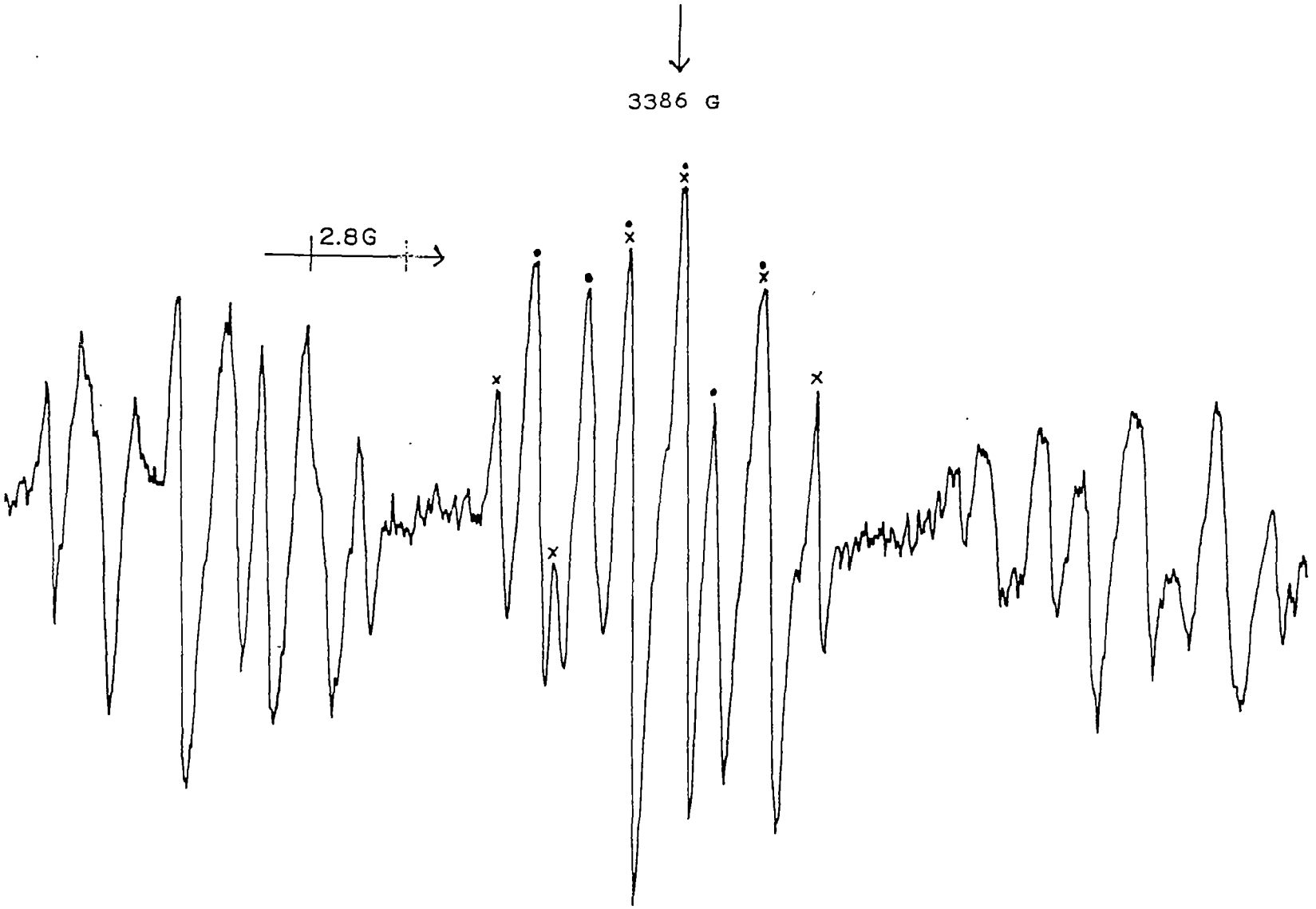


Fig. 14a. ESR Spectra obtained from the reaction of adenine, chloranil and nitrone in 1,4 dioxan. • (A); × (B)

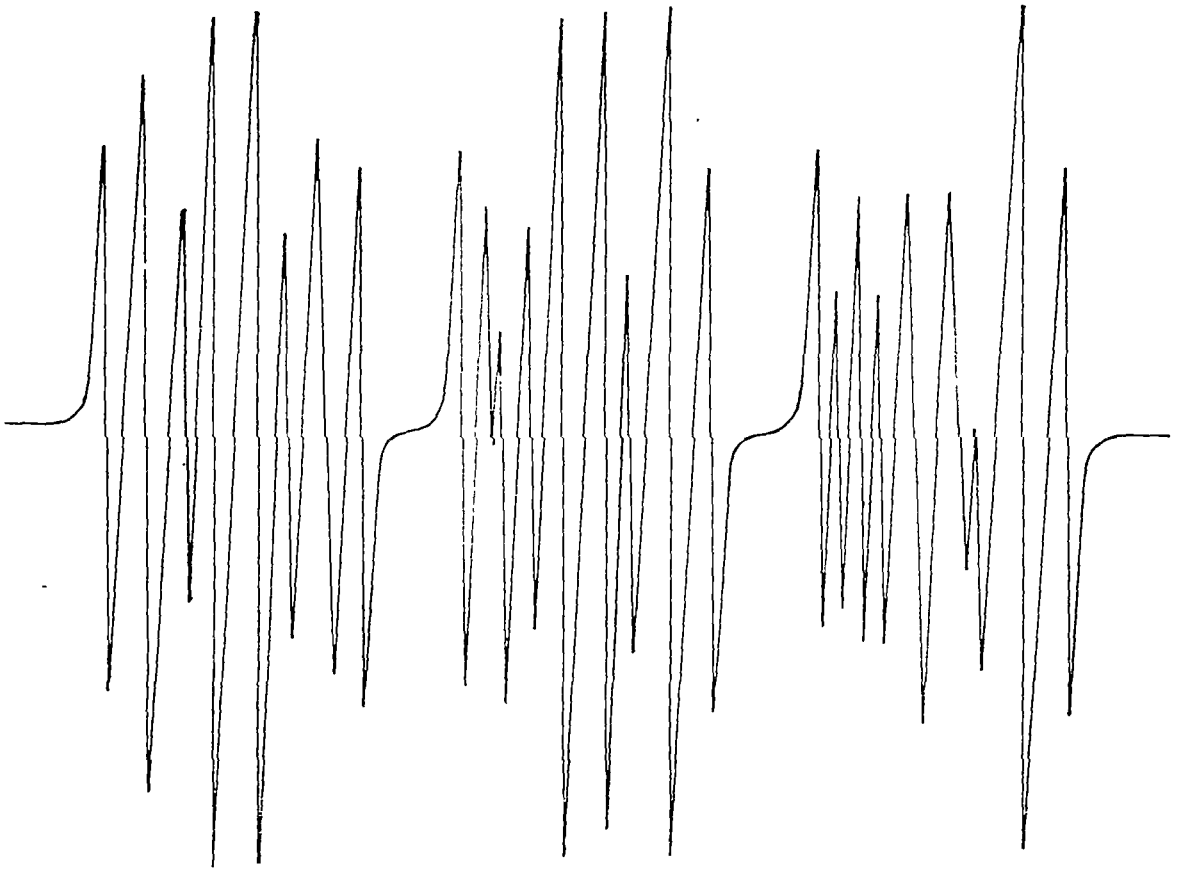


Fig. 14 b. Computer simulated Spectra of 14a, using the hyperfine parameters calculated from the experimental spectra ; (i) • L.W = 0.4 G ; (ii) × L.W = 0.4 G , ΔG = -0.2G

The parameters for A are similar to the adduct we assigned for Cytosine. We thus postulate a similar radical for Adenine too (as proposed be Steenken also, [10]). Also there exists a possibility of deprotonation from N 9 . If it were the case, the adduct formed would have a higher aN2 splitting value. The chloranil anion generated in this system will form a similar TS complex as shown for pyrimidine bases in Scheme II. The deprotonation will occur from both N (at C6) and N9 sites generating radicals (A) and (B). Both adducts A & B are fairly stable. The structures for adduct A and B are as shown in scheme VII

Fig. 14b shows a well simulated spectra with the hyperfine parameters obtained

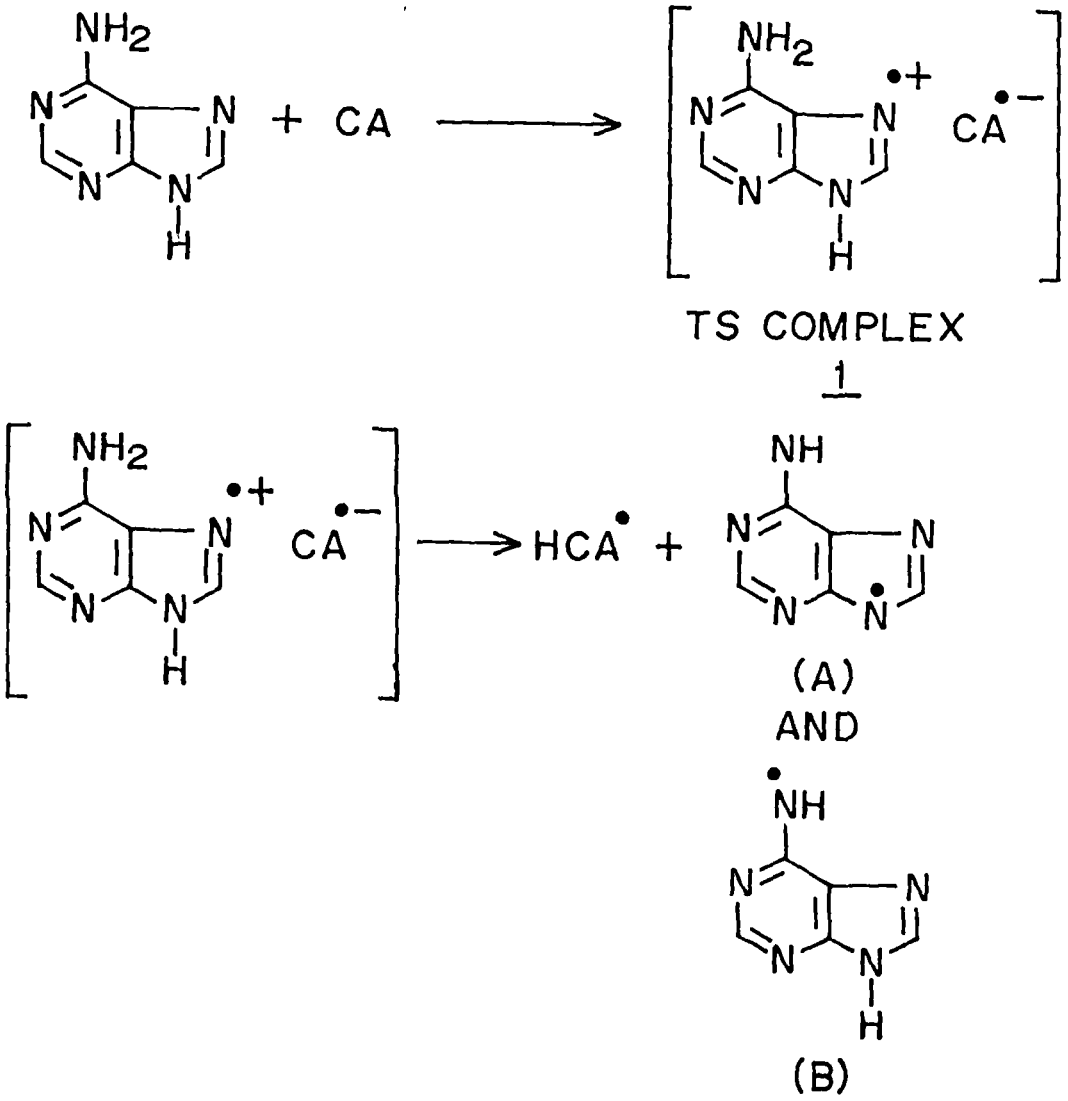
When one component of a triplet was scanned over a small field range at a very slow scan speed we did not observe any finer splitting, may be due to the overlapping of spectras.

ADENINE \ NITRONE \ CHLORANIL IN ALCOHOLS.

(i) Adenine \ Nitrone system in methanol.

In this set similar doublet of a triplet as with the pyrimidine bases was observed. Adenine \ Chloranil in Methanol.

This experiment produced interesting results The usual chloranil anion singlet along with another broad singlet with g = 2.0035 was observed. This broad singlet is observed for the first time, Fig. 15. The g value is very near to



Scheme-VII

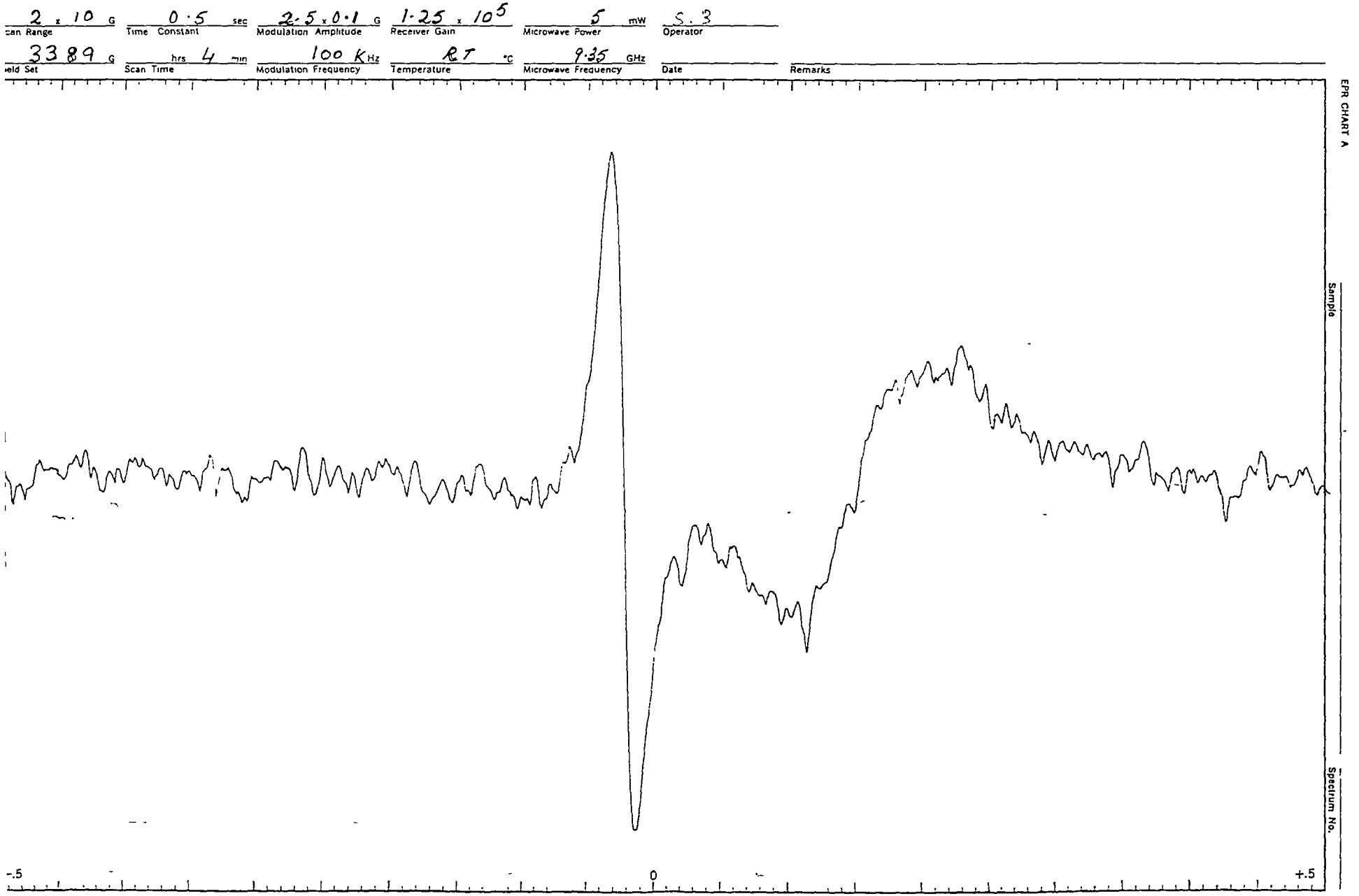


Fig. 15. ESR Spectra obtained from the reaction of adenine chloranil and nitrone in methanol.

DPPH a known carbon centered radical . From the g value we are inclined to postulate this radical as due to cation of adenine. This is the only assignment we can think of. The presence of the imidazole ring causes greater delocalisation and hence imparts stability.

(ii) Adenine \ Nitrone \ Chloranil system in Methanol.

The similar doublet of a triplet with aN = 14.40 G and aH = 2.80 G was observed, which has been assigned as RO -- PBN adduct due to hydrogen abstraction from alcohol molecules.

ADENINE \ CHLORANIL \ NITRONE IN ACETONITRILE.

When degassed solutions were mixed, the spectra immediately observed can be analysed as (i) singlet due to chloranil anion, (ii) a doublet of triplet with aN = 13.55 G and aH = 1.75 G, which has been assigned to hydroquinone radical adduct of PBN. (iii) Some weak signals which grew to a well resolved spectra. The spectra due to (ii) disappeared after some time. The spectra due to (iii) was analysed as overlapping due to two species A and B, Fig. 16. The hyperfine splitting for A ; aN = 14.00 G, aN2 = 3.90 G and aH = 1.80 G, for B ; aN = 14.10 G, aN2 = 2.80 G and aH = 2.00 G. The hyperfine parameters and splitting pattern suggests the trapping of the same radicals as with 1,4 dioxan. The slight differences in splitting constants are due to solvent effect. The observance of A and B in

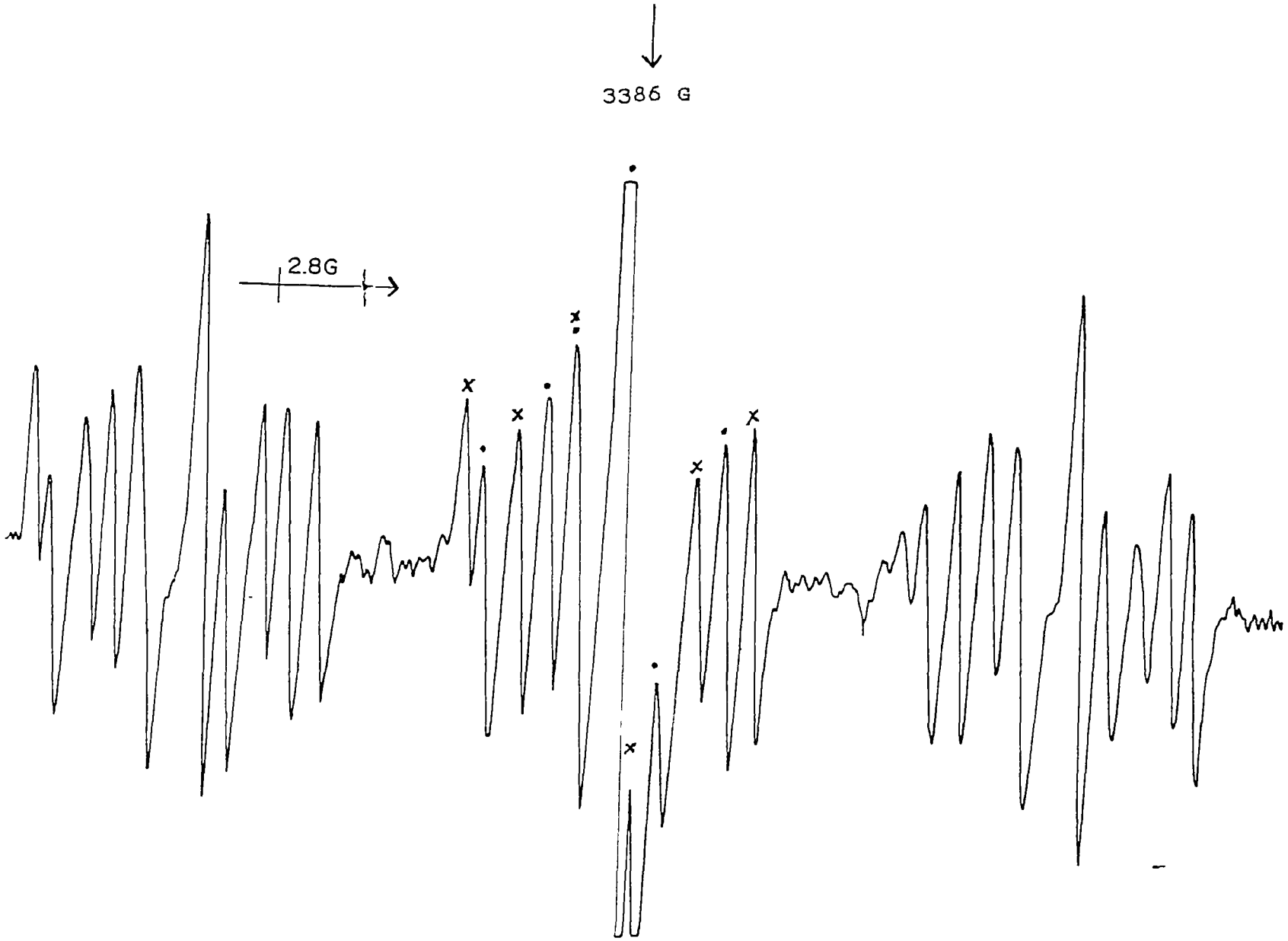


Fig. 16. ESR Spectra obtained from the reaction of adenine, chloranil and nitrone in acetonitrile× (A); • (B)

acetonitrile supports our postulation that the Adenine radical cation is relatively more stable than those of pyrimidine bases.

REACTION WITH DIBENZOYL PEROXIDE

Considerable attention has recently been focused on the mechanism through which cellular damage is induced by a large number of organic peroxides (such as benzoyl peroxide) and related compounds, which are used extensively in the chemical, pharmaceutical and cosmetic industries. In fact dibenzoyl peroxide is extensively used as a topical medication for the treatment of facial acne and was discovered to be a potent promoter of carcinogenesis. A number of these materials have been shown , in animal models, to act as tumour promoters in the multistage model of carcinogenesis, though they are not initiators or complete carcinogens. Evidence has been provided to support the hypothesis that these effects are mediated through the generation of free radicals from these compounds. Benzoyl peroxide can undergo one electron reduction , to give free radicals which can subsequently damage DNA.

The decomposition reactions of dibenzoyl peroxide with the pyrimidine bases was attempted by employing spin trapping technique [11]. We did not employ any photochemical means We

choose to study the reaction at room temperature without employing any means of excitation.The spin trap used is 2-methyl 2-nitrosopropane (MNP), the reason for its use shall be commented upon later.

(i) Pyrimidine bases \ MNP in acetonitrile

When degassed solution of the bases were mixed no spectra were observed even over a long period of time. The redox potential of MNP is 2.06 eV.

(ii) Pyrimidine bases \ dibenzoyl peroxide in acetonitrile

In this case too , no signals were observed. Although we presume that electron transfer might have occured , the short life time of the intermediates restriced its observance.

(iii) Benzoyl Peroxide \ MNP in Acetonitrile.

Here too, we did not observe any signal.

THYMINE \ BPO \ MNP IN ACETONITRILE

When degassed solutions were mixed, the immediate spectra, Fig. 17 observed consists of a triplet with aN = 15.62 G which is due to di-tert.butylnitroxide (DTBNO) and some weak signals which grew over time to a well resolved spectra. The triplet was unstable and disappeared within 30 mts. The spectra due to DTBNO is highly stable unless it reacts with some other species. The disappearance of DTBNO in the system containing BPO has been reported by Walton

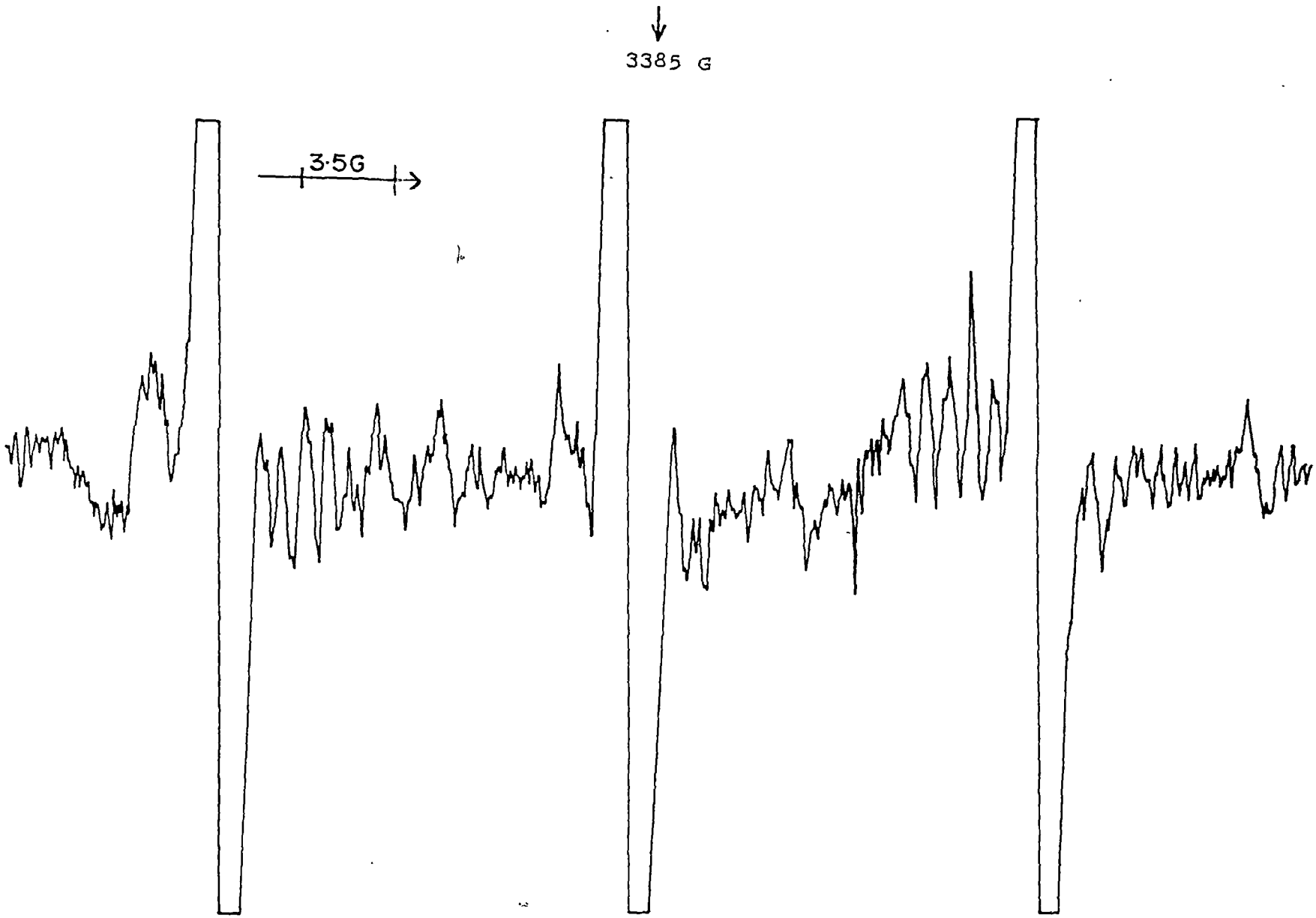


Fig.17. ESR Spectra obtained from the reaction of thymine, BPO, MNP in acetonitrile.

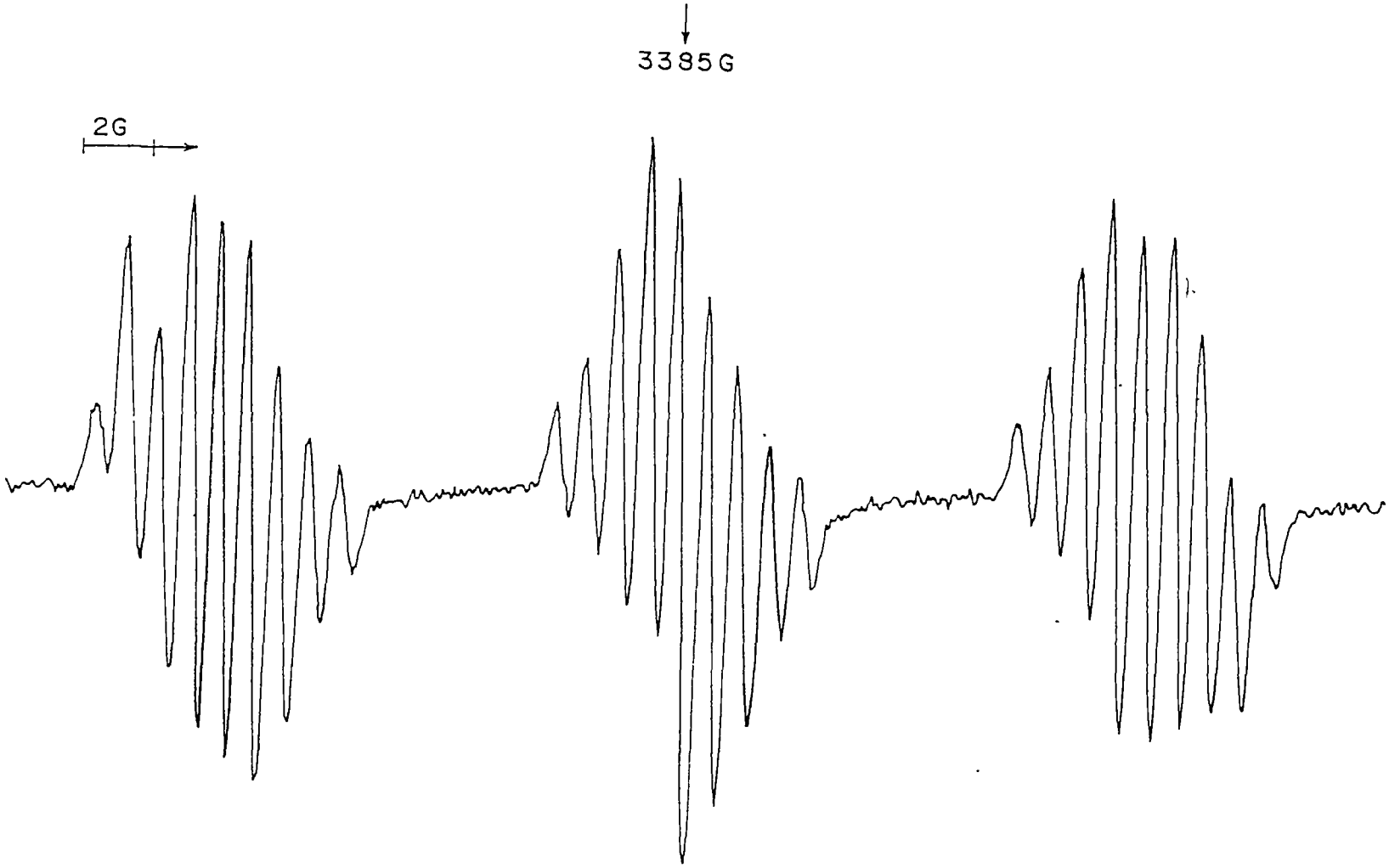


Fig.18a. ESR Spectra of the phenyl adduct of MNP, obtained from the reaction of thymine, BPO and MNP in acetonitrile.

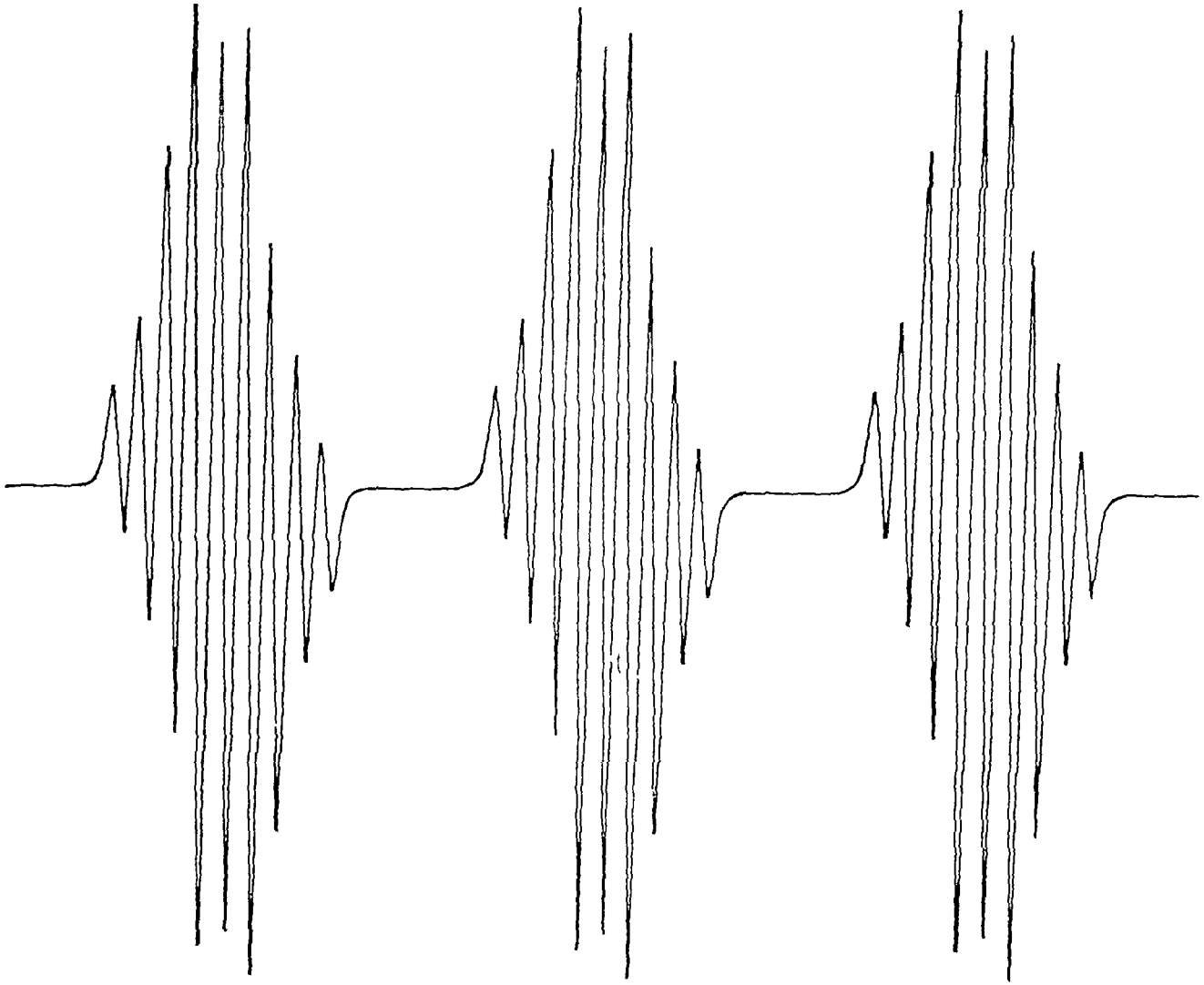
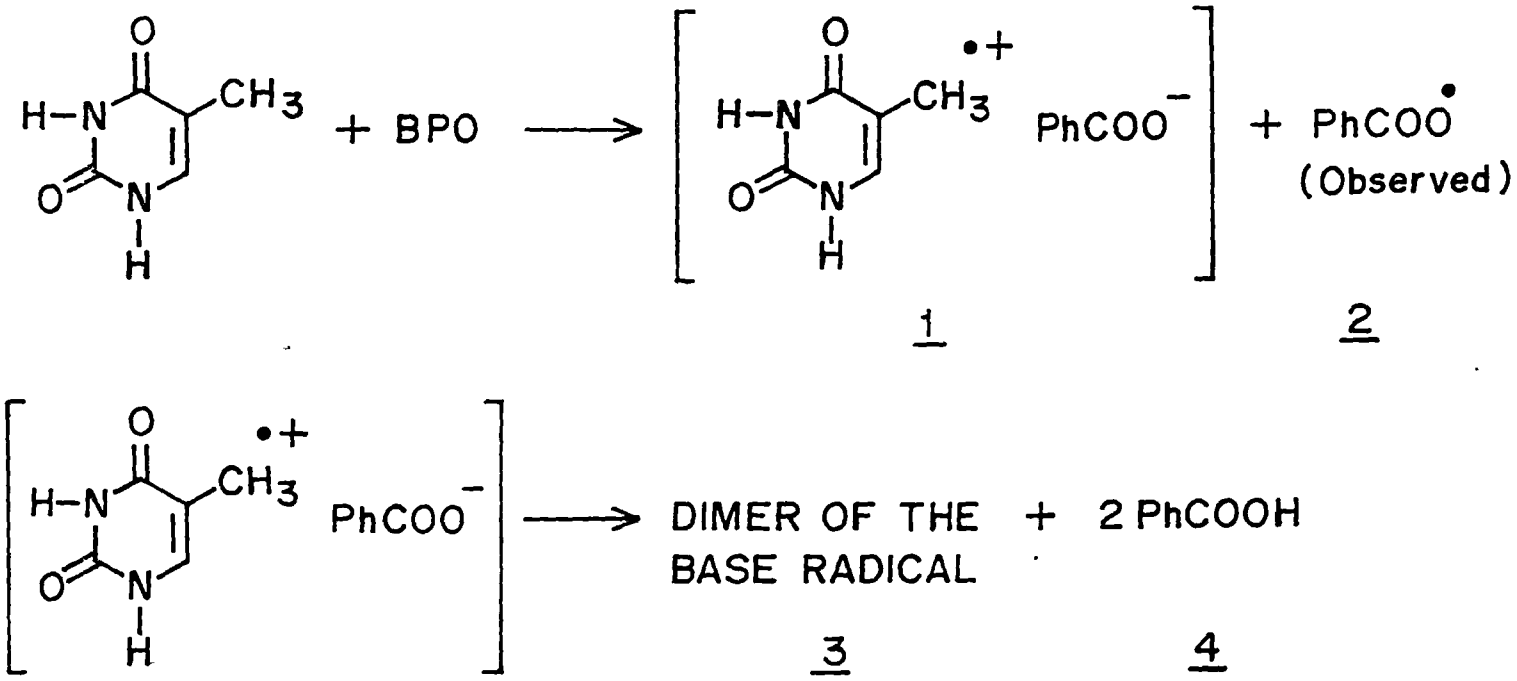


Fig.18b. Computer simulated spectra of 18a , using the hyperfine parameters calculated from the experimental spectra and L.W = 0.4 G.

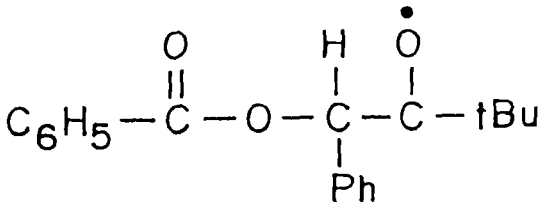

+ BPO
PhCOO⁻
+ PhCOO•
(Observed)
1
2
2
PhCOO⁻
DIMER OF THE BASE RADICAL
+ 2 PhCOOH
3
4
PhCOO• → Ph• + CO2
5
6
Ph• + MNP → MNP – Ph
7

Scheme – VIII

etal [12]. The second spectra has been assigned to phenyl adduct of MNP Fig. 18a. The hyperfine parameters are in good agreement with the reported value (walton). The spectra was very well simulated , Fig. 18b. The reaction mechanism is shown in scheme VIII.

THYMINE \ BPO \ NITRONE IN ACETONITRILE.

The blank experiment of BPO and Nitrone in acetonitrile gave a doublet of a triplet with aN = 13.50 G and aH = 1.80 G which developed slowly, Fig. 19. This has been assigned as due to benzoyloxyl radical adduct of PBN. This radical originates from the electron transfer from PBN to BPO, resulting in PBN cation and BPO anion in the primary act. Then BPO anion undergoes electron capture dissociation process, forming $C_6H_5COO^-$ and $C_6H_5COO^{\cdot}$. The latter reacts with PBN cation to give the adduct ;



We would like to comment that where we used MNP as a spin trap, the adduct identified was due to trapping of phenyl radical, rather than benzoyloxyl, whereas with nitrone

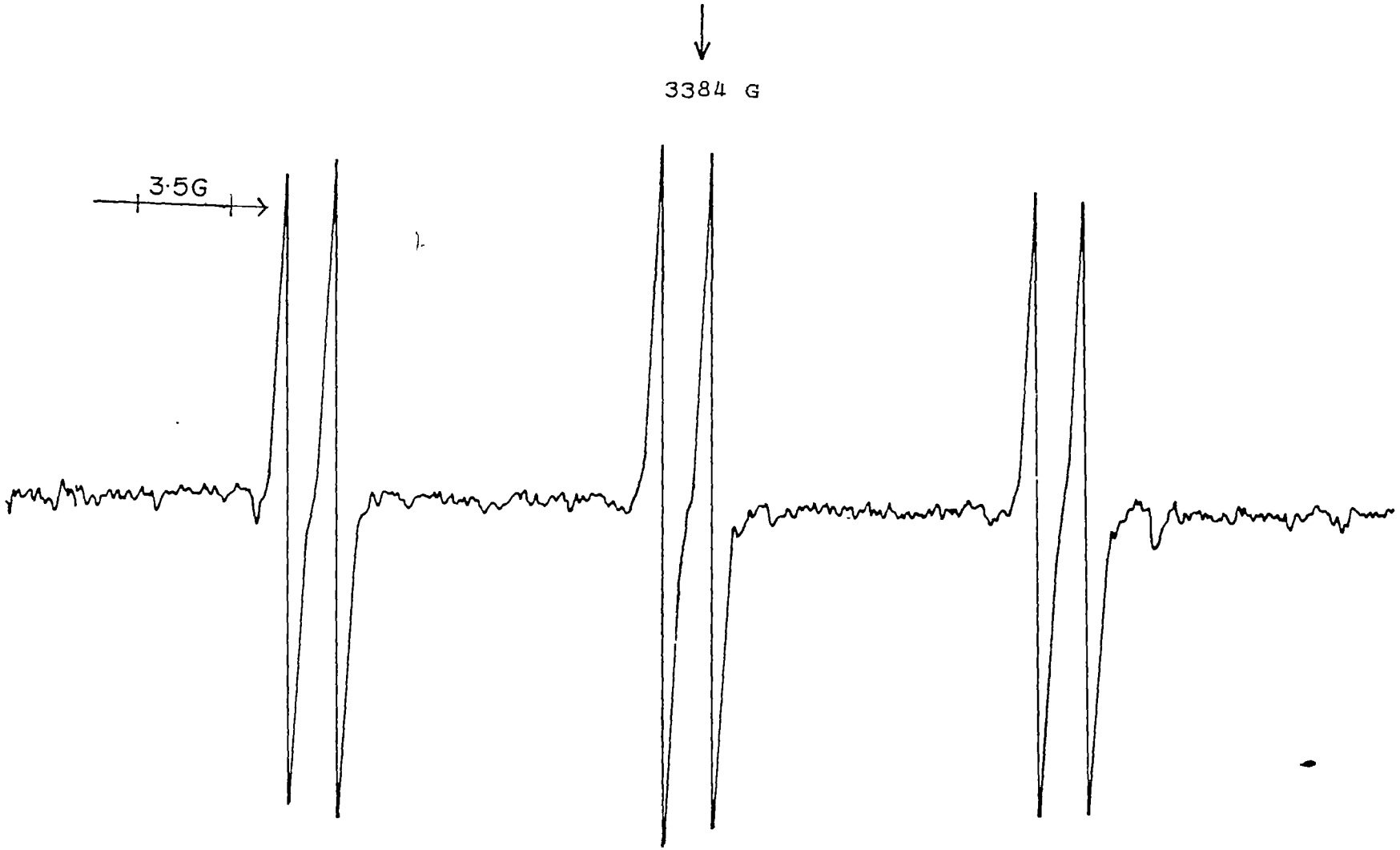


Fig. 19. ESR Spectra obtained from the reaction of thymine, BPO and nitrone in acetonitrile.

benzoyloxyl is trapped. It appears that the rate of addition of benzoyloxyl radical to MNP is lower, therefore, decarboxylation of benzoyloxyl occurs leading to the formation of phenyl radical.

When degassed solutions of all the three components were mixed, similar spectra, Fig. 19 with same hyperfine splittings was observed. The only difference in this case is that the spectra appeared immediately with good intensity. The appearance of similar spectra in both cases makes it difficult to assign whether electron transfer has occured from thymine or PBN. The red. pot of Thymine is 1.29 eV and of PBN is 1.75 eV, therefore, in this combination the electron in all probability would be given by Thymine with greater ease. Our failure to observe the spectra is due to the reported lower velocityconstant for the addition of the phenyl radical to PBN, than other typical spin trapping experiments [13]

URACIL \ BPO \ MNP SYSTEM IN ACETONITRILE

When the degassed solutions were mixed as usual, immediately no spectra was observed. After some time a well resolved, triplet of triplet, with aN = 16.44 G and aN2 = 1.81 G was observed. Fig. 20. The spectra is highly stable.

MECHANISM

On the basis of energetic consideration, we postulate that

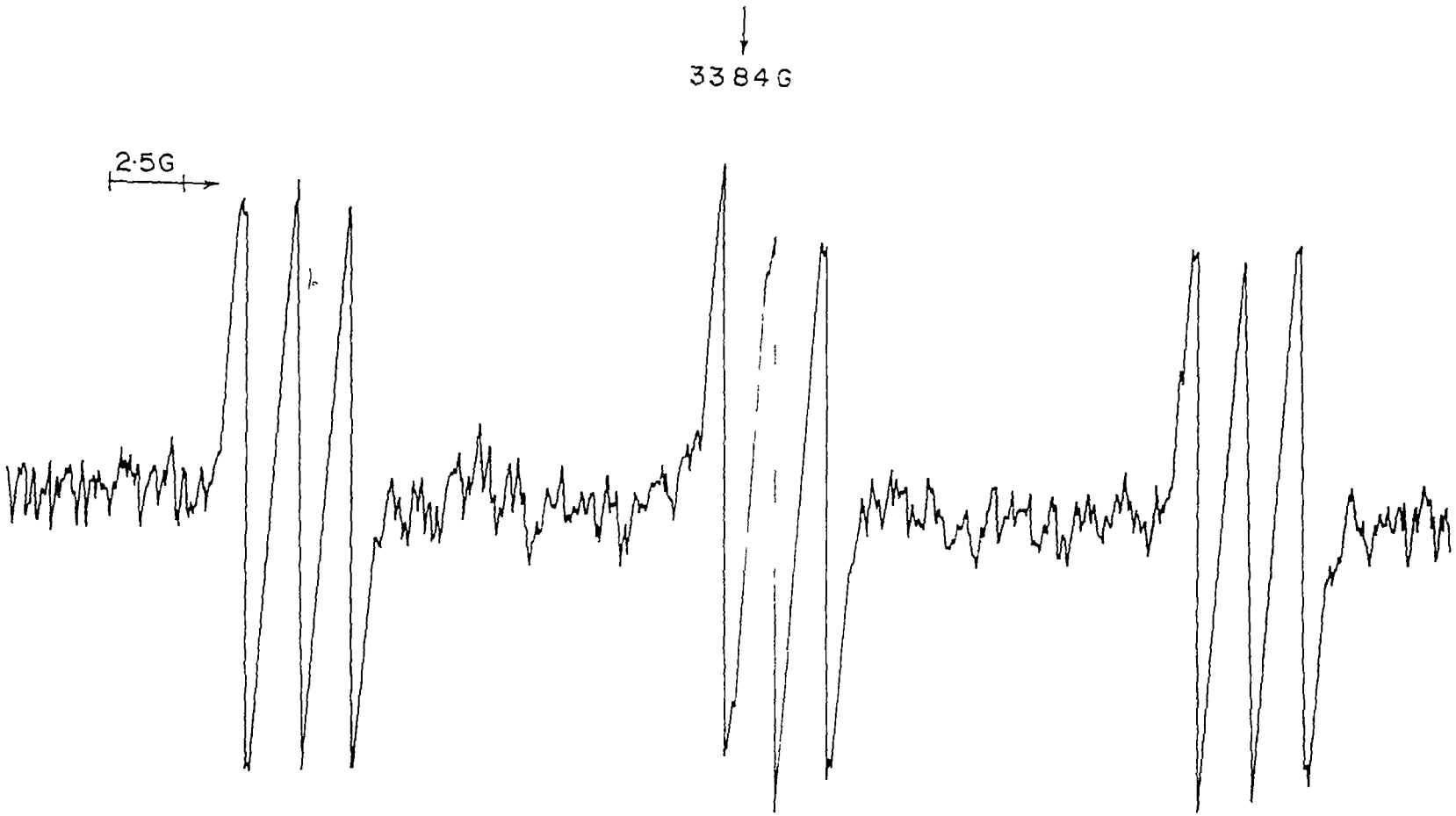


Fig. 20a. ESR Spectra obtained from the reaction of uracil, BPO and MNP in acetonitrile.

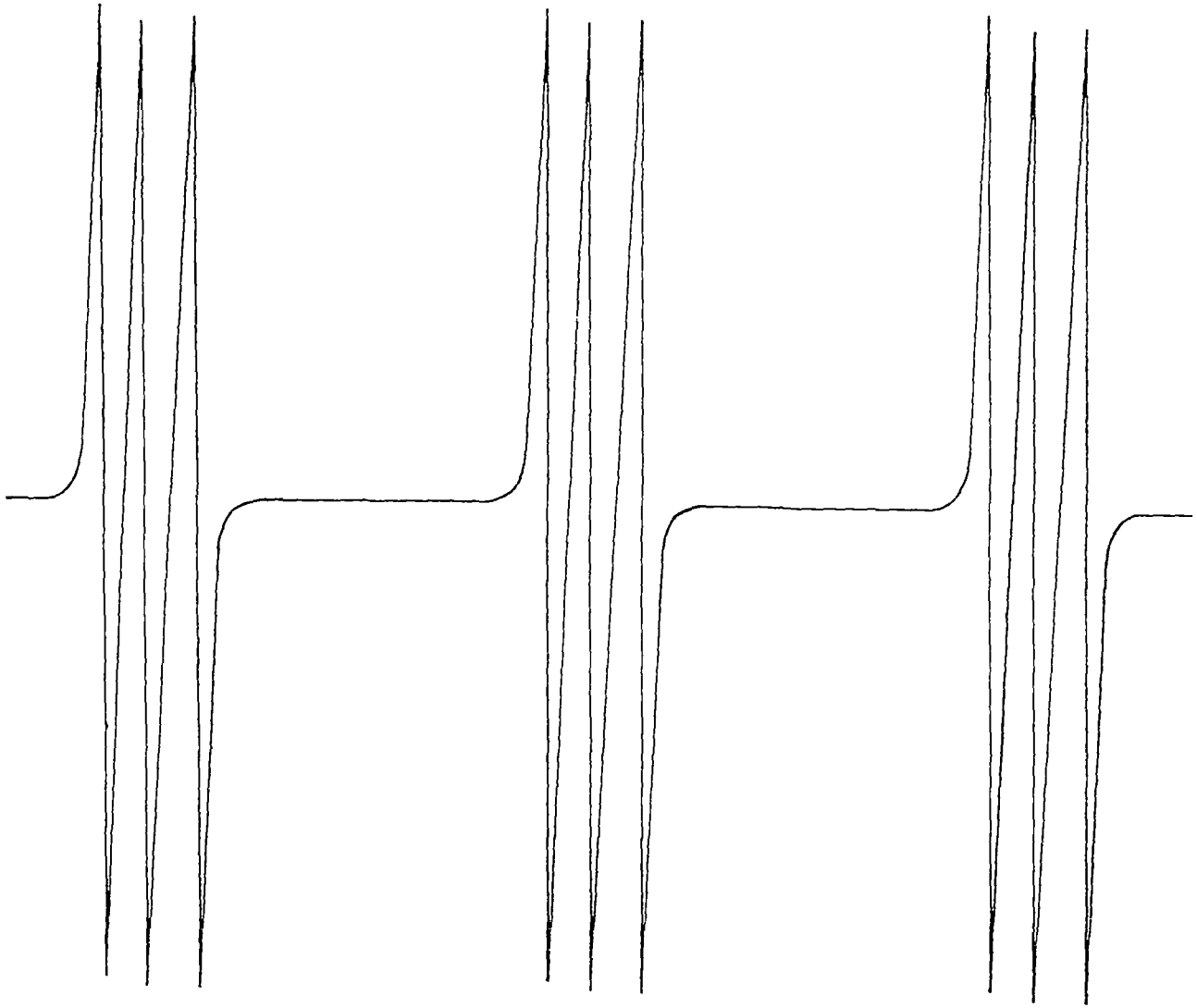


Fig.20b. Computer simulated spectra of 20a using the hyperfine parameters calculated from the experimental spectra and L.W = 0.45G.

in the primary act, an electron is transferred from Uracil to BPO. The resulting ionic species undergo reactions as shown in scheme VIII. The stable spectra due to adduct is observed. When one of the component was scanned over a small field range with lower scan speed no further splitting was observed. This prompted us to postulate that in this case it is the N (3) of the uracil cation which undergoes deprotonation. Had it been N1 site, we would have observed splitting from H at C6 as observed in earlier experiments with Chloranil and Nitrone. Another confirmatory evidence to our this postulation stems from the comparison of hyperfine splitting which are of the same order and magnitude as from succinimidyl adduct with MNP. In both the cases, the nitrogen trapped is flanked by two carbonyl groups, which tempts us to conclude that the N3 centered radical has been trapped. Fig. 20b shows the simulated spectra

URACIL \ BPO \ NITRONE IN ACETONITRILE

When degassed solutions were mixed the spectra observed was exactly similar to that obtained with thymine i.e the main adduct was the benzoyloxyl adduct of PBN.

CYTOSINE \ BPO \ MNP IN ACETONITRILE

When similar experiments were attempted with Cytosine, a very weak triplet with aN = 15.00 G developed after some time. Since signals were very weak, no attempt was made to

assign it. This could be due to high redox potential of Cytosine (1.44 eV), which makes electron transfer an inefficient process.

ESR STUDIES WITH NUCLEOSIDES

After gaining some understanding of the nucleobases , the next step was to study respective nucleosides where the H at N1 of pyrimidine bases and N9 of the purine bases is replaced by sugar moiety. It is well documented in the literature that the primary site of any damage to DNA is the heterocyclic base unit, which is then transferred to the sugar leading to the strand breakage - one of the major cause of DNA damage. It appears that sugar moiety acts as a bridge in the overall process of damage. Therefore, the study of nucleosides assumes significance.

PURINE BASES

A. GUANOSINE \ CHLORANIL \ NITRONE SYSTEM IN 1,4 DIOXAN

Since the solubility of Guanosine for ESR study is very low, attempts to study the reactions did not succeed. Even at the solubility limit, the concentration of the radicals was insufficient to produce detectable levels of Guanosine derived spin adducts.

B. ADENOSINE \ CHLORANIL \ NITRONE SYSTEM IN 1,4 DIOXAN

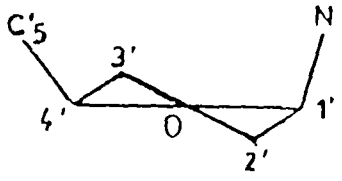
When the degassed solutions of adenosine and chloranil in

1,4 dioxan were mixed with the degassed solutions of nitrone in 1,4 dioxan, immediately no spectra was observed. After some time a well resolved a doublet of triplet, with aN = 14.40 G and aH = 17.20 G, was observed along with another doublet of a triplet which further splits up into a doublet (marked ii) with aN = 14.4 G, aH1 = 14.4 G and aH2 = 4 G

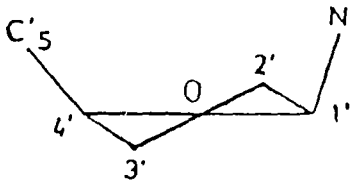
Fig 21.

The electron transfer from the adenosine can be pictured to occur as, scheme IX ;

The adduct formed by the trapping of (I) to PBN will be under a strain due to its bulkiness and will lead to a cleavage resulting in the formation of MNP. the MNP generated in the system will trap II , having hyperfine splittings analogous to no (1) values. For the furanose ring two conformations may be possible. However, NMR data shows that in the ribose nucleosides the furanose ring prefer conformation with C(3)' in endo position relative to C(5)' wheareas the C(2)' in exo position as shown in the Figure below[14].



a)



b)

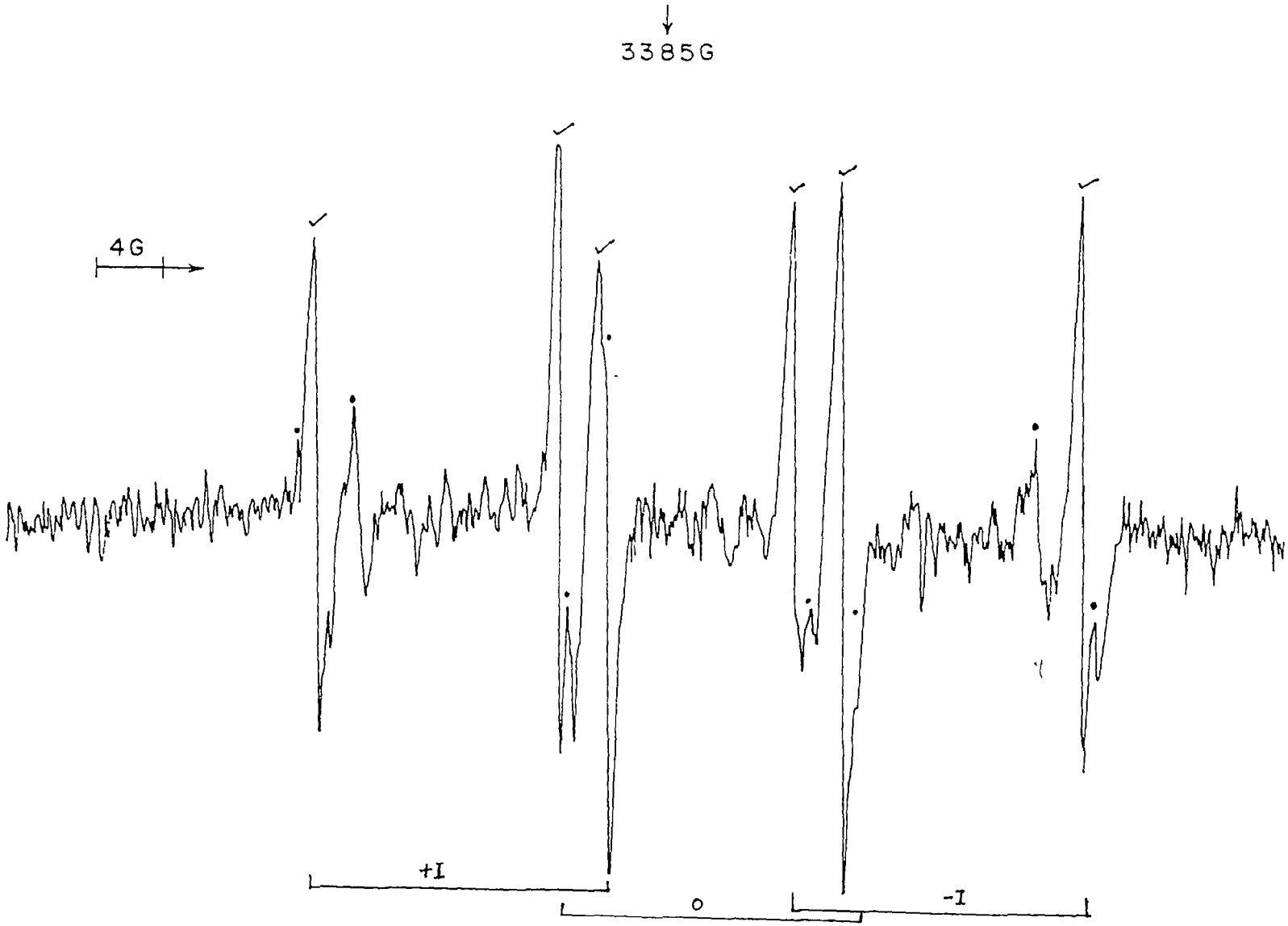


Fig.21a. ESR Spectra obtained initially from the reaction of adenosine, chloranil and nitrone in 1,4 dioxan. ✓ (i); • (ii)

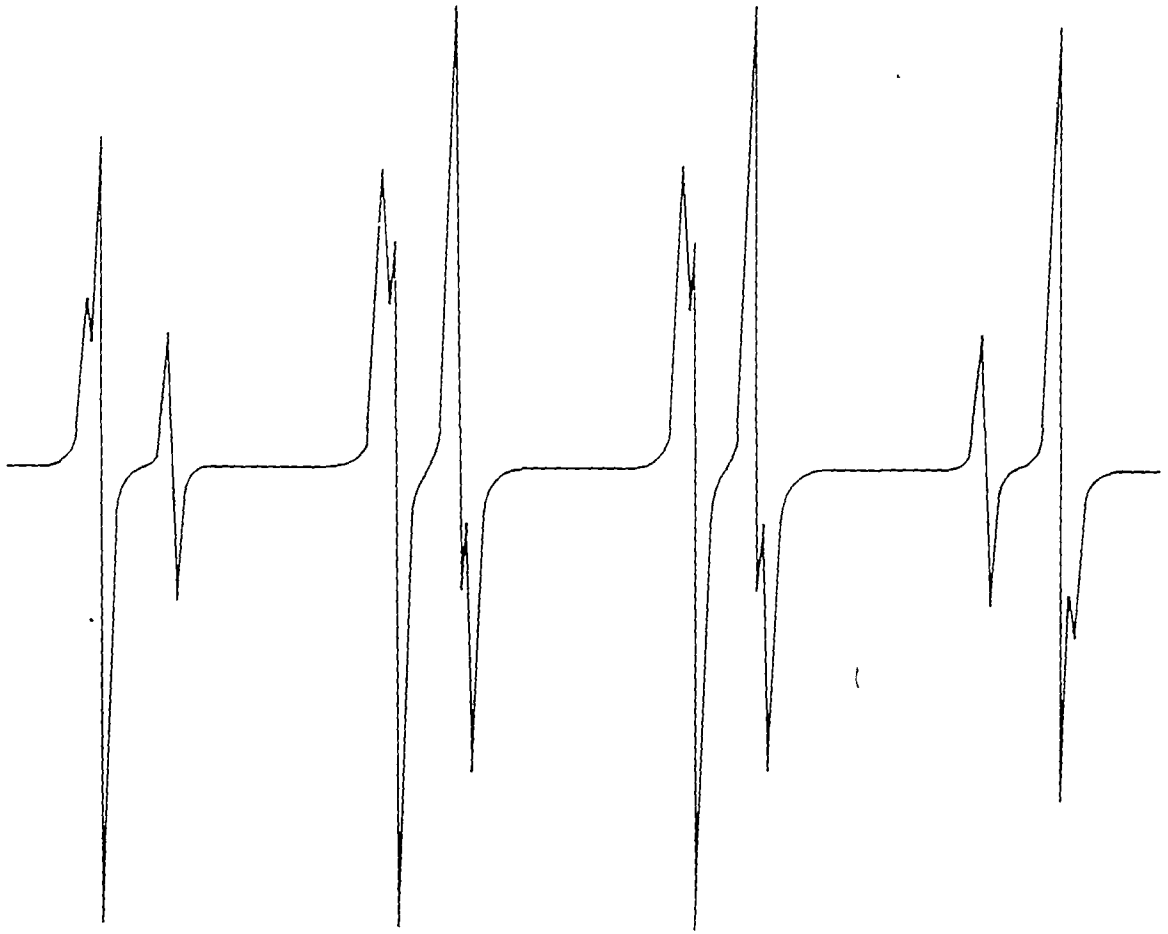
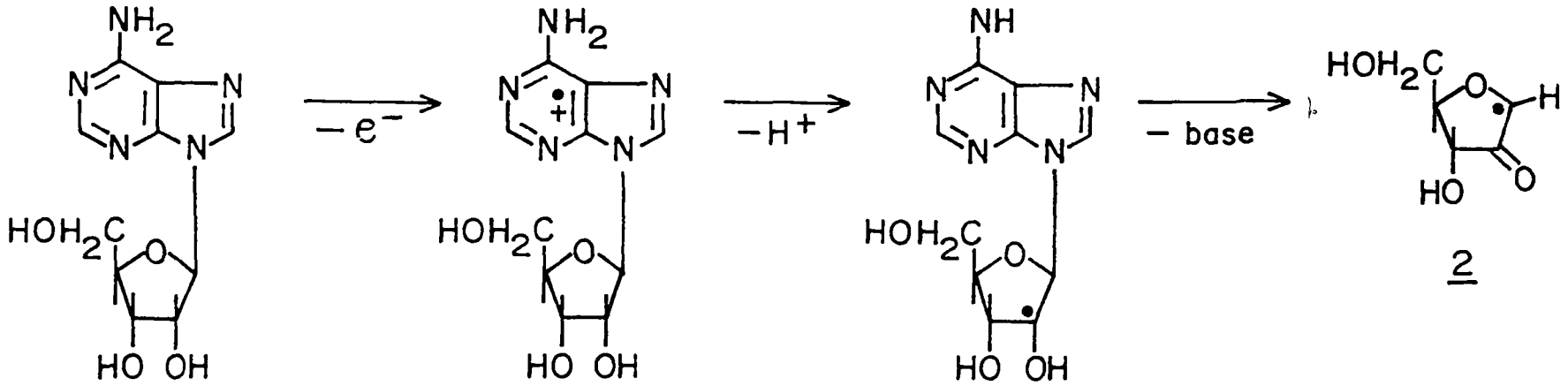


Fig. 21b. Computer simulated Spectra of 21a, using hyperfine parameters calculated from the experimental spectra.

(i) ✓L.W = 0.5 G, ΔG = 0 G (ii) • L.W = 0.5 G, ΔG = 0.6G


NH2
N
N
N
N
HOH2C
O
HO OH
−e−
NH2
N
N
N
N
HOH2C
O
HO OH
−H+
NH
N
N
N
N
HOH2C
O
HO OH
− base
HOH2C
O
H
HO
O

1 2

Scheme – IX

The trapping of a radical II have structures analogous to (a) is thus postulated.

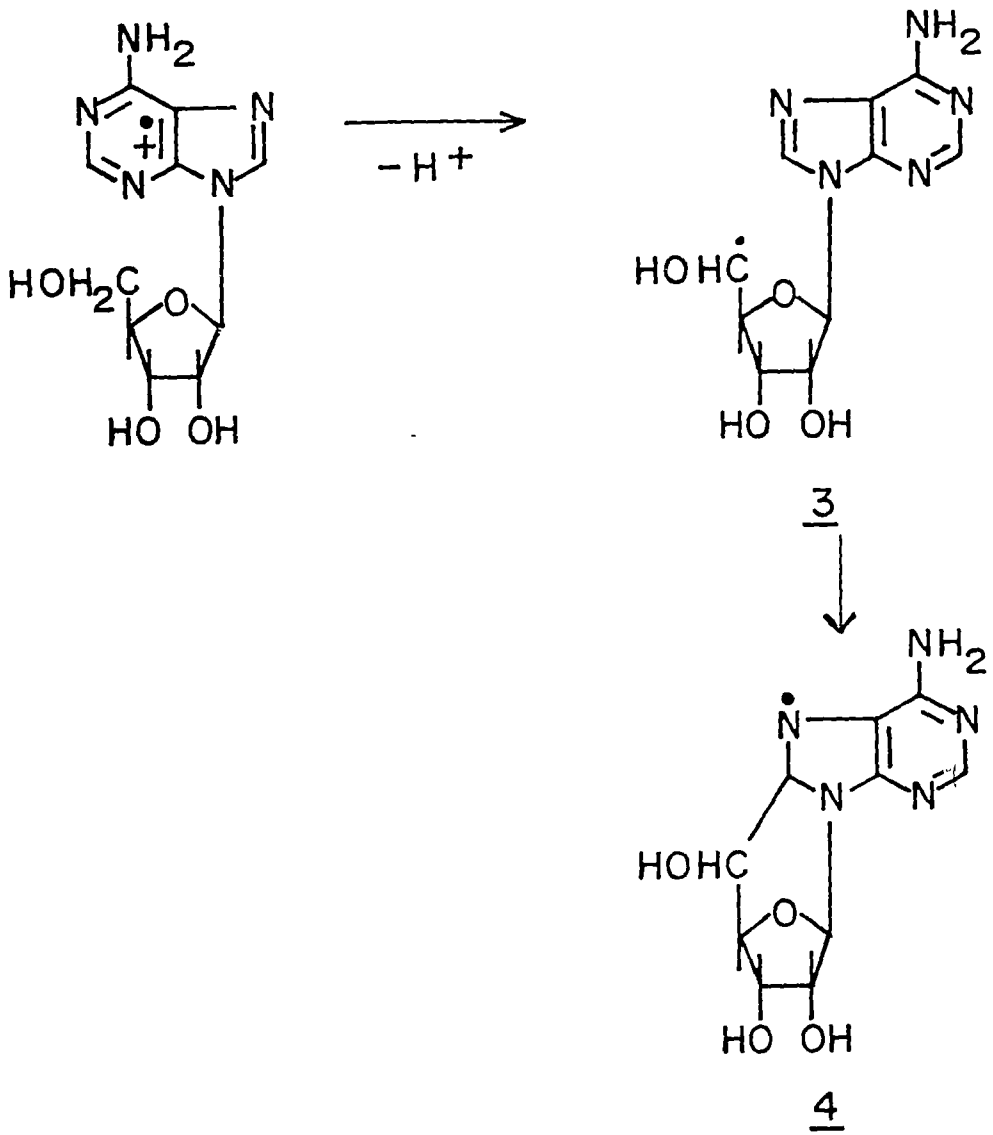
Further there exists another possibility of proton abstraction from the sugar moiety as shown in scheme X. Probably, the trapping of radical III to PBN is responsible for the splitting constants of (ii) . The concentration of this species is very low as a fast rearrangement to give a radical IV occurs. This trapped to PBN should show a doublet of a triplet which will further split into triplet. The experimental spectra observed over a period of time did in fact show such a spectra, Fig. 22a with splitting constants of (iii) aN = 11.69 G, aH = 4.0 G and aH2 = 2.3 G along with the first two set of signals, Fig. 22b shows a well simulated spectra.

THE PYRIMIDINE BASES:

The pyrimidine bases are in general electron deficient as compared to purine bases where the electron are more diffuse. Also the induction of the sugar moiety reduces the basicity further. Due to this reduced electron density probably the electron transfer would not be as facile as with the heterocyclic nucleic acid bases described in a preceeding section.

A. THYMIDINE / CHLORANIL / NITRONE SYSTEM IN 1,4 DIOXAN

When degassed solution of thymidine and chloranil were


NH2
N
N
N
N
HOH2C
O
HO OH
-H+
NH2
N
N
N
N
HOHC
O
HO OH
3
NH2
N
N
N
N
HOHC
O
HO OH
4

Scheme - X

mixed with degassed solution of nitrone we initially observed

(a) a doublet of a triplet with aN = 13.6 G and aH = 1,81 G

(b) a weak triplet with aN = 8G which decayed very quickly.

Over a period of time, only a triplet with aN = 15.5 G was observed.

The splitting constant of the first radical is consistent with that reaction between nitrone and chloranil. Thus it seems that electron transfer is not as facile as that with Thymine and thus electron transfer from nitrone also competes leading to two pathways. The deprotonated nucleoside adduct of PBN due to its bulky nature gets ruptured and the spectra observed with aN = 15.5 G due to di-tert.butyl nitroxide (DTBNO) is observed only.

B. URIDINE / CHLORANIL /NITRONE SYSTEM IN 1,4 DIOXAN :

With uridine too the dominant spectra consisted of that obtained from nitrone , chloranil system.

C. CYTIDINE \ CHLORANIL \ NITRONE SYSTEM IN 1,4 DIOXAN :

In this case too similar spectra as with cytosine was observed and is due to adduct, though signals were much weaker. The failure to observe the nucleoside adduct of PBN may either reflect lower possibility of electron transfer or the inefficiency of sugar trapped radicals

BASE PAIRS

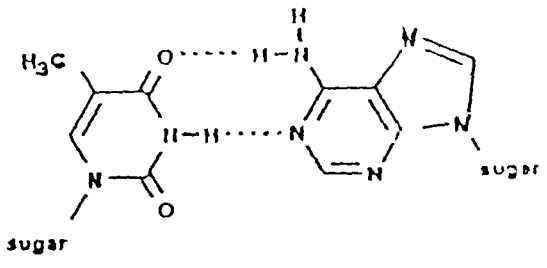
In DNA bases occur as pairs. It thus follows that, due to the pairwise existence of the bases, it is neccessary to treat the chemistry of the ionic base radicals as members of the pair, i.e the behaviour of the partner has to be considered as well.

Two natural base pairs existing are :

(1) Adenine - Thymine pair

(2) Guanine - cytosine pair

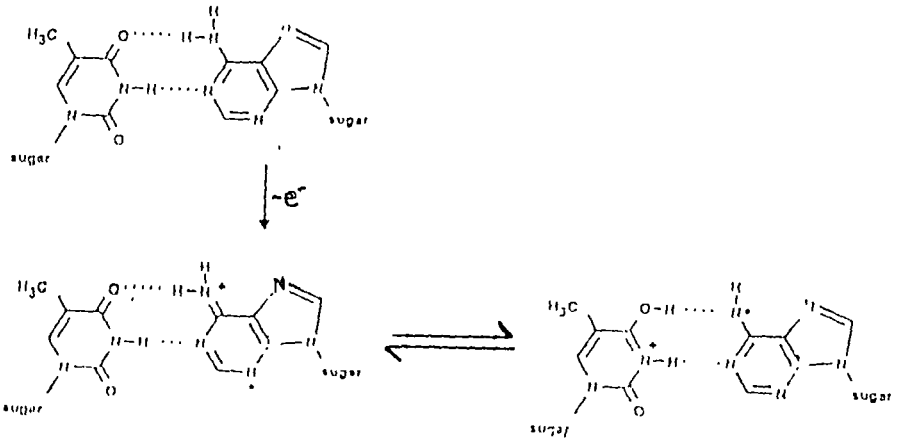
Due to very poor solubility of Guanine of the second pair the spectra observed had a very low signal to noise ratio. Thus all our attempts to study the system failed. We shall concentrate on the first pair only. This pair is believed to exist through hydrogen bonding as shown in the figure ;



Nucleosides were used for examining the base pairs as here the hydrogen at N1 in the pyrimidine bases and N9 for the

purine bases are substituted by the sugar moiety, and thus the reaction pathways may be different than that with the bases only and will very closely mimic the internal environment of DNA.

When the degassed solution of the base pair Thymidine and Adenosine,were mixed with chloranil and nitrone, the spectra was exactly identical to that observed with adenosine described above, Fig. 22. This may be explained on the basis of the following argument. The electron transfer from this particular pair may take place as :



If we accept that the primary attack of chloranil is directed towards the base moieties and then the site of free spin is transferred from the base radical to the sugar (shown in scheme, IX) [15], we then have to explain why this reaction takes place in the adenosine and not in thymidine or rather in the ribose but not in the

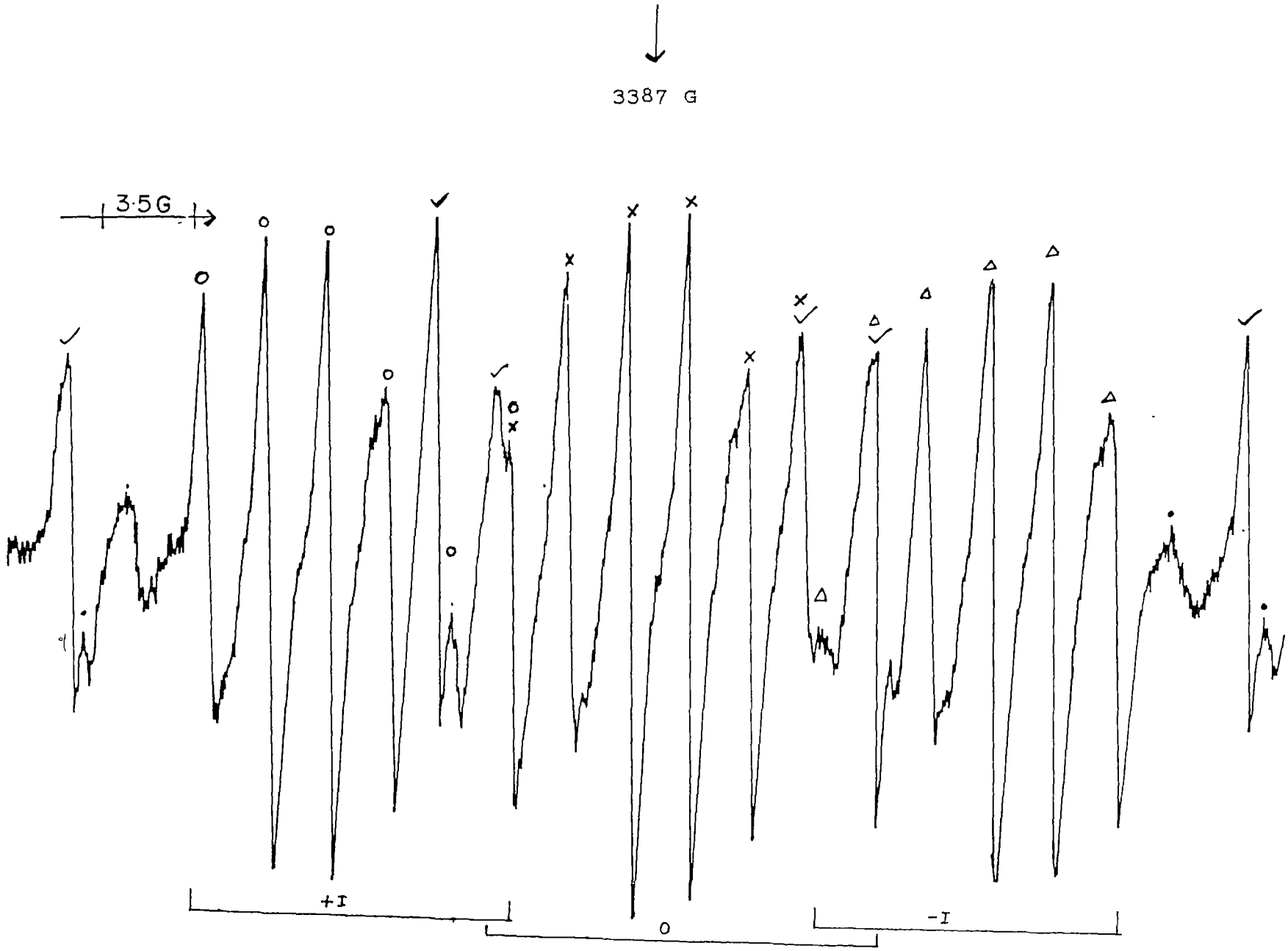


Fig. 22a. ESR Spectra from the reaction of adenosine, chloranil, and nitrone in 1,4 dioxan obtained after one hour. ✓(i); • (ii); o, x, △ (iii)

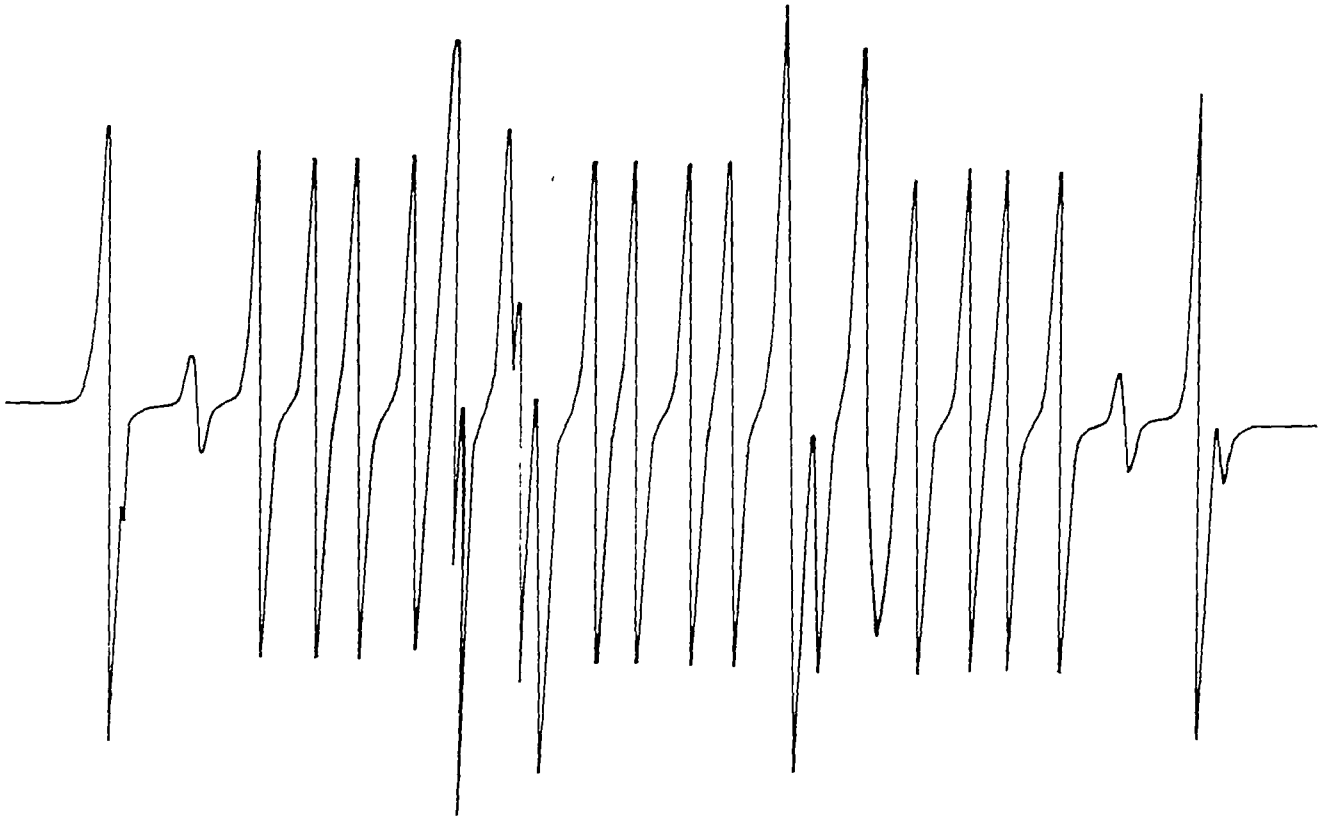
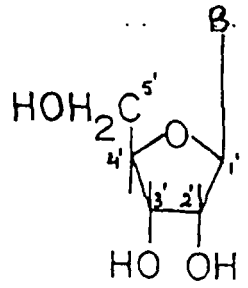
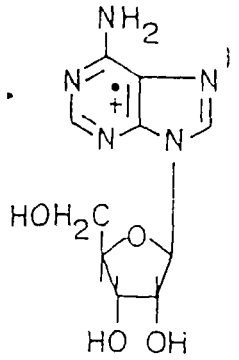


Fig. 22b. Computer simulated Spectra of 22a using the hyperfine parameters calculated from the experimental spectra. (i) ✓ L.W = 0.5 G, ΔG = -0.2 G (iii) • L.W = 0.5 G, ΔG = 0.6 G (iii) o, x, Δ L.W = 0.5 G, ΔG = O G.

deoxyribose. It may be mentioned here that the sugar associated with adenosine is ribose and that with thymine is deoxyribose. The most obvious reason for formation of sugar radicals from ribose nucleosides, seems to originate from activation of H (2'),(fig shows the numbering pattern)



The Chloranil abstracts H(2') from D-ribose but not from 2-deoxyribose. Accordingly , the base radicals formed might be able to attack 2'H from ribose compounds during the formation of the radical;



In agreement with our experimental results and those reported , this pathway is not feasible in the deoxyribose derivatives. 3'H can also be abstracted but would lead to ring opening. The reasons of abstraction of 3'H in the ribose but not in the deoxyribose derivatives are less evident.The electronic environment of 3'H is similar in the

two classes of compounds. It should be mentioned , however, that there are conformational differences which may be relevant to the hydrogen transfer. It is known that the furanose rings are not planer but exist in a variety of non planar conformations which interconvert by pseudorotation. From NMR data , it is known that they can exist as shown in fig a and b. i.e they prefer conformation with C3' in an endo position relative to C5'. In the deoxyribose the conformation equilibrium is shifted towards structures with C3' in exo position. from this situation it is concievable that fast intramolecular hydrogen abstraction from C3' is more fovourable in the ribose then the deoxyribose. One might argue that for the same reasons hydrogen abstraction from C2' should be hindered in ribose nucleosides. This was not observed. Possibly ,the effect of the sugar ring puckering is not strong enough to overcompensate the spatial conditions allowing rapid intramolecular hydrogen transfer from C2' to the base moiety. Thus for the base pair too, radicals from the adenosine moiety were observed exclusively. (consistent with Steenken's aqueous state result [10])

CONCLUSIONS

The major achievements of this project derived from ESR studies on the Nucleic acid bases are summarised below ; (1) The technique of spin trapping in ESR has been very successfully applied to trap the short lived intermediates formed as a result of transfer of a single electron from the nucleic acid bases to acceptors in non-aqueous solvents, under very mild conditions and at room temperature .

(2) The role of oxygen in line broadening (because of it being paramagnetic in the ground state) in ESR is well known . The crucial role which oxygen plays in free radical chemistry is also well documented. But the critical role which oxygen plays in electron transfer reactions have come across in this system both by uv and ESR. To our knowledge only few such instances exist.

(3) The role of polar and hydroxylic solvents in solvating the charged species and thus diverting the reaction pathways have been clearly observed.

(4) Deprotonation of the base radical cation have been shown to be the major pathway in contrast to hydration reaction, under the present experimental condition. We have observed that deprotonation occurs from N1 in thymine and uracil, and in rare case a proton abstraction from methyl group of thymine has been observed. For cytosine, the NH2 group at C4 was observed to be the site of deprotonation.

For adenine two sites of deprotonation were observed ; one at N1 and the other at N7.

(5) Reactions of bases particulary thymine with benzoyl peroxide under very mild condition led to the formation of benzoyloxyl and phenyl radical, both of which are known to act as damaging agent as a tumour promoter.

(6) Damage can be caused by strong oxidising agents like benzoyl peroxides and chloranil even under the mildest conditions.

(7) The primary radical site is the base and the site of free spin is transferred from the base radical to the sugar, a main cause of strand breakage and thus deactivation.

(8) Sugar derived radical could be obtained from ribose but not from deoxyribose.

(9) The strong evidence for the charge transfer complexation suggests that the electron transfer proceeds through an " Outer - sphere " mechanism.

(10) 1,4 dioxan and tetrahydrofuran have been found to be the most suitable solvent for SET studies of these bases under the present experimental conditions and results are identical in both the solvents.

We have thus, put forward a simple model for studying the Single Electron Transfer (SET) reactions of the nucleic acid bases in non-aqueous solvents employing the technique of spin trapping in ESR.

REFERENCES

(1) Faraggi,F.; Broitman,J.B.; Trent,J.B.; Klapper,M.H.; J.Phys.Chem, (1996), 100, 14751-14761.

(2) Catterall,H.; Davies,M.J.;Gilbert,B.C.; Polack,N.P.; J.Chem.Soc.Perkin Trans 2, 1993, 2039.

(3) Bockman,T.M.; Perrier,S.; Kochi,J.K.; J.Chem.Soc. Perkin trans.2, 1993, 595.

(4) Beuttner,R.Garry , Free Radical Biology and Medicine, Vol 3, p. 359 (1987).

(5) Fujita,S.; Steenken,S.; J.Am.Chem.Soc, 1981,103, 2540.

(6) Yabe,T.; Kochi,J.K.; J.Am.Chem.Soc. 1992,114,4491.

(7) Hazra,D.K.; Steenken,S.;J.Am.Chem.Soc, 1983,105, 4380.

(8) Solid State Radiation Chemistry of DNA : The Bases. Bernhard,W.A.;, Advances in Radiation Biology, Vol 9, 1984, p.208.

(9) Davies, M.J.; Gilbert,B.C.; Hazlewood,C.; Polack,N.P.; J.Chem.Soc.Perkin Trans. 2, 1995, p.13

(10) Steenken, S.; Chem.Rev., 1989, 89,503-520.

(11) Riesz,P.; Resenthal,I.; Can.J.Chem,60, (1982) 1478.

(12) Singh,H.; Teddar,J.M.; Walton,J.C.; J.Chem.Soc. Perkin Trans 2,1979) 1259.

(13) Janzen,E.G.; Evans, C.A.; J.Am.Chem.Soc., 1975,97,205.

(14) Frohlinde, Schulte D.; Hildenbrand, K.; In Free Radicals in Synthesis and Biology. Ed. Minisci.F.; NATO ASI series, Kluwer Academic Publication. Dordrecht. 1989,335.

CHAPTER - 6

CONCLUSIONS

6.1 SALIENT FEATURES OF THE PRESENT PROJECT

The major achievements of this project derived from UV and ESR studies on the Nucleic acid bases are summarised below ;

1. The uv spectra of the nucleic acid bases in organic solvents of varying polarity have been successfully recorded. To the best of our knowledge this is the first such studies.

2. The phenomenon of Keto - Enol tautomerism plays a very significant role in mutagenesis. This property of the nucleic acid bases have been theoretically predicted and experimentally proved in some cases mainly by employing IR and NMR in aqueous solvents. We have successfully employed uv spectroscopy to demonstrate the occurance of tautomerism even at very low concentrations. In bases with two exocyclic groups (e.g., guanine , cytosine and their corresponding nucleosides) both kinds of tautomerism ; keto - enol and amino - imine have been shown to occur. To the best of our knowledge, this is the first and fairly comprehensive report of tautomerism using UV spectroscopy.

3. Self - association of these bases have been reported, using IR, NMR and X- ray Crystallography etc. We have complimented these findings from uv studies. We are reporting a band for the first time due to dimeric formation at ca. 225 nm in low polarity solvents. The relative

associative capability have also been commented upon and has been generally found to be higher for the purine bases than the pyrimidine bases. This conclusion derived through UV studies are in agreement with those by IR and NMR.

(3) Charge transfer complexes are believed to be precursors to electron transfer processes. This has been very satisfactorily demonstrated . Charge transfer complexes through the appearence of isosbestic points were observed which are indeed a positive indication for the feasibility of electron transfer processes to take place.

(4) As mentioned in the objective , examples involving the transfer of a single electron are limited . The debate involving single electron transfer processes vs polar pathways is still on. To this controversy, we have added another example in favour of " Single Electron Transfer " processes, involving nucleic acid bases in organic solvents.

(5) The technique of spin trapping in ESR has been very successfully applied to trap the short lived intermediates formed as a result of transfer of a single electron from the nucleic acid bases to acceptors in organic solvents.

(6) The role of oxygen in line broadening (because of it being paramagnetic in the ground state)in ESR is well known. The crucial role which oxygen plays in free radical chemistry is also well documented. But the critical role which oxygen plays in electron transfer reactions have come

across in this system both by UV and ESR. To our knowledge only few such instances exist.

(7) The role of polar and hydroxylic solvents in solvating the charged species and thus diverting the reaction pathways have been clearly observed.

(8) Deprotonation of the base radical cation have been shown to be the major pathway in contrast to hydration reaction, under the present experimental condition. We have observed that deprotonation occurs from N1 in thymine and uracil, and in rare case a proton abstraction from methyl group of thymine has been observed. For cytosine, the NH2 group at C4 was observed to be the site of deprotonation. For adenine two sites of deprotonation were observed ; one at N1 and the other at N7.

(9) Reactions of bases particularly thymine with benzoyl peroxide under very mild condition led to the formation of benzoyloxyl and phenyl radical, both of which are known to act as damaging agent as a tumour promoter.

(10) Damage can be caused by strong oxidising agents like benzoyl peroxides and chloranil even under the mildest conditions.

(11) The primary radical site is the base and the site of free spin is transferred from the base radical to the sugar, a main cause of strand breakage and thus deactivation.

(12) Sugar derived radical could be obtained from ribose

but not from deoxyribose.

(13) The strong evidence for the charge transfer complexation suggests that the electron transfer proceeds through an " Outer - sphere " mechanism.

(14) 1,4 dioxan and tetrahydrofuran have been found to be the most suitable solvent for SET studies of these bases under the present experimental conditions.

As mentioned in brief in objective of this project, most of the work related to the DNA or its constituents have been done ; (i) at 77K solid state , (ii) by employing a powerful source of irradiation e.g., γ - rays, pulse radiolysis etc., (iii) in aqueous state , and (iv) reaction with strong oxidising agents like $SO_4^{\cdot -}$, $OH^{\cdot -}$ etc., as indirect source of damage.

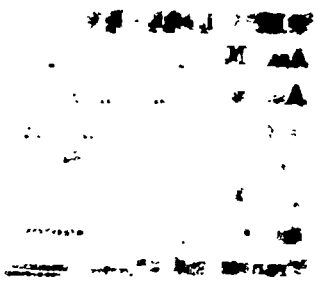
We, however, embarked upon this project with a deliberately different path. We studied the system at ;

(i) Ambient temperature, because human body is at that temperature.

(ii) Not employing any source of irradiation to generate radicals.

(iii) Under the mildest conditions, i.e., creating such favourable conditions where just one electron is transferred and reaction begins.

(iv) In non-aqueous solvents, as some fast two electron processes in aqueous phase show two step one electron



processes in non-aqueous solvents and it mimics the internal environment of the double helix.

We feel that the objective of the project has been quite satisfactorily met. We have developed a simple model for studying electron transfer reactions of the nucleic acid bases without employing any irradiating source.

6.2 SCOPE OF THE PRESENT WORK

Since, this is a first attempt of this kind at room temperature , in non-aqueous solvents under mildest conditions. The positive results appears to be quite promising. Therefore, it is certainly worth pursuing down the line to nucleotides and then ultimately to DNA and RNA macromolecule itself. The next stage of the work can be a study in the presence of those molecules which may form preferentially strong charge transfer complexes with nucleic acid bases and prevent the transfer of electron and thus blocking the reaction right in the beginning, the vitamins or anti-biotic molecule might prevent (?) the damage to these macromolecules.

CURRICULUM VITAE

NAME : SUCHANDRA BHATTACHARJEE

DATE OF BIRTH : 20.8.1970.

QUALIFICATIONS :

EXAM PASSED	UNIVERSITY	YEAR	DIV
B.Sc (PASS)	N.E.H.UNIV.	1989	I
B.Sc (HONS)	N.E.H.UNIV.	1990	I
M.Sc.	N.E.H.UNIV.	1992	I
B.Ed.	N.E.H.UNIV.	1993	I

PUBLICATIONS :

1. Radical cations from nitrone spin traps : reaction with water to give OH adducts. S.Bhattacharjee, Md.N.Khan, Harish Chandra and Martyn C.R.Symons.
   J.Chem.Soc. Perkin Trans 2 , Dec (1996), Pg 2631,
2. Electronic Spectroscopic Study of Self association of Guanine and Guanosine (on) Non aqueous media.
   S.Bhattacharjee, Md.N.Khan, Harish Chandra and Martyn C.R.Symons.(communicated), 1997 ?
3. Single Electron Transfer (SET) Reaction Studies.
   A possible New role for Nitrone Spin Trap.
   Md.N.Khan, S.Bhattacharjee, Harish Chandra and Martyn C.R.Symons.(communicated), 1997 ?