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THE STRUCTURES OF THE ISOMERIC THYMINE DIMERS AS DEDUCED FROM THEIR DIPOLE MOMENTS

D. WEINBLUM, F. P. OTTENSMEYER AND G. F WRIGHT

Department of Medical Biophysics and
Department of Chemistry, University of Toronto, Toronto (Canada)

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SUMMARY

Dipole moments of 3 isomeric tetramethyl-thymine dimers, designated by WEINBLUM AND JOHNS as A, B, and C were measured. The values obtained were 6.04, 2.79 and 5.75 Debye respectively. Calculated values for four structures, I, II, III and IV, thought possible by WULFF AND FRAENKEL were 6.1–6.8, 3.1–3.5, 5.1–5.6 and 0 Debye respectively. Accordingly Dimer A was assigned to Structure I, Dimer B to Structure II and Dimer C to Structure III. The identity of the three tetramethyl dimers with 3 fully methylated thymine dimers was established by comparison of their respective infrared spectra.

INTRODUCTION

WULFF AND FRAENKEL[1] have suggested that thymine dimers ($\widehat{\mathrm{TT}}$'s) can have 4 different steric arrangements (Fig. 1) or 6 if the D- and L-forms of Structures II and III are considered. Studies using chemical transformation[2] and NMR spectra[3,4] have indicated that the thymine dimer obtained by ultraviolet irradiation of frozen aqueous solutions of thymine corresponds to Structure I. The isolation of 5 different $\widehat{\mathrm{TT}}$'s, 2 being D- and L-forms of 1 dimer, was reported by WEINBLUM AND JOHNS[5]. On the basis of chemical stabilities, of infrared spectra and origin of the thymine dimers ($\widehat{\mathrm{TT}}$'s) WEINBLUM AND JOHNS tentatively assigned Structures I, II and III, and IV (Fig. 1) to their Dimers A, B, C(+, −), and D respectively. As a further test of the validity of these assignments the measurement of the dipole moments of the dimers was undertaken. Calculation of the dipole moments of the different structures by summation of the moments of individual atom pairs indicated that the total moments of the dimers differed to such an extent that a definite assignment of structure would be possible. This calculation could be performed with a high degree of confidence because the absence of conjugation in the structures of the dimers prohibits excessive delocal-

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Abbreviations: $\widehat{\mathrm{TT}}$, thymine dimer; TM$\widehat{\mathrm{TT}}$, N^1, N^3, $N^{1'}$, $N^{3'}$-tetramethyl-thymine dimer.

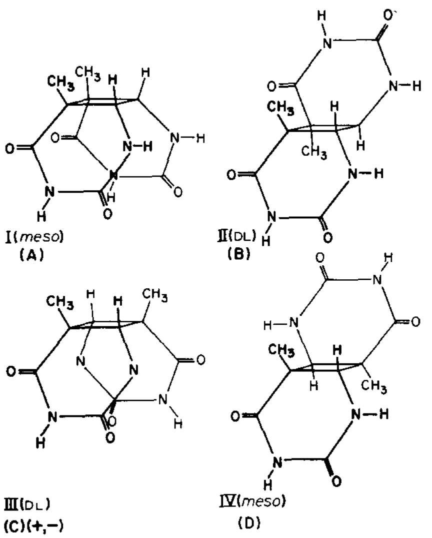


Fig. 1. Isomeric thymine dimers. I, II, III, and IV refer to the structures suggested by WULFF AND FRAENKEL[1], while A, B, C, and D refer to the dimers isolated by WEINBLUM AND JOHNS[5].

ization of electric charge and so virtually isolates the dipole moments of individual atom pairs. Moreover, in dihydrothymine a substance was available which represents almost exactly one half of a thymine dimer. With but minor corrections the calculation of the dipole moments of the dimers was then merely the vector addition of two such moments for dihydrothymine in different steric arrangements corresponding to the different dimer structures.

The measurement of the dipole moment of any substance requires that a relatively large amount of the material be dissolved in a non-polar solvent. Since the $\widehat{TT}$'s are essentially insoluble in benzene, the solvent used, or in any other non-polar solvent, the required solubility was attained by methylating the four *N*-positions of the dimers. Unfortunately methylation of the $\widehat{TT}$'s resulted in very low yields of tetramethyl-thymine dimers (TM$\widehat{TT}$'s). However, 3 different isomeric forms of TM$\widehat{TT}$'s can be produced easily and in quantity from other sources[1,6]. These tetramethyl dimers were then used for dipole moment measurements. The correspondence of these dimers with the TM$\widehat{TT}$'s obtained by direct methylation of the $\widehat{TT}$'s was established by use of infrared spectra.

MATERIALS AND METHODS

1. *Direct preparation of tetramethyl-thymine dimers*

1 (*a*) *Preparation of thymine dimers.* The thymine dimers were prepared as described in a previous publication[2].

1 (*b*) *Methylation of thymine dimers.* The most successful method of methylation was found to be a modification of the procedure described by WULFF AND FRAENKEL[1]. 10 mg of a given dimer, either A, B, C(−), or D, were dissolved in 0.8 ml of 1 M NaOH. 75 µl of dimethylsulphate were added under constant stirring. After all of the dime-

thylsulphate had dissolved in the solution, which had now become acidic, the methylated dimer was immediately extracted 3 times with 3-ml volumes of chloroform. The chloroform solution was dried by passage through dry filter paper. On removal of the chloroform by evaporation, the residue was dissolved in 20 ml of benzene. This solution was loaded on an alumina column (1 cm × 20 cm, acid alumina from Fisher Scientific) and eluted with successive 20-ml portions of the following solutions: benzene, benzene–ether (1:1 v/v), ether, ether with ethyl alcohol increasing from 1 % to 10 % alcohol in 1 % steps, and abs. alcohol. TMT͡T A appeared in the fractions containing 7–8 % ethyl alcohol, while TMT͡T's B and C appeared in the fractions containing 2–3 % and 3–4 % alcohol respectively; TMT͡T D eluted in the ether fraction. Incompletely methylated products from all dimers always eluted at higher alcohol concentrations than did the fully methylated dimers. The methylated products were extracted by evaporation of the solvent.

Yields for TMT͡T's A and B were 2.6 mg, for C 1.0 mg, and for D about 0.1 mg. Melting points were 251°, 268°, and 214° for TMT͡T's A, B, and C(−) respectively. The specific rotation $[\alpha]_{D,20}$ of TMT͡T C(−) was −136°. That of TMT͡T B could not be measured.

1 (*c*) *Assay for tetramethyl-thymine dimers.* Samples of methylated product were reirradiated at 254 mμ in aqueous solution. Under these conditions the TMT͡T's formed, 1,3-dimethylthymine. The ultraviolet spectra of this product are the same at pH 7 and 14, while the spectra of 1-methyl- and of 3-methylthymine, ultraviolet reversal products of incompletely methylated T͡T dimers, are not[7].

2. *Alternative preparation of tetramethyl-thymine dimers*

2 (*a*) *Dimers from 1,3-dimethylthymine.* Two tetramethyl-thymine dimers, shown below to be TMT͡T's A and C, were prepared as described by WULFF AND FRAENKEL[1], except that acid alumina obtained from Fisher Scientific was used. Under our conditions the two dimers were eluted from the alumina column at 3–4 % and 7–8 % ethyl alcohol.

2 (*b*) *Dimer from 1-methylthymine.* 500 mg of 1,1′-dimethylthymine dimer, obtained by irradiating crystalline 1-methylthymine, was a gift from Dr. R. STEWART. Conversion to the tetramethyl dimer, and its separation from partially methylated products was achieved by scaling the methods in 1(b) and 2(a) respectively. The yield of this tetramethyl dimer, shown in DISCUSSION to be TMT͡T B, was 400 mg. Analysis: m. p.: 255. Calculated: C = 54.53; H = 6.54; N = 18.17. Found: C = 54.72; H = 6.73; N = 18.02.

3. *Preparation of 5,6-dihydro- 1,3-dimethylthymine*

1 g of 1,3-dimethylthymine, prepared according to DAVIDSON AND BAUDISH[8], was dissolved in 60 ml of 10 % acetic acid. 2 g of 5 % rhodium on alumina (Engelhard Ind. Inc., Newark, N.J.) was added as a catalyst for hydrogenation, as proposed by JANION AND SHUGAR[9]. Under vigorous agitation, hydrogen was passed into the mixture at a pressure of 15 $\text{lb} \cdot \text{inch}^{-2}$. After 2 h hydrogen uptake was complete. Following removal of the catalyst by filtration the acetic acid was flash-evaporated using a bath temperature of 25°. The residue, a yellow oil, was dissolved in 100 ml of

benzene and loaded on an alumina column and eluted as described above (2a). Dihydrodimethylthymine eluted in the ether fraction. On evaporation of the ether the yellow oily residue was taken up in 3 ml of ethyl acetate. The addition of about 50 ml cold petroleum ether caused the precipitation of a white solid. After keeping the mixture at −20° for 24 h the precipitate was filtered off, redissolved in 3 ml of ethyl acetate and precipitated with petroleum ether as above.
Analysis: m. p.: 44°. Calculated: C = 53.84; H = 7.75; N = 17.93. Found: C = 54.18; H = 7.64; N = 17.70.

4. *Infrared spectra, ultraviolet spectra, melting points and specific rotations*

Infrared spectra were recorded using pressed pellets containing 0.5 mg of any particular dimer and 200 mg KBr. The spectrophotometers used were the Beckman IR 9 or Perkin Elmer 237B infrared spectrometers. Ultraviolet spectra were recorded with a Cary Model 14 recording spectrophotometer. Uncorrected melting points were determined with a Thomas Hoover melting point apparatus. An ETL-NPL automatic polarimeter (Bendix Ericson Ltd.) was used to measure specific rotations.

5. *Dipole moment measurements*

Dielectric constants and specific volumes of benzene solutions containing TMT͡T A, B or C as well as solutions containing dihydrodimethylthymine were determined by use of equipment described by MEREDITH AND WRIGHT[10]. Electrical polarizations, P_T, (Table I) were calculated by the method of HALVERSTADT AND KUMLER[11] in which ε, $\bar{v}$ and ω are dielectric constant, specific volume and weight fraction respectively. The dielectric constants, ε, and densities, d, of the crystalline substances were determined according to BEDARD *et al.*[12] (Table II).

TABLE I

ELECTRICAL POLARIZATIONS OF TETRAMETHYL-THYMINE DIMERS A, B, AND C, AND OF DIHYDRODIMETHYLTHYMINE

Soln. benzene; 20°.

Substance	$d\varepsilon/d\omega$*	$d\bar{v}/d\omega$*	*Dielectric constant*		*Specific volume*		*Total polarization*
			Extrapolated	*Pure solvent*	*Extrapolated*	*Pure solvent*	
Dimer A	13.66	0.392	2.2853	2.2849	1.13777	1.13782	852
Dimer B	3.10	0.406	2.2847	2.2849	1.13780	1.13782	245
Dimer C	12.53	0.383	2.2847	2.2849	1.13784	1.13782	788
Dihydrodimethylthymine	9.93	0.306	2.2842	2.2850	1.13786	1.13781	321

* ε, dielectric constant; $\bar{v}$, specific volume; ω, weight fraction.

RESULTS AND DISCUSSION

Comparison of dimers

Table III summarizes the different ways in which the various isomeric forms

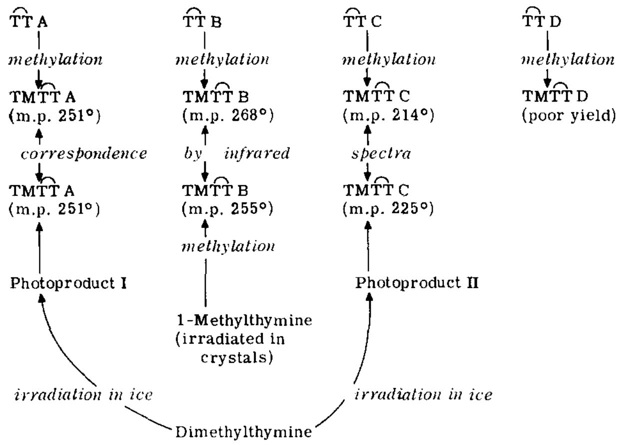
TABLE II

DISTORTION POLARIZATIONS AND DIPOLE MOMENTS OF TETRAMETHYL-THYMINE DIMERS AND DIHYDRODIMETHYLTHYMINE AT 23°

Substance	*Density* d	*Dielectric constant* ε	*Distortion polarization* P_D (*cm*^{3})	*Molecular refraction* MR_D (*cm*^{3})	*Atomic polarization* P_A	*Dipole moment* μ *from* P_D	μ *from* MR_D
Dimer A	1.363	2.706	82.0	76.0	6.0	6.04	6.07
Dimer B	1.413	2.783	81.4	76.0	5.4	2.79	2.84
Dimer C	1.331	3.008	92.8	76.0	16.8	5.75	5.82
Dihydrodimethylthymine	1.232	2.756	46.2	39.7	6.5	3.61	3.65

TABLE III

SOURCES OF TETRAMETHYL-THYMINE DIMERS



of the tetramethyl dimers were obtained. All 4 isomers were produced directly by methylation of the corresponding thymine dimer.

WULFF AND FRAENKEL[1] obtained 2 different dimers by irradiating 1,3-dimethylthymine in frozen aqueous solution. One of these, their Photoproduct II (m.p. 250–251°), was shown by them to be identical to the fully methylated thymine dimer from frozen thymine solution (designated as $\widehat{TT}$ A by WEINBLUM AND JOHNS[5]) using mixed melting points and NMR spectra. This identity was confirmed by us using the infrared spectra of these compounds. By this same method we showed that WULFF AND FRAENKEL's other dimethylthymine photoproduct (m.p. 224–225°) was identical with the methylation product of dimer C(—). Mixed melting points could not be used to establish the identity of these latter compounds because the methylated thymine dimer C(—) is one pure enantiomer while the corresponding dimethylthymine photoproduct is in the form of a racemic mixture.

Another tetramethyl dimer was obtained by methylation of the photoproduct produced by ultraviolet irradiation of crystalline 1-methylthymine. Its identity with tetramethyl dimer B was established by infrared spectroscopy. As in the case of dimer

C mixed melting points produced inconclusive results. The TMT͡T's from both T͡T B and 1-methylthymine are mixed enantiomers. However in the product from 1-methylthymine the enantiomers are in equal proportions, while in the product from Dimer B either the D- or the L-form predominates. Sufficient material had not been isolated to determine by specific rotation which form was the major constituent of the mixture. The difference in composition of the mixtures accounts for the difference in their melting points.

TMT͡T D could not be obtained in large quantities for a number of reasons. It was difficult to produce thymine dimer D. Moreover the yield on methylation of T͡T D was less than 5 %, probably on account of the alkaline instability of this compound[13]. Methylation with diazomethane at neutral conditions was even less successful possibly due to the high pK of this dimer[13]. Since no other method is known to produce TMT͡T D in quantities sufficient to measure the dipole moment, this compound was not further considered in this investigation.

Dipole moments

Calculations. The approximate dipole moments of individual pairs or groups of atoms were taken from various texts[14,15]. The dimensions of the thymine ring was obtained from the crystallographic data on thymidylic acid[16], and used as an approximation to the dimensions of the corresponding rings in the tetramethyl dimers. The magnitudes and directions of the individual dipole moments along molecular bonds, and of their sum in one 1,3-dimethyl ring of a tetramethyl dimer are shown in Fig. 2.

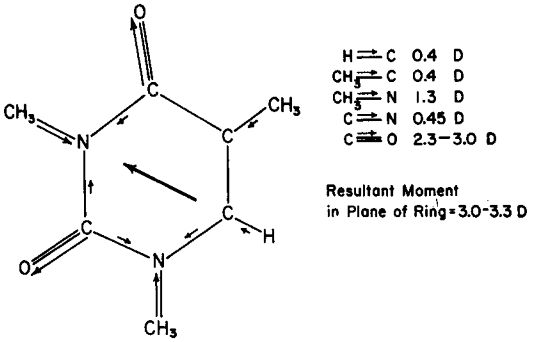


Fig. 2. One half of a tetramethyl-thymine dimer in the plane of the 6-membered ring. The dipole moments along individual bonds are indicated drawn to scale. The heavy arrow in the middle of the ring indicates the direction and magnitude of the resultant moment in the plane of the ring.

The C–C pair of atoms was assumed to have no dipole moment, while for the C=O moment the extreme values found, 2.3 and 3.0 Debye (D), were both used in the calculations. $C^2{=}O$ and $C^4{=}O$ moments were considered to have the same magnitude. It was assumed that on the average the resultant moment would be in the plane of the ring, even though in the solid[16] the 2,4 oxygens, and consequently the dipole moment, are constrained to be slightly out of the plane of the ring. Using an angle of 115° between the thymine ring and the cyclobutane ring as measured from framework molecular models (Prentice-Hall Inc., Englewood Cliffs, N.J.) of the dimers the resultant moments of the four dimer structures were obtained by summation as shown in Fig. 3. The range of values of the resultants reflect the range of the C=O moments.

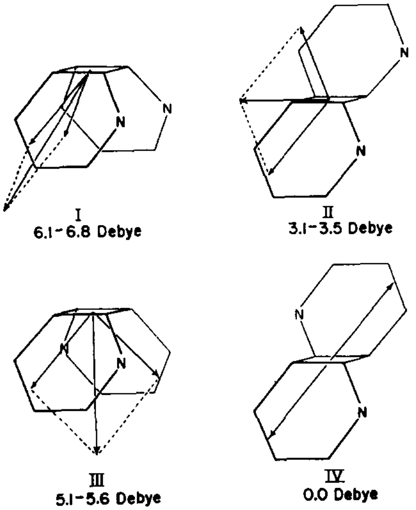


Fig. 3. Vector addition of the dipole moments of dimer halves for the different dimeric structures. Stylized dimer skeletons have been added to aid visualization. Only the 1,1′ nitrogens are shown to indicate the steric arrangements of the dimer halves.

Measurements. The dielectric constants, ε, and densities, *d*, of the crystalline substances are shown in Table II as are distortion polarizations (P_D) together with the dipole moments calculated both from P_D and from the less meaningful molar refraction (MR_D), derived from atom or bond refractivities.

Included also in Table II are values for the atom polarization (P_A) assuming that MR_D is equivalent to electron polarization (P_E). It may be seen that the atom polarization ($P_D - P_E$) of dimer C is abnormally high. No explanation for this abnormality is apparent at the present time.

The calculated and the measured dipole moments of the tetramethyl dimers are summarized in Table IV. As a control on the calculation the moments obtained for dihydrodimethylthymine are included in the table. The agreement between the calculated and measured values for this substance is surprisingly good. Consequently

TABLE IV

COMPARISON OF DIPOLE MOMENTS

Dipole moments calculated		*Dipole moments measured*	
Structures	*Debye*	*Dimer*	*Debye*
I	6.1–6.8	A	6.04
II	3.1–3.5	B	2.79
III	5.1–5.6	C	5.75
IV	0.0	D	Not measured
Dihydrodimethyl-thymine	3.3–3.6	Dihydrodimethyl-thymine	3.61

the dipole moments calculated for Structures I, II, and III were matched with those measured for Dimers A, B, and C respectively.

The slight disagreement between the measured values for Dimers B and C and the values calculated for Structures II and III suggests that the moment for $C^4{=}O$ is smaller than for $C^2{=}O$. Alternatively, the larger than expected dipole moment for Dimer C can be explained by an angle between the thymine rings smaller than the 50° assumed in the calculations.

Yet even without such ameliorating arguments the agreement between calculated and measured values of the dipole moments is highly satisfactory. Therefore the tentative assignments made by WEINBLUM AND JOHNS[5] are correct. Dimers A, B, and C correspond to Structures I, II, III respectively.

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