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## **Infrared Assignment and Formation Mechanism of Trans-Anti Thymine Dimer**

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

Keywords : Molecular vibrations, Force constants, Hyperfine coupling constants, Cyclobutadithymine.

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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, respectively.

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

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  $120^\circ$  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  $1 \times 10^{-2} \text{M}$  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 vacuum, 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 cylindrical 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  $\text{cm}^{-1}$  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  $\text{cm}^{-1}$  (Fig.1). The absorptions at 3300 and 3180  $\text{cm}^{-1}$  are assigned to the N-H stretching vibration ( $\nu_{\text{NH}}$ ) 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  $\text{cm}^{-1}$ ) is complicated by strong Fermi resonance [11]. The bands centered at 3090 and 2980  $\text{cm}^{-1}$  are assigned to the cyclobutyl-type CH stretching mode ( $\nu_{\text{CH}}$ ) while the absorptions at 2940 and ca. 2837  $\text{cm}^{-1}$  are attributable to the asymmetric and symmetric stretching vibrations of the  $\text{CH}_3$  group ( $\nu_{\text{as}}\text{CH}_3$  and  $\nu_{\text{s}}\text{CH}_3$ ), in agreement with reported data on thymine [9].

In thymine, two bands at 1750 and 1620  $\text{cm}^{-1}$  were assigned to the C=O stretching vibrations ( $\nu_{\text{CO}}$ ) [9]. As a consequence of an electronic redistribution, the dimer formation is accompanied by a coalescence of these bands. A doublet near 1690  $\text{cm}^{-1}$  (Fig.1) is readily attributable to  $\nu_{\text{CO}}$ .

It might be mentioned that an empirical assignment for the spectral region between 1400 and 1000  $\text{cm}^{-1}$  is almost impossible because of the pronounced coupling between several coordinates ( $\nu_{\text{ring}}$ ,  $\nu_{\text{C-CH}_3}$  and deformation modes).

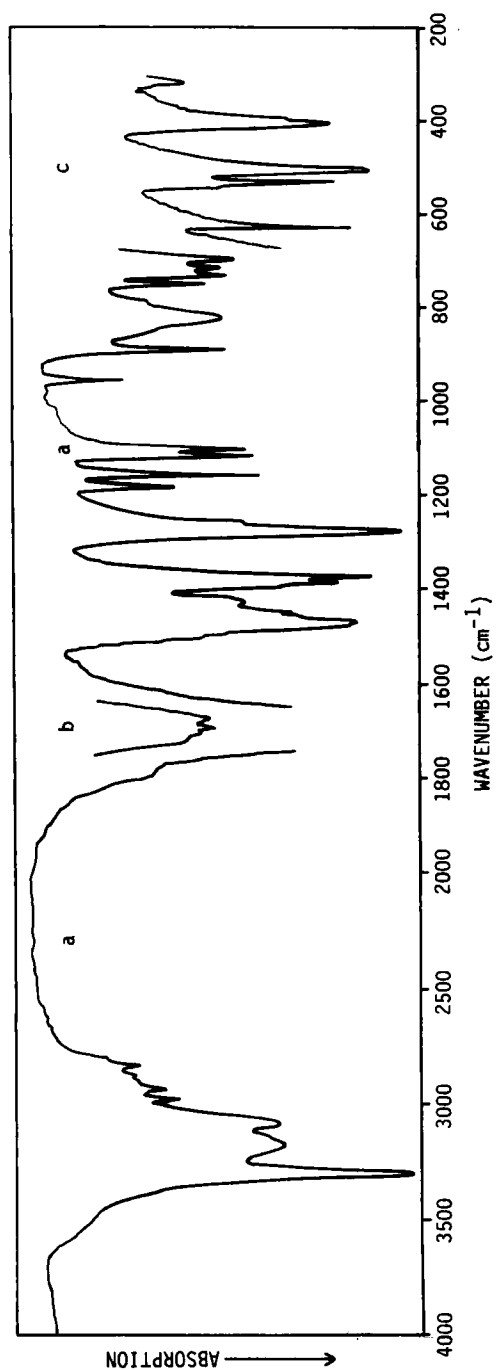


FIG.1. Infrared spectrum of trans-anti thymine dimer

- a) medium concentration,
- b) low concentration,
- c) high concentration.

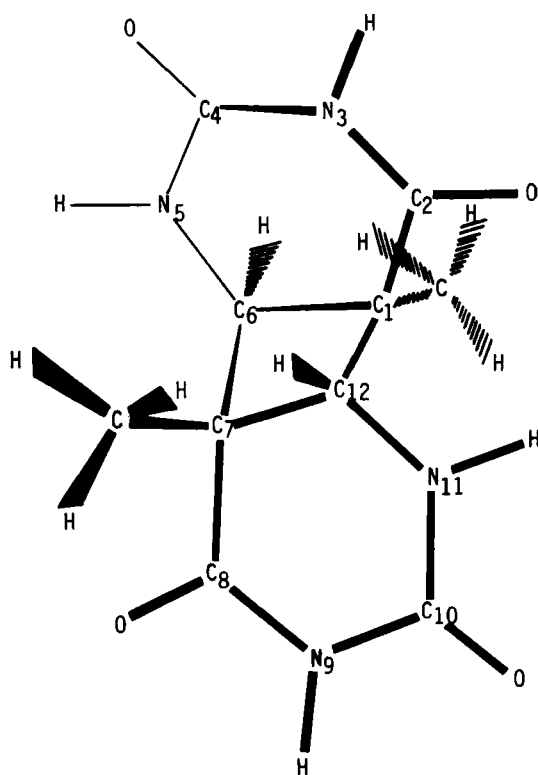


FIG.2. Stereochemical structure of trans-anti thymine dimer.

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

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:  $\text{C}^1\text{-C}^{12}(\text{C}^6\text{-C}^7)$  and  $\text{C}^1\text{-C}^6(\text{C}^7\text{-C}^{12})$ . The asymmetric broad band at  $1275\text{ cm}^{-1}$  and one of the maxima of the double peak band at

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

The  $\nu(\text{C-CH}_3)$  mode is attributable to the band at  $1180\text{ cm}^{-1}$ . In thymine this mode, which is highly coupled with  $\nu_{\text{ring}}$  vibrations, is assigned to the absorption at  $1203\text{ cm}^{-1}$  [9]. The  $\nu(\text{C-CH}_3)$  mode is energetically 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  $1425\text{ cm}^{-1}$  to the NH bending modes ( $\delta\text{NH}$ ) in agreement with results in the series of uracil derivatives [10,12]. According to the normal coordinate treatment for the pyrimidine derivatives [9], the bands at  $1450$  and  $1383\text{ cm}^{-1}$  are ascribed to  $\delta\text{CH}_3$ . The  $\delta\text{CH}$  vibrations may be overlapped at ca.  $1270$  and  $1150\text{ cm}^{-1}$  by other bands related to  $\nu_{\text{ring}}$  modes [9].

The absorption bands due to the hydrogen rocking deformation ( $\rho\text{CH}$ ) and skeletal deformation vibrations appear in the region between  $900$  and  $250\text{ cm}^{-1}$  (Fig.1). The band at  $955\text{ cm}^{-1}$ , which is observed in thymine at  $984\text{ cm}^{-1}$ , can be assigned to the  $\text{CH}_3$  rocking vibration ( $\rho\text{CH}_3$ ). Banhegyi et al. [13] assigned the calculated frequency  $891\text{ cm}^{-1}$  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  $870\text{ cm}^{-1}$ . These results allow us to propose the band at  $888\text{ cm}^{-1}$  as due to the  $\delta(\text{C}^1\text{C}^6\text{C}^7)$  mode (Fig.2). The broad band at about  $820\text{ cm}^{-1}$  might be assigned, as in pyrrole ( $826\text{ cm}^{-1}$ ) [8], to the  $\delta\text{NCC}$  mode. Four absorptions observed at  $785$ ,  $750$ ,  $730$  and  $712\text{ cm}^{-1}$  have been attributed to the in-plane ring deformation modes ( $\delta_{\text{ring}}$ ). The band at  $630\text{ cm}^{-1}$  is readily assigned to an  $\delta\text{CO}$  vibration in concordance with results in pyrimidines [9,10] and cyclopropanone [15]. The bands at  $505$  and  $530\text{ cm}^{-1}$  are likely to be  $\delta_{\text{ring}}$  vibrations such as the  $506$  and  $521\text{ cm}^{-1}$  bands in thymine [16]. The band at  $407\text{ cm}^{-1}$  in the dimer probably corresponds to the that observed in cytosine ( $422\text{ cm}^{-1}$ ), uracil ( $427\text{ cm}^{-1}$ ) and thymine ( $433\text{ cm}^{-1}$ ),

which have been assigned to the out-of-plane oxygen wagging ( $\rho\text{CO}$ ) [16]. The shoulder at  $395\text{ cm}^{-1}$  is probably predominantly due to an out-of-plane ring deformation ( $\chi\text{ring}$ ). The well-defined band at  $318\text{ cm}^{-1}$ , which does not occur in uracil [10] and cytosine spectra [16], is associated with the torsional modes of the methyl group ( $\tau\text{CH}_3$ ). The puckering mode is assigned to a vibration below  $200\text{ cm}^{-1}$  [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 derivatives [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  $\text{C}^1\text{-C}^{12}$ ,  $\text{C}^6\text{-C}^7$  bond lengths, the diedral angle of the cyclobutane-type skeleton and the angles between the plane of the thymine bases and the cyclobutyl group ( $\text{C}^2\text{C}^1\text{C}^{12}$  and  $\text{N}^5\text{C}^6\text{C}^7$ ) (Fig.2): the optimized values are  $1.561\text{ \AA}$ ,  $26^\circ$ ,  $112.8^\circ$  and  $114.3^\circ$  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 stretching, bending and puckering modes in cyclobutane. The internal coordinate definition for the rocking deformation mode ( $\rho$ ) was taken from an explicit



TABLE 1.

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

Freq.	Assignment	Freq.	Assignment
3300	$\nu\text{NH}$	1155	$\nu\text{CC}(\text{cyclobutyl}), \delta\text{CH}$
3180	$\nu\text{NH}$	1115	$\nu\text{CC}(\text{cyclobutyl})$
3090	$\nu\text{CH}(\text{ring})$	1100	$\nu\text{CC}(\text{cyclobutyl})$
2980	$\nu\text{CH}(\text{ring})$	955	$\rho\text{CH}_3$
2940	$\nu\text{CH}_3$	888	$\delta\text{CCC}(\text{cyclobutyl})$
2837	$\nu\text{CH}_3$	820	$\delta\text{NCC}$
1700	$\nu\text{CO}$	785	$\delta\text{ring}$
1680	$\nu\text{CO}$	750	$\delta\text{ring}$
1470	$\delta\text{NH}$	730	$\delta\text{ring}$
1450	$\delta\text{CH}_3$	712	$\delta\text{ring}$
1425	$\delta\text{NH}$	694	$\delta\text{CCC}(\text{cyclobutyl})$
1390	$\nu\text{C-CO}$	630	$\delta\text{CO}$
1383	$\delta\text{CH}_3$	530	$\delta\text{ring}$
1370	$\nu\text{CN}$	505	$\delta\text{ring}$
1275	$\nu\text{CC}(\text{cyclobutyl})$	407	$\rho\text{CO}$
1255	$\delta\text{CH}$	395	$\chi\text{ring}$
1180	$\nu\text{C-CH}_3$	318	$\tau\text{CH}_3$

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

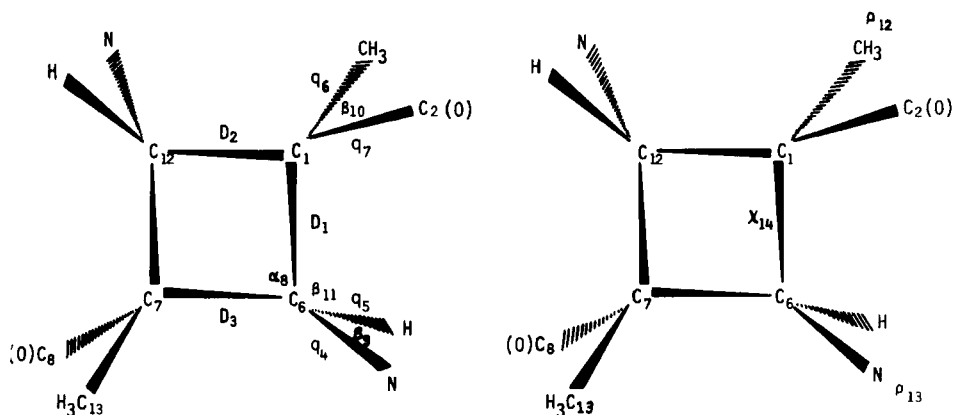


FIG.3. Molecular model and internal coordinates.

changes in 7 bonds, 3CC(D) and 4(q), one ring bending CCC( $\alpha$ ), three deformations, NCH,  $C^{13}C^1C^2$  and  $C^1C^6N$  ( $\beta$ ), two rocking coordinates ( $\rho$ ), and one internal torsion (puckering) corresponding to the diedral angle  $C^{12}C^1C^6 \sim C^1C^6C^7$  ( $x$ ). 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 obtained values are consistent with reported force constants in related molecules with similar chemical groups [8,15,17,26,27]. The diagonal deformation 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  $F_{ij} = \Lambda_i/G_{ij}$ , where  $\Lambda_i = (w_i/1303.16)^2$  with  $w_i$  the experimental 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 vibrations 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 characteristic 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  $3.6 \text{ mdyn } \text{\AA}^{-1}$  for the  $\text{C}^1\text{C}^6$  equilibrium distance  $1.537 \text{ \AA}$  is in agreement with the correlation between CC force constants and the bond length proposed by Majoube in 9-methylguanine [17]. Unfortunately  $\nu\text{C}^1\text{C}^6$  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  $4.5 \text{ mdyn } \text{\AA}^{-1}$  are in good accordance with reported data in cyclobutane ( $4.57 \text{ mdyn } \text{\AA}^{-1}$ ) [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  $\delta\text{NCH}$  to the mode near  $1313 \text{ cm}^{-1}$ .

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

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

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

$F(\rho_{12}) \simeq 0.4 \text{ mdyin } \text{\AA} \text{ rd}^{-2}$  is considered characteristic for methyl rocking vibrations [28]. With such a value it was impossible to adjust the experimental frequency 955  $\text{cm}^{-1}$ ; a good approach is found when  $F(\rho_{12})$  is about 2.0  $\text{mdyn } \text{\AA} \text{ rd}^{-2}$ . 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  $F(D_2) = 4.5 \text{ mdyin } \text{\AA}^{-1}$  and the equilibrium distance  $r_e = 1.598 \text{ \AA}$  by using the formulation given by Lippincott and Schroeder [29]. This model, which has been successfully 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 ( $\sim 83$  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  $\text{\AA}^{-1}$ ; stretch-bend, mdyn  $\text{rad}^{-1}$ ; bend, mdyn  $\text{\AA} \text{rad}^{-2}$ 

\*\* 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  $\text{cm}^{-1}$ ,

TABLE 3

Proposed assignments for some bands of trans-anti thymine dimer

$\nu_{\text{exp.}} (\text{cm}^{-1})$	$\nu_{\text{calc.}}$	Assignment *
3090	3089	$\nu\text{CH}$
1390	1412	$\nu\text{C-CO}$ , $\nu\text{C-CH}_3$
1275	1313	$\nu_{\text{as}}\text{C}^6\text{C}^7$ , $\nu\text{CN}$
1180	1208	$\nu\text{C-CH}_3$ , $\delta\text{CH}$ , $\nu\text{C}^6\text{C}^7$
1155	1142	$\delta\text{CH}$ , $\nu\text{C-CO}$ , $\nu\text{C}^1\text{C}^6$
1100	1035	$\nu_{\text{s}}\text{C}^6\text{C}^7$ , $\nu\text{CN}$
955	910	$\rho\text{CH}_3$ , $\nu\text{C}^6\text{C}^7$
888	832	$\delta\text{C}^1\text{C}^6\text{C}^7$ , $\nu\text{C}^1\text{C}^6$
712	711	$\delta\text{NC}^6\text{C}^1$
694	664	$\delta\text{CH}_3$
530	568	$\rho\text{CH}$
505	455	$\delta\text{C}^1\text{C}^6\text{C}^7$
395	426	$\delta\text{C}^2\text{C}^1\text{C}^{13}$ , $\rho\text{CH}_3$
--	93	puckering

\* For numbering of atoms, see Fig.2.

highly coupled with  $\nu\text{CN}$  vibrations. The normal modes form matrix allowed to distinguish the symmetry of these modes (Table 3).

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

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

The band at  $505\text{ cm}^{-1}$ , calculated at  $455\text{ cm}^{-1}$ , is due to a ring bending vibration in agreement with Beetz et al. [16]. These authors assigned the band at  $530\text{ cm}^{-1}$  to the same mode; nevertheless our calculations suggest that this vibration is attributable to an  $\rho\text{CH}$  mode in concordance with the result of a normal coordinate analysis for pyrrole [8]. Finally our calculations suggest that the shoulder at  $395\text{ cm}^{-1}$  is an out-of-plane ring deformation. This mode, which involves the CO group, appears slightly coupled with  $\rho(\text{CH}_3)$ .

#### 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,  $\dot{\text{T}}^+$  and  $\dot{\text{T}}^-$ , 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 ( $\dot{\text{T}}$ ).

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 ( $1.327\text{ \AA}$ ) by a C-C single bond length ( $1.54\text{ \AA}$ ). The relative stability follows the order  $\dot{\text{T}}^- > \dot{\text{T}} > \dot{\text{T}}^+$ . Net charges, spin densities

TABLE 4

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

Atom*	$\dot{T}^\cdot$			$\dot{T}^-$			$\dot{T}^+$		
	q	$\rho^\pi$	a	q	$\rho^\pi$	a	q	$\rho^\pi$	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	-1.63	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	-1.18	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 molecular structure of  $\dot{T}$ ,  $\dot{T}^-$  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  $\gamma$ -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 constants are in accordance with experimental data [31]; particularly, the



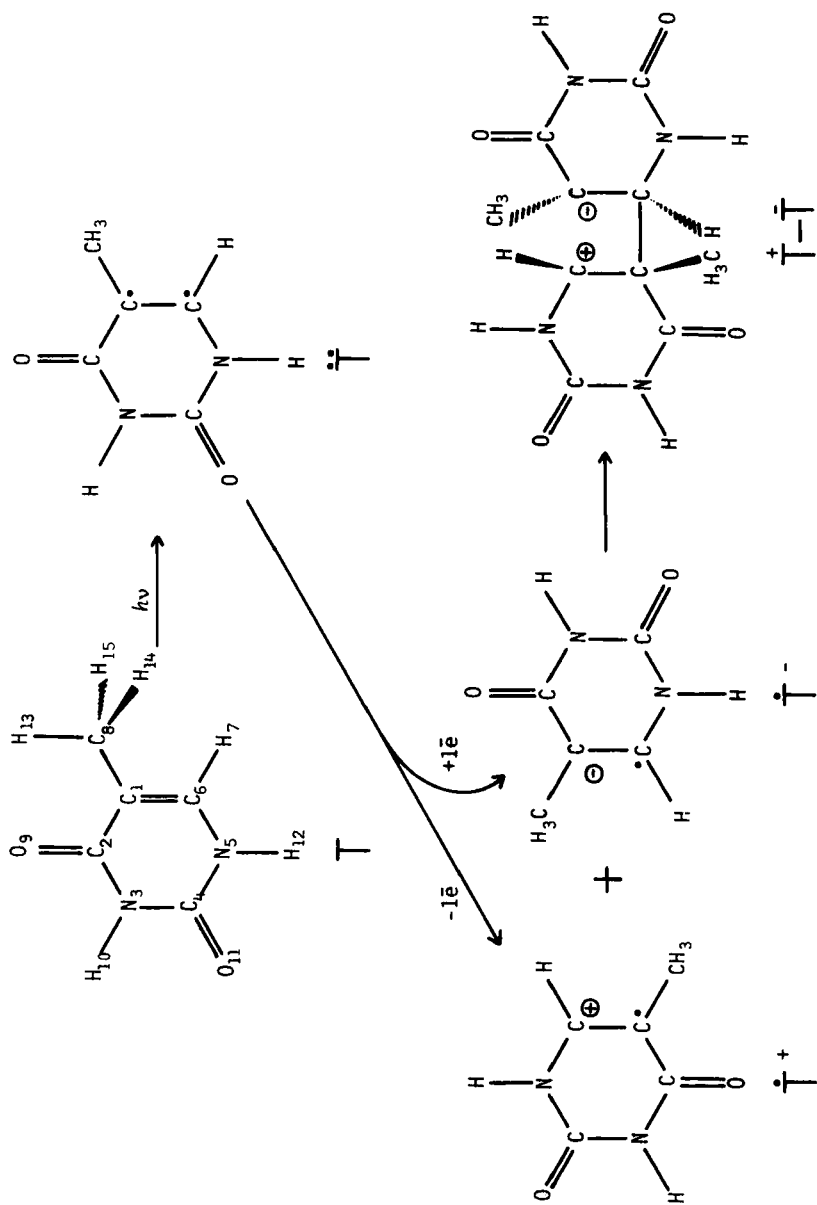


FIG. 4. Proposed mechanism for the trans-anti dimer formation.

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 predominated. This result suggests that the reaction does not proceed by way of a concerted attack of excited triplet thymine  $\dot{\text{T}}$  on ground state monomer. In fact, such a mechanism is unacceptable on a theoretical viewpoint 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  $\dot{\text{T}}^+$  and the result of the electron attachment is an anion radical  $\dot{\text{T}}^-$ . It is also expectable that the electron reacts with the  $\pi$  cation to form thymine. It seems that the next step is the recombination of reactants  $\dot{\text{T}}^+$  and  $\dot{\text{T}}^-$  to form  $\text{T}^+-\text{T}^-$  in the trans-anti or trans-syn conformations (Fig.4). These precursors are more stable than monoteritary radicals  $\dot{\text{T}}-\dot{\text{T}}$ .

The most probable structure for the  $\text{T}^+-\text{T}^-$  trans conformers was found when  $\text{C}^6\text{C}^1\text{C}^{12}$  and  $\text{C}^7\text{C}^{12}\text{C}^1$  angles and  $\text{C}^1\text{C}^{12}$  bond length (Fig.2) were equal to  $95^\circ$  and  $1.56 \text{ \AA}$  respectively. Calculated dipole moments ( $\mu$ ) are 2.14 D for  $\text{T}^+-\text{T}^-$  trans-anti and 7.52 D for  $\text{T}^+-\text{T}^-$  trans-syn. As in a conformational equilibrium the most polar form is favoured by increasing the dielectric constants of the medium [37], one would expect to obtain more trans-syn dimer ( $\mu = 7.73 \text{ D}$ ), as observed at low sensitizer concentration (high dielectric constants of the solution) [2]. Thus, the trans-anti dimer ( $\mu = 1.92 \text{ D}$ ) 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.

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