INFRARED ASSIGNMENT AND FORMATION MECHANISM OF TRANS-ANTI THYMINE DIMER.

G.I.Cárdenas J., K.A.Figueroa., V.Vargas C., J.S.Gómez-Jeria and M.M.
Campos-Vallette.
Department of Chemistry, Faculty of Sciences, University of Chile. P.O.
BOX 653 Santiago-CHILE.

ABSTRACT

Thymine dimers were obtained under ultraviolet irradiation. The

major product is a trans-anti dimer linked by a cyclobutane ring.   The

infrared assignments and the mechanism of formation of this dimer are

discussed in terms of an approximate force field and INDO data, respec-

tively.

INTRODUCTION

The radiation chemistry of pyrimidines bases in aqueous solution

has been extensively studied [1-6]. Cyclobutadipyrimidines represent

the major class of DNA lesion induced by the action of far and near ul-

traviolet light on biological systems. However, data concerning the

geometrical parameters and vibrational analysis of the thymine dimers

are scarce and its formation mechanism seems to be very complex.

Thymine dimers exist in four different stereochemical configurations

trans-anti, cis-anti, trans syn and cis-syn [1], depending upon the expe-

rimental conditions of preparation [2]. Weinblum and Johns [3] succeeded

107

in ascribing the correct form to the dimers, however they did not report the whole infrared (IR) spectra and the bands assignment is only partially discussed. Extended Huckel calculations [4] indicate that the preferred dimer conformation corresponds to planar bases forming an angle of 120° with the cyclobutane ring. A different quantum-mechanical study [5] using the frontier orbitals predicted that the most stable conformer is the cis-syn dimer. Due to the complexity of the reaction mechanism for the dimer formation, it is still no clear if the dimerization is induced either by cation-anion recombination or through bimolecular combination of excited states [6,7].

In this work we present preliminary information about the characteristics of the chemical bond in the thymine-thymine trans-anti conformation in terms of their IR fundamental frequencies, force constants and hyperfine coupling constants. These data will serve to systematize the normal coordinate treatment and the photoinduced mechanism of formation in the thymine dimers series.

EXPERIMENTAL

Thymine dimers were prepared as described in a previous publication [2]. Grade A thymine from Calbiochem was used after recrystallization. The solution 1×10^{-2}M in thymine, diluted with sensitizer acetone at 83 percent, was degassed by subjection to five freeze-pump-thaw cycles at pressures below 10^{-4} torr. The tubes, sealed under vaccum, were placed on an air cooled merry-go-round and irradiated at room temperature for 1, 2 and 12 days periods by a high pressure mercury vapor lamp, fitted with a cyclindrical Pyrex light filter. The precipitates from several samples were mixed, filtered, washed with acetone and water and dried (ca. 35 percent yield). Their routine infrared and NMR spectra were identical to those published for the trans-anti form [3,6].

The IR spectrum was recorded between 4000 and 200 cm^{-1} at room temperature by using a Perkin Elmer grating spectrometer model 621. The sample was used in the solid state. The absorption frequencies are listed in Table 1 together with their experimental assignments, and the actual spectrum is given in Fig.1.

VIBRATIONAL STUDY

Infrared bands assignment.

The adopted C_1 geometry for the trans-anti dimer is shown in Fig.2. The 84 fundamental vibrations present IR and Raman activity.

The IR spectrum of the trans-anti dimer displays a series of bands between 3500 and 2000 cm^{-1} (Fig.1). The absorptions at 3300 and 3180 cm^{-1} are assigned to the N-H stretching vibration (νNH) in good concordance with IR and Raman results and theoretical data in pyrrole, thymine and uracil, and its deuteroderivatives [8-10]. The C-H stretching frequencies region (3100-2950 cm^{-1}) is complicated by strong Fermi resonance [11]. The bands centered at 3090 and 2980 cm^{-1} are assigned to the cyclobutil-type CH stretching mode (νCH) while the absorptions at 2940 and ca. 2837 cm^{-1} are attributable to the   asymmetric and symmetric stretching vibrations of the CH_3 group ($\nu_{as}CH_3$ and ν_sCH_3), in agreement with reported data on thymine [9].

In thymine, two bands at 1750 and 1620 cm^{-1} were assigned to the C=O stretching vibrations (νCO) [9]. As a consequence of an electronic redis tribution, the dimer formation is accompained by a coalescence of these bands. A doublet near 1690 cm^{-1} (Fig.1) is readely attributable to νCO.

It might be mentioned that an empirical assignment for the spectral region between 1400 and 1000 cm^{-1} is almost impossible because of the pronounced coupling between several coordinates (ν_{ring}, νC-CH_3 and defor- mation modes).
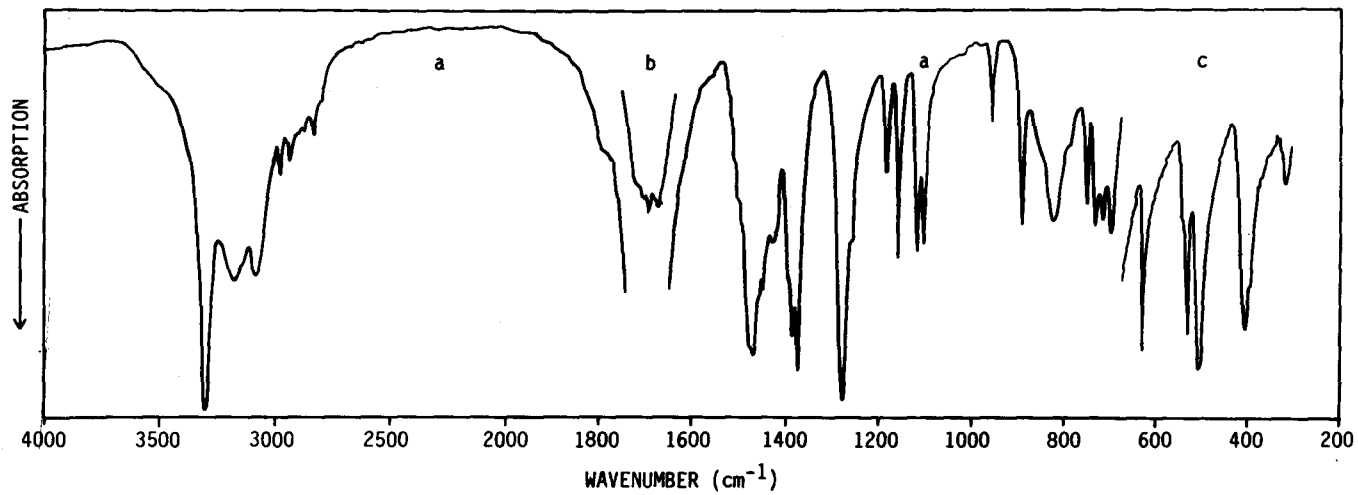
FIG.1. Infrared spectrum of trans-anti thymine dimer

a) medium concentration,

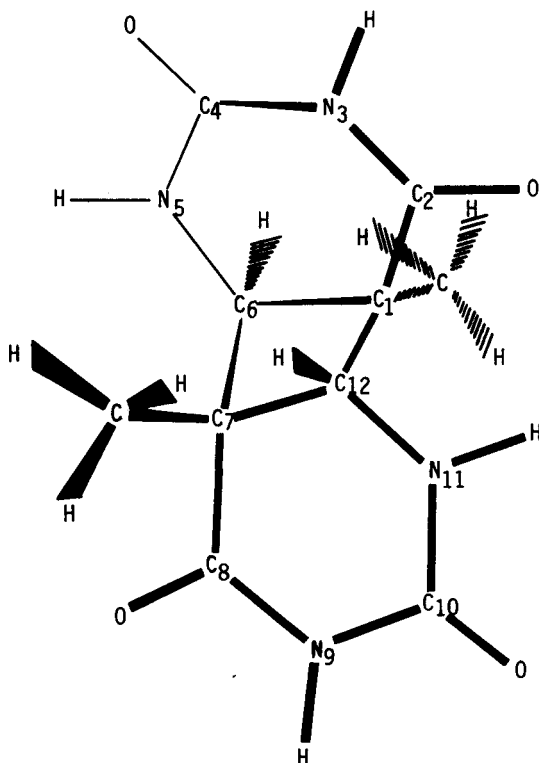b) low concentration,

c) high concentration.

FIG.2. Stereochemical structure of trans-anti thymine dimer.

Two ring stretching modes are expected in the region around 1370 cm^{-1} (Fig.1) [8-10] . The shoulder at about 1390 cm^{-1} is tentatively assigned to νC-CO while the absorption observed at 1370 cm^{-1} is attributable to νCN.

From a structural view point one can distinguish four class of C-C bonds in the dimer (Fig.2). At least two pairs of them are expected to be energetically comparable: C^1-C^{12}(C^6-C^7) and C^1-C^6(C^7-C^{12}). The asymmetric broad band at 1275 cm^{-1} and one of the maxima of the double peak band at

about 1110 cm^{-1} reveal the dimer formation and, in consequence, they are assigned to the cyclobutil stretching modes.

The $\nu(C-CH_3)$ mode is attributable to the band at 1180 cm^{-1}. In thymine this mode, which is highly coupled with ν_{ring} vibrations, is assigned to the absorption at 1203 cm^{-1} [9] . The $\nu(C-CH_3)$ mode is ener- getically more favoured in monomer than in dimer because of electronic contribution of double C=C bond and the proximity of the C=O group (Fig.2).

We assign the bands at 1470 and 1425 cm^{-1} to the NH bending modes (δNH) in agreement with results in the series of uracil derivatives [10,12] . According to the normal coordinate treatment for the pyrimidine derivati- ves [9] , the bands at 1450 and 1383 cm^{-1} are ascribed to δCH_3. The δCH vibrations may be overlapped at ca. 1270 and 1150 cm^{-1} by other bands related to ν_{ring} modes [9] .

The absorption bands due to the hydrogen rocking deformation (ρCH) and skeletal deformation vibrations appear in the region between 900 and 250 cm^{-1} (Fig.1). The band at 955 cm^{-1}, which is observed in thymine at 984 cm^{-1}, can be assigned to the CH_3 rocking vibration (ρCH_3). Banhegyi et al. [13] assigned the calculated frequency 891 cm^{-1} to a ring bending of the cyclobutane molecule. Berends et al. [14] observed that the infrared spectrum of a trans thymine dimer contains the small peaks of a cyclobutane ring system at about 870 cm^{-1}. These results allow us to propose the band at 888 cm^{-1} as due to the $\delta(C^1C^6C^7)$ mode (Fig.2). The broad band at about 820 cm^{-1} might be assigned, as in pyrrole (826 cm^{-1}) [8] , to the δNCC mode. Four absorptions observed at 785, 750, 730 and 712 cm^{-1} have been attributed to the in-plane ring deformation modes (δ_{ring}). The band at 630 cm^{-1} is readily assigned to an δCO vibration in concordance with results in pyri- midines [9,10] and cyclopropenone [15] . The bands at 505 and 530 cm^{-1} are likely to be δ_{ring} vibrations such as the 506 and 521 cm^{-1} bands in thymine [16] . The band at 407 cm^{-1} in the dimer probably corresponds to the that observed in cytosine (422 cm^{-1}), uracil (427 cm^{-1}) and thymine (433 cm^{-1}),

which have been assigned to the out-of-plane oxygen wagging (ρCO) [16] . The
shoulder at 395 cm^{-1} is probably predominantly due to an out-of-plane ring
deformation (χring). The well-defined band at 318 cm^{-1}, which does not occur
in uracil [10] and cytosine spectra [16] , is associated with the torsional
modes of the methyl group (τCH$_3$). The puckering mode is assigned to a
vibration below 200 cm^{-1} [13] .

The infrared frequencies and the experimental assignments of trans-
anti thymine dimer are given in Table 1.

Normal Coordinate treatment

Normal-mode calculations have been carried out for pyrimidine deri-
vatives [9,10,17] and cyclobutane molecules [13] but none has been published
for the thymine dimers series. Therefore we tried to obtain approximate
force constants which account for the experimental assignment for the trans-
anti dimer. The initial force field will be the basis to systematize the
normal coordinate treatment in the series.

a) Structure and coordinates.

As the structure of the dimers is not known, we have taken geometrical
parameters from related molecules: thymine [18] , cyclobutane [13] and
psoralen-thymine monoadduct [19] . The approximate geometry of the trans-
anti dimer was locally modified in order to obtain CNDO/2 energy minima
for the C^1-C^{12}, C^6-C^7 bond lengths, the diedral angle of the cyclobutane-
type skeleton and the angles between the plane of the thymine bases and
the cyclobutyl group ($C^2C^1C^{12}$ and $N^5C^6C^7$) (Fig.2): the optimized values
are 1.561 Å, 26°, 112.8° and 114.3° respectively.

According to Pulay et al. [20] , internal coordinates were
used because they allow a direct comparison with experimental results.
These are identical to those used by Banhegyi et al. [13] for the stret-
ching, bending and puckering modes in cyclobutane. The internal coordinate
definition for the rocking deformation mode (ρ) was taken from an explicit

TABLE 1.

Infrared frequencies (cm^{-1}) and experimental assignments for trans-anti thymine dimer.

Freq.	Assignment	Freq.	Assignment
3300	νNH	1155	νCC(cyclobutyl),δCH
3180	νNH	1115	νCC (cyclobutyl)
3090	νCH (ring)	1100	νCC (cyclobutyl)
2980	νCH (ring)	955	ρCH$_3$
2940	νCH$_3$	888	δCCC (cyclobutyl)
2837	νCH$_3$	820	δNCC
1700	νCO	785	δring
1680	νCO	750	δring
1470	δNH	730	δring
1450	δCH$_3$	712	δring
1425	δNH	694	δCCC (cyclobutyl)
1390	νC-CO	630	δCO
1383	δCH$_3$	530	δring
1370	νCN	505	δring
1275	νCC (cyclobutyl)	407	ρCO
1255	δCH	395	χring
1180	νC-CH$_3$	318	τCH$_3$

formulation given by Aroca et al. [21]. As a model, we considered only the cyclobutane-type skeleton with the same dimer geometrical parameters. The internal coordinates were chosen to satisfy the requirements that they must be the most complete, representative and symmetrically compatible; the elimination of certain groups of symmetrically equivalent internal coordinates does not result in a loss of generality [22]. In the present case, the fourteen internal coordinates were chosen as follows : the
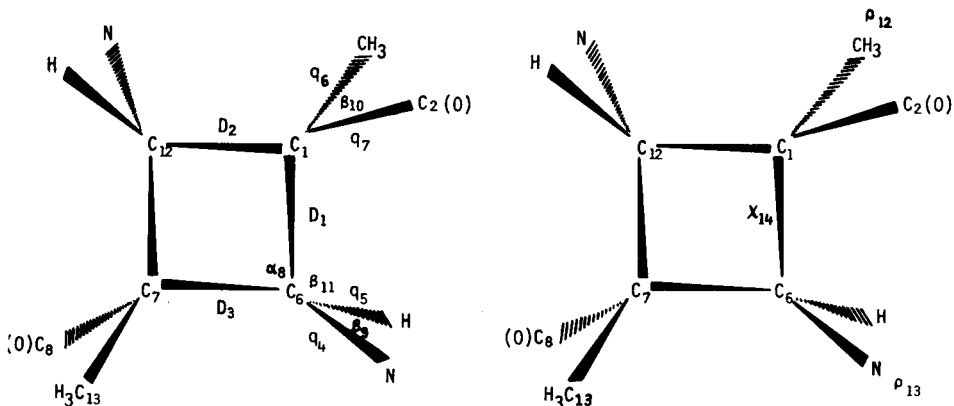
FIG.3. Molecular model and internal coordinates.

changes in 7 bonds, 3CC(D) and 4(q), one ring bending CCC(α), three defor-
mations, NCH, $C^{13}C^1C^2$ and C^1C^6N (β), two rocking coordinates (ρ), and one
internal torsion (puckering) corresponding to the diedral angle
$C^{12}C^1C^6 \frown C^1C^6C^7$ (χ). The adopted molecular model, a non planar one of C_1
symmetry, and the internal coordinates are shown in Fig.3.

b) Force field.

        The calculation was carried out using the method of Wilson et al.
[23] and a valence force field.   We took the force constants from
different sources. The valence force constants were estimated from
CNDO/2 [24] data (total charges and Wiberg's index) by a simple method
derived from a point charge model for the chemical bond [25] ; the ob-
tained values are consistent with reported force constants in related mo-
lecules with similar chemical groups [8,15,17,26,27] . The diagonal defor-
mation constants were transferred from the ones proposed for cyclobutane
[13] and pyrimidine derivatives [9,10] . The force constants involving
the rocking deformation coordinates were obtained from direct computation

of the equation $F_{ii} = \Lambda_i/G_{ii}$, where $\Lambda_i = (w_i/1303.16)^2$ with w_i the expe-
rimental frequency, and published data [8,28]. We added some interaction
constants which exist in the force fields of some of the above mentioned
molecules [9,10,13,26]. The initial force field is built-up using the
same approximation as the kinetic energy, i.e. interactions between vibra-
tions not involving a common atom are assumed to be zero.

Some initial force constants were modified in order to obtain the
experimental wavenumbers and potential energy distribution (PED) of charac-
teristic bands whose assignment is obvious in the trans-anti molecule:
$F(q_5)$, $F(\alpha_8)$, $F(\rho_{13})$ and $f(q_5,\alpha_8)$.

Our $F(D_1)$ value of 3.6 mdin $\overset{\circ}{A}^{-1}$ for the C^1C^6 equilibrium distance
1.537 $\overset{\circ}{A}$ is in agreement with the correlation between CC force constants
and the bond length proposed by Majoube in 9-methylguanine [17]. Unfor-
tunately νC^1C^6 appears highly coupled with other ring stretching modes.
We tried, without success, to remove this coupling from the PED by
modifying the interactions $f(D_1,D_2)$ and $f(D_1,D_3)$ introduced by Banhegyi
et al. in cyclobutane [13].

The most interesting force constants in the present study are those
concerning thymine-thymine bonds $F(D_2)$ and $F(D_3)$. The corresponding force
constant values 4.5 mdyn $\overset{\circ}{A}^{-1}$ are in good accordance with reported data in
cyclobutane (4.57 mdyn $\overset{\circ}{A}^{-1}$) [13]. In order to reduce the difference
between calculated and experimental frequencies we added $f(D_2,\beta_{10})$,
$f(D_3,\beta_9)$ and $f(D_3,q_4)$, which also allows to eliminate the contribution of
δNCH to the mode near 1313 cm^{-1}.

We assumed $F(q_4) = 6.0$ mdyn $\overset{\circ}{A}^{-1}$ as proposed for 9-methylguanine [17].
To remove the vibrational coupling of νCN with stretching modes we introduced
$f(D_3,q_4)$. However, the PED does not allow to distinguish νCN from other
vibrational modes.

When $F(q_7) = 5.6$ mdyn $\overset{\circ}{A}^{-1}$ [10,15], the calculated frequency for the
C-CO stretching mode was originally higher than the experimental wavenumber

(1390 cm^{-1}): to obtain good agreement, without modifying the diagonal value, we were constrained to reduce $f(q_7, \beta_{10})$ and to introduce $f(q_7, \rho_{12})$.

$F(\rho_{12}) \simeq 0.4$ mdyn $\overset{\circ}{A}$ rd^{-2} is considered characteristic for methyl rocking vibrations [28]. With such a value it was impossible to adjust the experimental frequency 955 cm^{-1}; a good approach is found when $F(\rho_{12})$ is about 2.0 mdyin $\overset{\circ}{A}$ rd^{-2}. There is no obvious physical reason to explain this high value.

Proposed values for $F(q_5)$, $F(q_6)$, $F(\alpha_8)$, $F(\beta_9)$, $F(\beta_{10})$, $F(\beta_{11})$ and $F(\rho_{13})$ are in concordance with published data [8,17,26,28]. The interaction constants $f(D_1, q_5)$, $f(D_1, q_7)$, $f(D_1, \alpha_8)$, $f(D_1, x_{14})$, $f(D_2, q_7)$, $f(D_{3}, \alpha_8)$ and $f(q_5, x_{14})$ were set to zero because they had no effect on the frequencies or on the potential energy distribution.

We calculated the thymine-thymine bond dissociation energy on the basis of the force constant value $F(D_2) = 4.5$ mdyn $\overset{\circ}{A}^{-1}$ and the equilibrium distance $r_e = 1.598$ $\overset{\circ}{A}$ by using the formulation given by Lippincott and Schroeder [29]. This model, which has been succesfully used in low polar polyatomic molecules, leads to a dissociation energy for the trans-anti dimer of 82.1 Kcal/mol. This value is in good agreement with experimental data for single C-C bonds (~ 83 Kcal/mol).

The approximate force field obtained is given in Table 2. The mean difference between calculated and experimental frequencies is less than 5 percent (Table 3). We did not try to improve the agreement because of the small amount of experimental data compared to the number of force constants. In fact, by assuming that the frequency differences between the trans-isomers are essentially due to the kinetic energy, experimental frequencies from the trans-syn dimer will serve to refine the proposed approximate force field. Thus, various force constants, mainly those involving the non-diagonal terms, could be modified.

TABLE 2

Most relevant force constants[*]

Coordinates involved[**]	Force constants	Coordinates involved[**]	Force constant
$F(D_1)$	3.60	$f(D_1,D_2)$	0.35
$F(D_2)$	4.50	$f(D_1,D_3)$	0.55
$F(D_3)$	4.50	$f(D_1,\rho_{12})$	-0.45
$F(q_4)$	6.00	$f(D_2,\beta_{10})$	-0.85
$F(q_5)$	5.18	$f(D_2,\rho_{12})$	0.20
$F(q_6)$	4.95	$f(D_3,q_4)$	-0.50
$F(q_7)$	5.60	$f(D_3,\beta_9)$	-0.49
$F(\alpha_8)$	2.15	$f(q_4,\beta_9)$	0.45
$F(\beta_9)$	1.05	$f(q_5,\alpha_8)$	0.20
$F(\beta_{10})$	2.20	$f(q_6,q_7)$	0.42
$F(\beta_{11})$	2.10	$f(q_6,\beta_{10})$	0.40
$F(\rho_{12})$	2.00	$f(q_7,\beta_{10})$	0.55
$F(\rho_{13})$	0.40	$f(q_7,\rho_{12})$	-0.33
$F(x_{14})$	0.45	$f(\beta_9,\beta_{11})$	0.37

[*] Units: stretch, mdyn $\overset{\circ}{A}^{-1}$; stretch-bend, mdyn rad^{-1}; bend, mdyn $\overset{\circ}{A}$ rad^{-2}

[**] For numbering of coordinates, see Fig.3.

c) Spectral assignment.

A good agreement was found   between the most probable assignment obtained by the normal mode calculation (Table 3) and the experimental one (Table 1), except for some bands.

Numerous normal modes involve several coordinates. The thymine-thymine stretching vibrations were calculated at 1313 and 1035 cm^{-1},

TABLE 3

Proposed assignments for some bands of trans-anti thymine dimer

$\nu_{exp.}$ (cm^{-1})	$\nu_{calc.}$	Assignment [*]
3090	3089	νCH
1390	1412	$\nu C-CO$, $\nu C-CH_3$
1275	1313	$\nu_{as} C^6 C^7$, νCN
1180	1208	$\nu C-CH_3$, δCH, $\nu C^6 C^7$
1155	1142	δCH, $\nu C-CO$, $\nu C^1 C^6$
1100	1035	$\nu_s C^6 C^7$, νCN
955	910	ρCH_3, $\nu C^6 C^7$
888	832	$\delta C^1 C^6 C^7$, $\nu C^1 C^6$
712	711	$\delta NC^6 C^1$
694	664	δCH_3
530	568	ρCH
505	455	$\delta C^1 C^6 C^7$
395	426	$\delta C^2 C^1 C^{13}$, ρCH_3
--	93	puckering

[*] For numbering of atoms, see Fig.2.

highly coupled with νCN vibrations.   The normal modes form matrix allowed to distinguish the symmetry of these modes (Table 3).

The observed band at 888 cm^{-1} is calculated at 832 cm^{-1} and assigned to the cyclobutane-type ring bending mode δCCC in accordance with Berends et al. [14] . This mode is coupled with $\nu C^1 C^6$ (Fig.2). The absorption at

712 cm^{-1}, calculated at 711 cm^{-1}, is preferentially a δNCC mode. We have empirically ascribed the band at 694 cm^{-1} to δCCC in concordance with Banhegyi et al. [13] . However the PED shows that this frequency, which is calculated at 664 cm^{-1}, is mainly a δCH$_3$.

The band at 505 cm^{-1}, calculated at 455 cm^{-1}, is due to a ring bending vibration in agreement with Beetz et al. [16] . These authors assigned the band at 530 cm^{-1} to the same mode; nevertheless our calculations suggest that this vibration is attributable to an ρCH mode in concordance with the result of a normal coordinate analysis for pyrrole [8]. Finally our calculations suggest that the shoulder at 395 cm^{-1} is an out-of-plane ring deformation. This mode, which involves the CO group, appears slightly coupled with $\rho(CH_3)$.

REACTION MECHANISM FOR THE TRANS-ANTI DIMER FORMATION.

The formation mechanism of the thymine dimers has been studied by means of ESR and pulse radiolysis; although Infante et al. [30] reported that the mechanism involves radical species, they neither submitted any real evidence about the dimer formation nor its specific configuration. It has been proposed that the thymine cation and anion radicals, $\overset{\cdot+}{T}$ and $\overset{\cdot-}{T}$, are formed by a biphotonic process [31] . Jia-Shan et al. [6] suggested that the dimerization may be induced either by cation-anion recombination or through bimolecular combination of excited states $(\overset{\cdot\cdot}{T})$.

In order to give further insight into the thymine dimers formation, we carried out a preliminar theoretical study on the formation mechanism of the trans conformers. INDO method [32] was used to characterize the structure of the most probable thymine radicals involved in different dimer formation mechanisms [2,6,30,31,33-36] . Input geometries were taken similar to the proposed one in thymine [18] except for the replacement of the C=C double bond (1.327 Å) by a C-C single bond length (1.54 Å). The relative stability follows the order $\overset{\cdot-}{T}$> $\overset{\cdot\cdot}{T}$ >$\overset{\cdot+}{T}$. Net charges, spin densities

TABLE 4

Net charges (q), spin densities (ρ^π) and hyperfine coupling constants
(a) (Gauss) for precursors of the trans-anti dimer formation

Atom*	$\ddot{T}$			$\dot{T}^-$			$\dot{T}^+$		
	q	ρ^π	a	q	ρ^π	a	q	ρ^π	a
1	-0.055	0.030	24.36	-0.326	-0.001	-0.40	0.035	0.026	21.56
2	0.370	0.017	-14.25	0.407	-0.001	-0.38	0.399	-0.017	-13.84
3	-0.284	0.002	0.81	-0.299	0.001	0.51	-0.236	-0.004	-1.64
4	0.504	-0.005	-3.81	0.496	-0.001	-0.72	0.517	-0.007	-5.75
5	-0.134	0.011	4.03	-0.198	0.001	0.29	-0.018	0.021	7.88
6	-0.036	0.030	24.89	-0.072	0.033	27.30	0.215	-0.011	-8.86
7	0.002	0.033	17.64	-0.061	-0.027	-14.54	0.069	0.003	1.77
8	0.062	0.012	10.09	0.132	-0.002	-1.68	0.032	-0.009	-7.27
9	-0.290	0.009	8.14	-0.501	0.002	1.76	-0.215	0.009	8.02
10	0.126	0.002	0.84	0.069	-0.002	-1.13	0.176	0.001	0.46
11	-0.378	0.002	1.48	-0.479	-0.000	-0.00	-0.293	0.003	2.92
12	0.122	0.013	6.88	0.064	-0.003	-1.74	0.194	-0.016	-8.82
13	-0.020	0.002	0.79	-0.068	-0.001	-0.74	0.024	0.003	1.36
14	0.006	0.058	31.28	-0.080	0.010	5.35	0.054	0.044	23.73
15	0.006	0.058	31.28	-0.080	0.010	5.35	0.054	0.044	23.73

* For numbering of atoms, see Fig.4.

and hyperfine coupling constants are collected in Table 4. Schematic mo-
lecular structure of $\ddot{T}$, $\dot{T}^-$ and $\dot{T}^+$ are shown in Fig.4.

Net charges and spin densities explain quite well the structure of
radicals displayed in Fig.4. Graslund et al. [35] indicated that in
γ-irradiated DNA the relation between the amount of anionic and cationic
species should be in the range 1:1 to 3:1. The hyperfine coupling cons-
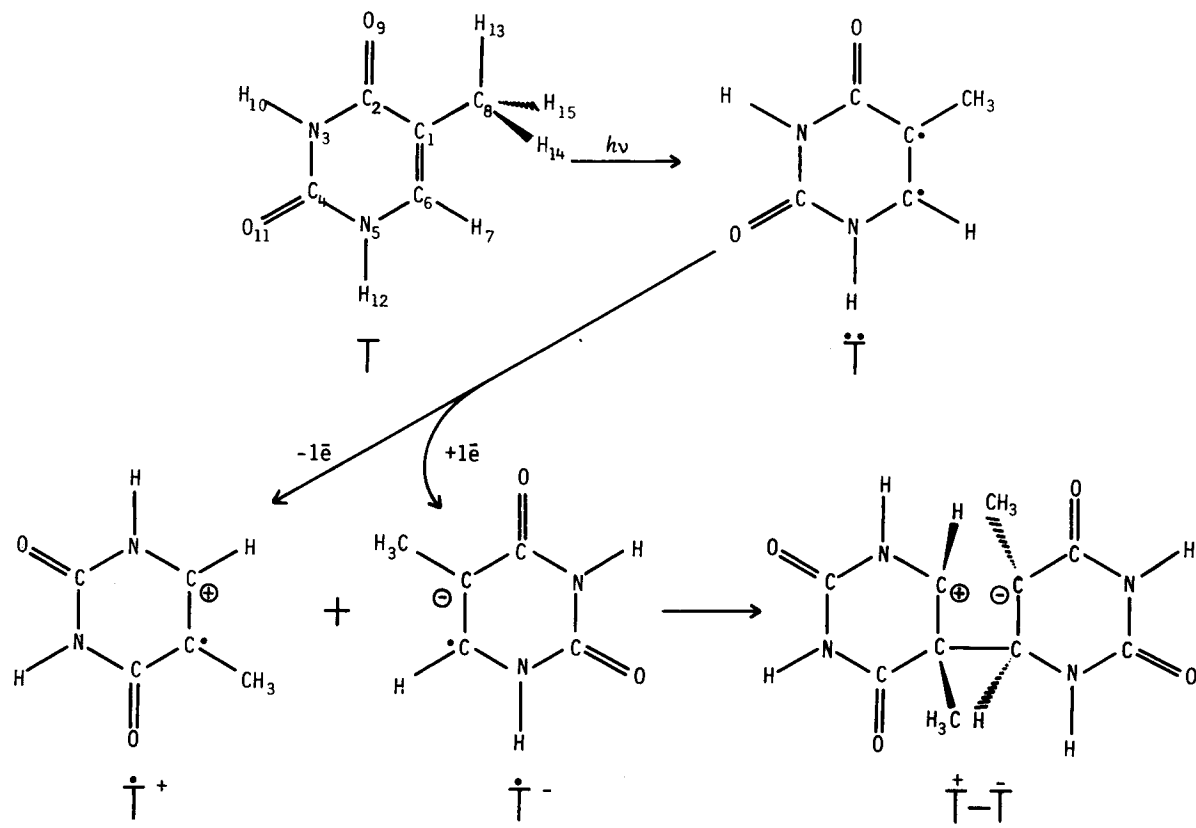tants are in accordance with experimental data [31] ; particularly, the

FIG.4.   Proposed mechanism for the trans-anti dimer formation.

width of the singlet observed in DNA (27 G) [33] equals the width of the
thymine anion doublet (Table 4).

Under experimental conditions studied, thymine trans-anti dimer pre-
dominated. This result suggests that the reaction does not proceed by
way of a concerted attack of excited triplet thymine $\overset{..}{T}$ on ground state
monomer. In fact, such a mechanism is unacceptable on a theoretical view-
point for any photo-induced cycloaddition involving the triplet state.
A reasonable alternative mechanism seems to be the photoionization of
thymine from its excited triplet state [31] : the species produced by loss
of an electron is considered a cation radical $\overset{.+}{T}$ and the result of the
electron attachment is an anion radical $\overset{.-}{T}$. It is also expectable that the
electron reacts with the π cation to form thymine. It seems that the next
step is the recombination of reactants $\overset{.+}{T}$ and $\overset{.-}{T}$ to form $\overset{+\ -}{T\text{-}T}$ in the trans-
anti or trans-syn conformations (Fig.4). These precursors are more stable
than monotertiary radicals $\overset{.\ .}{T\text{-}T}$.

The most probable structure for the $\overset{+\ -}{T\text{-}T}$ trans conformers was found
when $C^6C^1C^{12}$ and $C^7C^{12}C^1$ angles and C^1C^{12} bond length (Fig.2) were equal to
95° and 1.56 Å respectively. Calculated dipole moments (μ) are 2.14 D for
$\overset{+\ -}{T\text{-}T}$ trans-anti and 7.52 D for $\overset{+\ -}{T\text{-}T}$ trans-syn. As in a conformational equi-
librium the most polar form is favoured by increasing the dielectric cons-
tants of the medium [37] , one would expect to obtain more trans-syn dimer
(μ = 7.73 D), as observed at low sensitizer concentration (high dielectric
constants of the solution) [2] . Thus, the trans-anti dimer (μ = 1.92 D)
will be structurally favoured under experimental conditions here used i.e.
solution of lower dielectric constant (83% acetone).

The above results allowed us to propose the photochemical mechanism
of thymine trans dimers formation displayed in Fig.4.

REFERENCES

1. D.L.WULFF and G.FRAENKEL, Biochim, Biophys. Acta 51 (1961) 332.

2. B.H.JENNINGS, S-CH.PASTRA and J.L.WELLINGTON, Photochem. Photobiol.,
    11 (1970) 215.

3. D.WEINBLUM and H.E.JOHNS, Biochim. Biophys. Acta,114 (1966) 450.

4. F.JORDAN and B.PULLMAN,Theoret. Chim. Acta, 10 (1968) 423.

5. C.NAGATA, A.IMAMURA, Y.TAGASHIRA, M.KODAMA and N.FUKUDA, J.Theoret.
    Biol.,9 (1965) 357.

6. Z.JIA-SHAN, Q.GUO-LIANG and Z.WEN-LONG, Radiat. Phys. Chem., 17 (1981)
    207.

7. V.A.SHARPATYI, J.CADET and R.TEOULE, Int. J. Rad. Biol.,33 (1978) 419.

8. C.A.ACEVEDO, M.CAMPOS-VALLETTE and R.E.CLAVIJO C., Spectrochimica Acta,
    42A (1986) 919.

9. H.SUSI and J.S.ARD, Spectrochimica Acta,30A (1974) 1843.

10. H.SUSI and J.S.ARD, Spectrochimica Acta,27A (1971) 1549.

11. F.A.MILLER, K.J.CAPWELL, R.C.LORD and D.G.REA, Spectrochimica Acta,28A
    (1972) 603.

12. H.T.MILES, T.P.LEWIS, E.D.BECKER and J.FRAZIER, J.Biol.Chem., 248
    (1973) 1115.

13. G.BANHEGYI, G.FOGARASI and P.PULAY, J.Mol.Structure, 89 (1982) 1.

14. R.BEUKER and W.BERENDS, Biochim. Biophys. Acta, 41 (1960) 550.

15. G.DIAZ F., B.N.CYVIN and S.J.CYVIN, Spectroscopy Lett., 12 (1979) 151.

16. C.P.BEETZ, Jr. and G.ASCARELLI, Spectrochimica Acta, 36A (1979) 299.

17. M.MAJOUBE, Biopolymers, 24 (1985) 1075.

18. J.E.Del BENE, J. Chem. Phys., 76 (1982) 1058.

19. S.PECKLER, B.GRAVES, D.KANNE, H.RAPOPORT, J.HEARST and S.H.KIM, J.
    Mol. Biol., 162 (1982) 157.

20. P.PULAY, G.FOGARASI, F.PANG and J.E.BOOGS, J. Am. Chem. Soc., 101
    (1979) 2550.

21. R.AROCA M., Y.N.PANCHENKO and A.S.MAKHNIOV, Chem. Phys., 7 (1975)
    457.

22. S.J.CYVIN, J.BRANVEL and B.N.CYVIN, Mol. Phys., 14 (1968) 43; B.
    VIZI and S.J.CYVIN, Acta Chem. Scand., 22 (1968) 2012.

23. E.B.WILSON, J.C.DECIUS and P.C.CROSS, "Molecular Vibrations". Ed.
    Mc Graw-Hill Co., N.Y. (1955).

24. J.A.POPLE and D.L.BEVERIDGE "Approximative Molecular Orbital Theory"
    Mc Graw Hill, N.Y. (1970).

25. W.KOSMUS, Z. Naturforsch., 33A (1978) 1056.

26. J.JAKES and S.KRIMM, Spectrochim. Acta, 27A (1971) 19.

27. M.ASAI, K.NODA and A.SADO, Spectrochim. Acta,30A (1974) 1147.

28. D.W.SCOTT, J. Mol. Spectrosc., 31 (1969) 451; G.MILLE, J.METZGER,
    C.POUCHAN and M.CHAILLET, Spectrochim. Acta, 31A (1975) 1115.

29. E.R.LIPPINCOTT and R.SCHROEDER, J. Am. Chem. Soc., 78 (1956) 5175.

30. G.A.INFANTE, P.JIRATHANA, J.H.FENDLER and E.J.FENDLER, J.C.S.Faraday Trans.I., 69 (1973) 1586.

31. M.D.SEVILLA, J.Phys. Chem., 75 (1971) 626.

32. J.A.POPLE, D.L.BEVERIDGE and P.A.DOBOSH, J.Am.Chem.Soc., 90 (1968) 421; ibid., J. Chem. Phys., 47 (1967) 2026.

33. A.D.LENHERR and M.G.ORMEROD, Biochim. Biophys. Acta, 166 (1968) 298.

34. S.GREGOLI, M.OLAST and A.BERTINCHAMPS, Radiat. Res., 65 (1976) 202.

35. A.GRASLUND, A.EHRENBERG, A.RUPPRECHT and G.STROM, Biochim. Biophys. Acta, 254 (1971) 172.

36. M.N.KHATTAK and S.YI WANG, Tetrahedron, 28 (1972) 945.

37. M.CAMPOS-VALLETTE and M.REY-LAFON, J.Mol.Struct., 101 (1983) 23.