Theoret. chim. Acta (Berl.) 10, 423—434 (1968)

Extended Hückel Calculations on the Conformation and Structure of Thymine Photodimers*

FRANK JORDAN** and BERNARD PULLMAN
Institut de Biologie Physico-Chimique, 13, rue Pierre Curie, Paris 5e, France

Received March 6, 1968

Extended Hückel calculations have been performed for the structure of C_5–C_6 dihydrouracil and thymine and for the uracil and thymine photodimers. The results indicate, in agreement with experiment, that the most stable conformation of the dihydropyrimidines should be the half-chair one. The preferred conformations of the dimers should correspond to planar bases forming an angle of 120° with the cyclobutane ring. The most stable dimers, *per se*, appear to be the *trans-anti* and the *trans-syn* ones in the case of uracil and the *cis-anti* and the *trans-anti* ones in the case of thymine. Other factors than inherent stability must play a decisive role in the production of the dimers under specific conditions. When a semi-empirical reduction is carried out upon their absolute values the dipole moments predicted for the different conformers of the thymine dimer agree very satisfactorily with experiment.

Die Strukturen von 5,6-Dihydrouracil, Thymin und den Photodimeren von Uracil und Thymin wurden mit der erweiterten Hückelmethode untersucht. In Übereinstimmung mit dem Experiment ist die stabilste Konformation der Dihydropyrimidine die Halbsesselform. Die bevorzugte Konformation der Dimeren sollte einer planaren Base in einem Winkel von 120° gegenüber dem Cyclobutanring entsprechen, und zwar beim Uracil in der *trans-anti* und *trans-syn*-Form, beim Thymin in der *cis-anti-* und *trans-anti*-Form. Andere Faktoren als die eigene Stabilität müssen bei der Entstehung der Dimeren unter bestimmten Bedingungen vorherrschend sein. Nach semiempirischer Korrektur stimmen die berechneten Dipolmomente der verschiedenen Konformeren des Thymindimeren befriedigend mit dem Experiment überein.

La méthode de Hückel étendue est utilisée pour l'étude de la structure de C_5–C_6 dihydro-uracile et thymine et des photodimères de l'uracile et de la thymine. Le calcul prévoit, en accord avec l'expérience, que la conformation la plus stable des dihydropyrimidines devrait être en demi-chaise. Quant aux dimères, les conformations préférentielles correspondent aux bases planes formant un angle de 120° avec le noyau du cyclobutane. Les dimères les plus stables *per se* s'avèrent être le *trans-anti* et le *trans-syn* pour l'uracile et le *cis-anti* et le *trans-anti* pour la thymine. Des facteurs autres que la stabilité intrinsèque des dimères doivent jouer un rôle décisif dans leur obtention, dans des condition déterminées. Lorsqu'une correction empirique est apportée à leurs valeurs absolues, les moments dipolaires evalués pour les différents conformères du dimère de la thymine sont en accord très satisfaisant avec les valeurs expérimentales.

The extended Hückel theory [1], which appears quite successful [1, 2, 3] in investigations on molecular conformations is employed in this paper for the study of the structure and properties of the possible geometrical isomers of uracil and thymine photodimers. As a preliminary, the structure and properties of the 5–6 saturated monomers, dihydrouracil (DHU) and dihydrothymine (DHT) are also investigated. The method of calculations and the parameters employed have been indicated in our previous paper on the conformation of purine and pyrimidine nucleosides [3] and will not be repeated here.

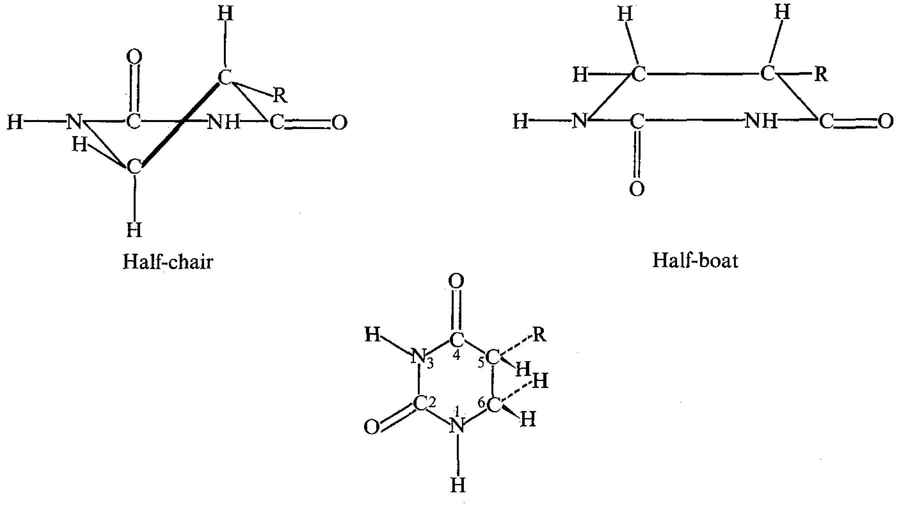
---

* This work was supported by grant n° CR-66-236 of the Institut National de la Santé et de la Recherche Médicale (Intergroupe Cancer et Leucémie).

** NATO Postdoctoral Fellow in Science 1967–1968.

I. The Dihydropyrimidines

The bond angles and bond distances, being unknown in the dihydropyrimidines at the time of the calculations *(vide infra)*, had to be allotted reasonable values. We have been greatly helped in this respect by the elegant structural studies carried out on these molecules by Nofre and associates [4] using infra-red and nuclear magnetic reasonance spectroscopy together with a simple theoretical investigation based upon a combination of classical energy terms for the ring strain with a Hückel approach for the calculation of delocalization energy based upon variations in the resonance integrals with the cosine of the bond angles.



Half-chair

Half-boat

Planar

Fig. 1. Possible types of conformation in DHU and DHT ($R = H$, Dihydrouracil; $R = CH_3$, Dihydrothymine)

Three types of typical conformations have been considered represented by the half-chair, half-boat and planar model (Fig. 1). In order to make the conformational studies possible in a rather simple way, all C–N bond distances in the planar portion of the molecules were assumed equal to 1.38 Å. The other distances adopted were $N_1{-}C_6 = 1.45$ Å, $C_4{-}C_5 = 1.50$ Å, $C_5{-}C_6 = 1.525$ Å, $N_1{-}H = N_3{-}H = 1.00$ Å, $C_4{=}O = 1.214$ and $C_2{=}O = 1.237$ Å. The C–H bond distances were uniformly assumed at 1.10 Å with H–C–H angles normal tetrahedral ones of 109° 28′, the hydrogens on C always situated in planes perpendicular to the X–C–X plane, this H–C–H plane bisecting the X–C–X angle. Lacking any better information, we assumed that the bond angles involving the environment around the two nitrogens and the two carbonyl carbons ($\mathrm{X{-}}\underset{\substack{|\\ \mathrm{H}}}{\widehat{\mathrm{N}}}\mathrm{{-}X}$ and $\mathrm{X{-}}\underset{\substack{\|\\ \mathrm{O}}}{\widehat{\mathrm{C}}}\mathrm{{-}X}$) undergo, in pairs, identical changes. Another, perhaps *a priori* a more drastic assumption was that the puckering emanating from atoms 1 and 4 had identical angles. Anticipating upon very recent experimental X-ray study of dihydrothymine [5], the results of which became available only after our calculations were performed *(vide infra)*, we may already say that this assumption was proven acceptable by the experimental findings which indicate that the saturated C atoms are almost equally displaced on either side of the best plane that can be drawn through the four other atoms of the skeleton [5].

Table 1 shows the variation of the total orbital energy in DHU as a function of the variation in the internal angles. It is obvious from the results that *the most stable conformation is the half-chair one and the least stable the half-boat one.*

Table 1. *Variation of total orbital energy with internal angles in dihydrouracil*

Type of structure	angle in ° $\underset{\underset{\mathrm{H}}{\mid}}{\mathrm{X{-}N{-}X}}$	angle in ° $\underset{\underset{\mathrm{O}}{\parallel}}{\mathrm{X{-}C{-}X}}$	Displacement of the saturated C atoms from the plane of the skeleton (Å)		Total orbital energy (kcal/mole)
			Atom 5	Atom 6	
planar	125.3	118.3	0.00	0.00	−20,227.04
half-chair	124	117	0.31	−0.28	−20,231.14
half-boat	124	117	−0.52	−0.47	−20,221.82
half-chair	123	116	0.40	−0.36	−20,229.31
half-boat	123	116	−0.68	−0.61	−20,220.84
half-chair	121	116	0.47	−0.43	−20,225.71
half-boat	121	116	−0.79	−0.73	−20,219.75
half-chair	124	116	0.36	−0.32	−20,229.66
half-chair	124	118	0.24	−0.22	−20,231.13
half-chair	123	117	0.36	−0.33	−20,230.98
half-chair	123	118	0.31	−0.28	−20,231.88
half-chair	125	117	0.25	−0.22	−20,229.92
half-chair	125	116	0.31	−0.28	−20,229.53

The best half-chair structure (for 123° $\mathrm{C{-}\underset{\underset{H}{\mid}}{\widehat{N}}{-}C}$ and 118° $\mathrm{X{-}\underset{\underset{U}{\parallel}}{\widehat{C}}{-}X}$ angles) is shown to be preferred to the planar one by about 6 kcal/mole, a result in very good agreement with the estimates of Nofre *et al.* [4]. Coincidentally internal angles are very close to the desirable unstrained values in this structure also, the values being 111°18′ at atom 5 and 111°3′ at atom 6.

These differences in stability between the planar and the best half-chair conformation can, at least in part, be attributed to the removal of the eclipsing of the methylene hydrogens and to the decrease in strain around the 5 and 6 carbon atoms.

Table 2 indicates the results of similar although somewhat less extensive evaluations for DHT. The best half-chair conformation is again found to be the most stable one, in this case by about 8 kcal/mole (2 kcal/mole more than DHU) more than the planar one, with the methyl group slightly preferring the equatorial positions over the axial ones. Although the crudeness of the method prohibits any too detailed conclusions it may nevertheless be interesting to remark that the NMR results of Nofre *et al.* [4] show that whereas DHU exists in constant interconversion, DHT seems to exist exclusively in the equatorial form. Since interconversion presumably proceeds via the planar form, a difference in energy between the best puckered form and the planar form may be partially responsible for this phenomenon along with the less favorable energy of axial methyl as compared to the equatorial one.

Table 2. *Variation of total orbital energy with internal angles in dihydrothymine*

Type of structure	angle (°) $X\text{–}\underset{\substack{\vert\\ H}}{N}\text{–}X$	angle (°) $X\text{–}\underset{\substack{\Vert\\ O}}{C}\text{–}X$	Displacement of the saturated atoms (Å) from the plane of the skeleton		Total orbital energy (kcal/mole)
			Atom 5	Atom 6	
Planar	125.3	118.3	0.00	0.00	−22,627.18
Best half-chair axial Me	123	118	0.31	−0.28	−22,635.30
Best half-chair, equatorial Me	123	118	0.31	−0.28	−22,635.55
Experimental geometry	121.6 126.3	116.6 113.6	0.42	−0.31	−22,628.06

The last line of Table 2 referring to calculations obtained with the experimental geometry of DHT deserves special mention. In fact, our calculations were already terminated when there appeared an X-ray study of the structure of DHT [5]. It confirms the half-chair conformation and altogether indicates a distribution of bond lengths and angles close to the one corresponding to our best half-chair conformation, with however some differences (Fig. 2). One major discrepency is the length of the N_1–C_2 bond for which we assumed 1.38 Å and which in fact is only 1.32 Å. A further difference concerns the length of the C–H bond, for which we have adopted a uniform value of 1.1 Å while the experimental lengths seem to be generally smaller than 1 Å. The incidence of these differences, which is altogether limited (it may perhaps be worthwile indicating that in the experimental work the deviation of C_5 and C_6 from the best least square plane is 0.422 Å and −0.310 Å, respectively while the corresponding values for "best half-chair" are +0.305 Å and −0.280 Å) can be seen particularly in some aspects of the results concerned with the charge distribution and the overlap populations in the molecules studied.

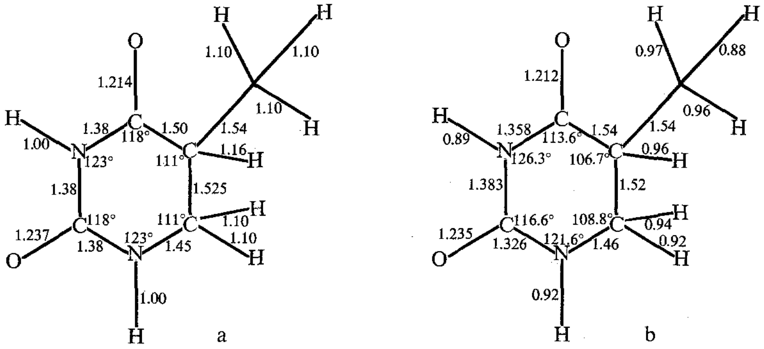


Fig. 2. Geometry of (a) assumed (best half-chair) and (b) experimental DHT

These are given in Figs. 3 and 4 and one may notice e.g. the appreciable differences in the net charges on the methyl group obtained in DHT with the assumptions of the "best half-chair" and with the experimental geometries. But generally speaking the main features of the distribution of the electronic indices are very similar in both calculations. An aspect of the general results which deserves stressing is perhaps the appreciable increase in the net electronic charge of N_1 of uracil and thymine upon the saturation of the C_5–C_6 bond. It is tempting to relate this evolution to the well known increased facility of acidic hydrolysis of the nucleosides of the dihydropyrimidines with respect to the nucleosides of the pyrimidines. Although in these calculations we do not deal with the nucleosides, we showed in our previous publication that the electronic charge of the glycosidic nitrogen does not greatly differ in the free base from that in the nucleoside.

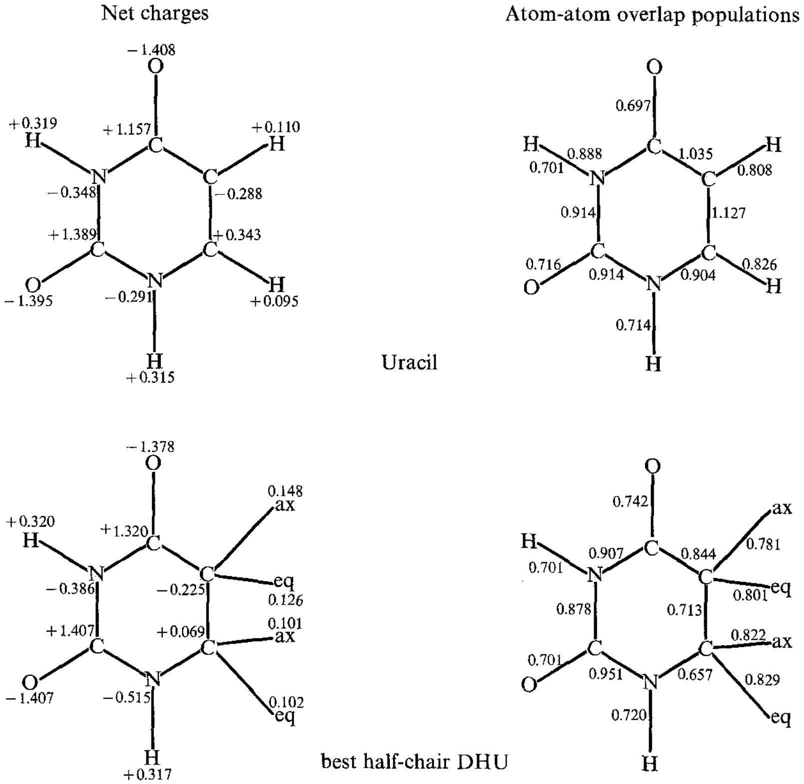


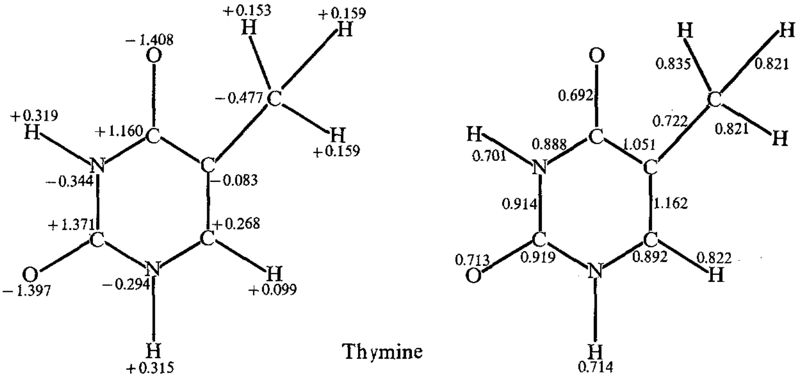
Fig. 3. Electronic indices in uracil and in best half-chair DHU

II. The Photodimers

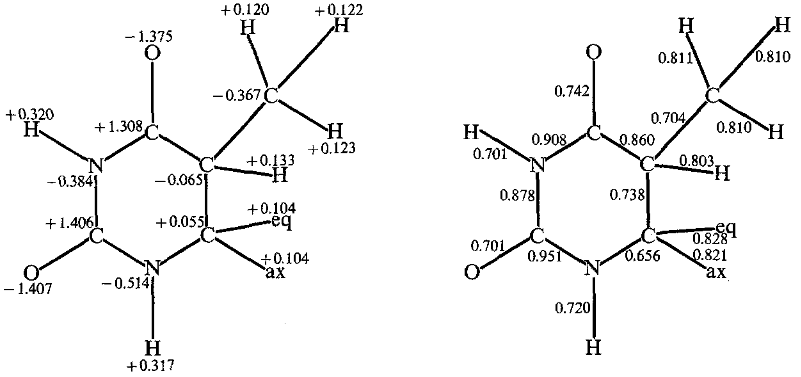
Since the original discovery by Beukers and Berends [6] in 1960 of the induced photodimerization of thymine in frozen aqueous solution, the subsect has become a central one in photobiology. It has also been realized since then that photodimers can also be obtained from uracil and cytosine [7]. A quantum-mechanical

Net charges

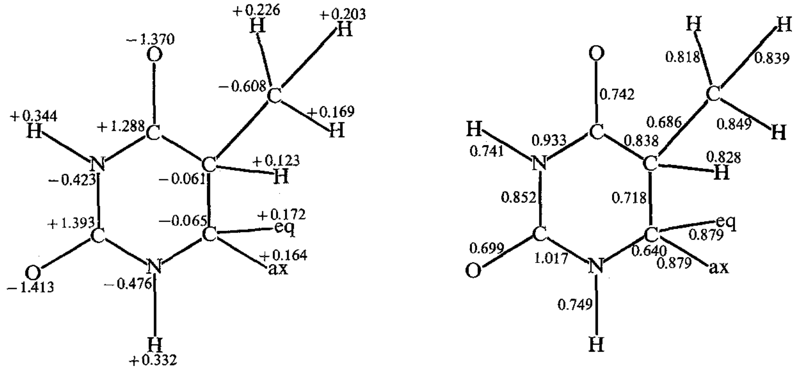
Atom-atom overlap populations



Thymine



best half-chair DHT (equatorial CH_3)



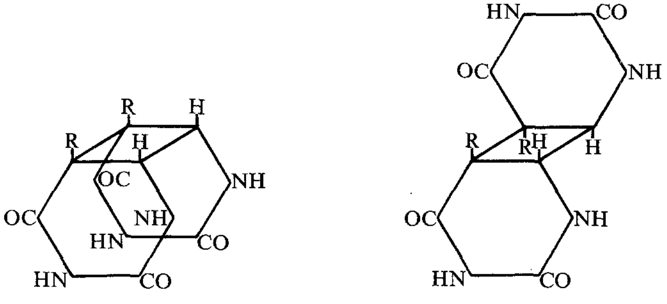
experimental geometry DHT (equatorial CH_3)

Fig. 4. Electronic properties of thymine and DHT

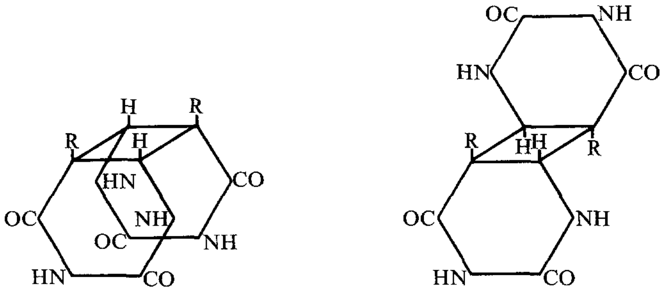
investigation has been carried out on the possible mechanism of the photodimerization (Mantione and Pullman [8]). Presently we are interested in the structural properties of the dimers.

It was originally suggested by Wulff and Fraenkel [9] that thymine dimers can exist in four different stereochemical configurations. Figure 5 shows the possibilities and the accompanying nomenclature.

It is now confirmed that all these possible forms may be formed and it appears moreover that the nature of the dimer(s) formed depends upon the experimental conditions of their preparation. Thus, Weinblum and Johns [10] have obtained by irradiation of thymidyl-(3′-5′)-thymidine or of frozen solutions of thymidine five thymine type dimers, one of the four originally proposed existing in two optically active forms. They seemed to have succeeded in ascribing the correct forms to their molecules; they do not indicate however the ratios of the products although they do give their relative acid stabilities. Thus, they indicate the *cis-syn* and *trans-syn* to be acid stable whereas the *cis-anti* and *trans-anti* to be acid unstable. K. C. Smith [11] reported the production in frozen solution of thymine of the *cis-syn* and *cis-anti* products, the ratio being dependent on the presence or absence of adenine. Lamola and Mittal [12] in acetonitrile obtained mainly *trans-anti* type dimer. Similarly, Ben Hur *et al.* [13] find that whereas in frozen



cis-syn *trans-syn*



cis-anti *trans-anti*

Fig. 5. Possible conformations of thymine and uracil dimers ($R = CH_3$ Thymine dimers; $R = H$ Uracil dimers)

solutions 86% *cis* type form is produced (38% *cis-syn*, 48% *cis-anti*) and 14% *trans-anti*, 61% *trans* (6% *trans-syn*, 55% *trans-anti*) and 39% *cis* (26% *cis-syn* and 13% *cis-anti*) forms are produced with acetone sonsitization. The irradiation of DNA yields essentially the *cis-syn* form [14]. Thus the dimerization depends on the solvent and on the sensitizer. The course of the dimerization has recently been reviewed also on the basis of available evidence as to whether the reaction takes place *via* an excited triplet or singlet state [15].

Since the extended Hückel theory cannot easily be used for the study of reaction kinetics, we cannot hope to be able to gain insight into why one type of geometrical arrangement should be favored over the others under the different conditions of solvent or mode of irradiation. What we propose, however, is to investigate the ground state properties of the dimers, once they are formed.

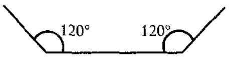
Table 3 sums up the results obtained about the total orbital energies of uracil and thymine dimers in different conformations. The C–C bond lengths in the cyclobutane rings have been assumed to be equal to 1.525 Å. Although they may be somewhat off the exact value, this should not influence the relative outcome of the results. The hydrogen atoms (or methyl groups in the thymine dimers)

Table 3. *The total orbital energy of photodimers*

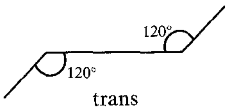
		Angle α between base and cyclobutane (°)	Total orbital energy (kcal/mole)
Uracil dimers			
Planar bases	*cis-syn*	120	−38,832.87
	trans-syn	120	−38,835.70
	cis-anti	120	−38,834.03
	trans-anti	120	−38,836.88
	cis-syn	150	−38,750.16
	trans-syn	150	−38,752.33
	planar syn	180	−38,574.32
	planar anti	180	−38,588.04
"Best" half-chair bases	*cis-syn*	120	−38,725.50
	trans-syn	120	−38,726.95
	cis-anti	120	−38,728.11
	trans-anti	120	−38,727.98
"Best" half-boat bases	*cis-syn*	120	−38,826.32
	trans-syn	120	−38,828.02
	cis-anti	120	−38,826.73
	trans-anti	120	−38,830.59
Thymine dimers			
Planar bases	*cis-syn*	120	−43.634.40
	trans-syn	120	−43.641.45
	cis-anti	120	−43,643.93
	trans-anti	120	−43,642.54
"Best" half-chair bases	*cis-syn*	120	−43,520.58
	trans-syn	120	−43,532.60
	cis-anti	120	−43,543.51
	trans-anti	120	−43,543.94

on the cyclobutane skeleton were placed as suggested by similar substituents in planar cyclobutane, i.e. in the plane perpendicularly bisecting the C–C–C bond angles, in appropriate directions depending on the angle formed by the bases with the cyclobutane plane. The principal conclusions which may be drawn for Table 3 are:

1. As illustrated in the case of the uracil dimer constructed out of the planar bases, the preferred angle between the planes of the bases and the plane of cyclobutane is 120°, both for the *cis* and for the *trans* conformations. These can therefore by diagramatically schematized as follows:



cis



trans

This result is not really surprising. First of all, it is at this angle that the rings minimize the strain on the cyclobutane structure. Secondly, in a previous study by one of us [2] on a number of highly strained hydrocarbons in bicyclic and tricyclic molecules this angle (or its environment) was preferred when cyclobutane or cyclopropane rings were involved in the bicyclic or tricyclic skeletons.

2. The most stable conformations of both uracil and thymine dimers are obtained with planar bases. The least stable (by about 80–100 kcal/mole) are those derived from the half-chair model.

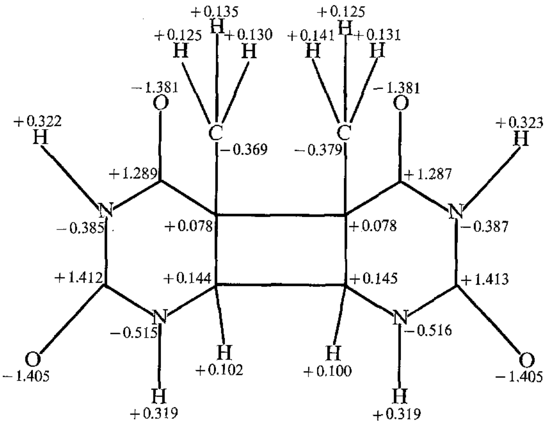
3. The most stable dimers appear to be the *trans-anti* and the *trans-syn* in the case of uracil dimer and the *cis-anti* and the *trans-anti* ones in the case of thymine dimer. The *cis-syn* dimers which appear to be formed preferentially in particular upon irradiation of DNA and RNA, seem to be the least stable *per se*. But it must be recognized that the calculated energy differences between the different dimers formed with planar bases are very small. Other effects than inherent stabilities may therefore play a decisive role in the production of these dimers ander specified conditions. It may also be mentioned that a different quantum mechanical study [16] using the "frontier electrons" approach to reactivities implying the calculation of "delocalization energies", predicted the *trans-syn* and *cis-syn* products to be more stable (delocalization energy $=0.7008\,\gamma$) than the two *anti* forms (d.e. $=0.5678\,\gamma$ each). On the other hand this work proposes a $\sim +100$ kcal/mole heat of reaction, which we roughly confirm i.e.

$$2\mathrm{U} \rightarrow \widehat{\mathrm{UU}} \sim +120\ \mathrm{kcal/mole}\,,$$
$$2\mathrm{T} \rightarrow \widehat{\mathrm{TT}} \sim +125\ \mathrm{kcal/mole}\,.$$

In the dimers the distribution of the essential electronic indices is very similar in the four dimers of each pyrimidine except for the cyclobutane portion of the molecules. Fig. 6 represents the distribution of the net electronic charges and of the overlap populations in one conformer of thymine dimer, the *cis-syn*. Fig. 7 indicates the same indices in the cyclobutane portion of the remaining conformers.

In spite of the similarity in electronic distribution, the geometry of the molecules may induce an appreciable difference in some physico-chemical properties of the conformers depending upon these indices. Such should be e.g. the case for

Net atomic charges



Atom-atom overlap populations

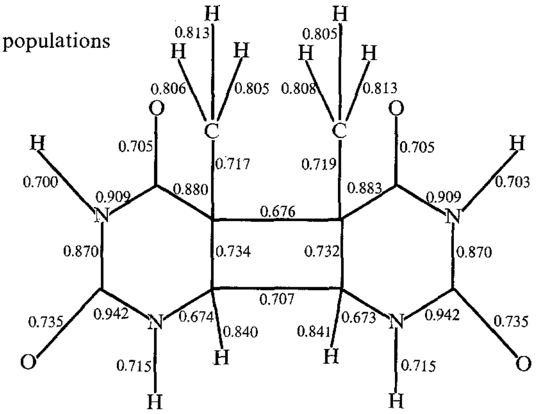


Fig. 6. Net charges and overlap populations in *cis-syn* thymine dimer

the dipole moments. As is known [17] the values of the net electronic charges computed by the extended Hückel method are generally exaggerated and lead thus to overestimated dipole moments, although their general orientations are in agreement with those of more refined computations. By reducing empirically the calculated dipole moment of uracil (12.3 D) to the experimentally determined one (3.90 D for N_1–N_3-dimethyluracil) and applying the same correction to the moments predictable for the dimers, values are obtained which are expected to be reasonable. That such is actually the case may be seen in Table 4, where the "corrected" dipole moments computed in this way are compared with the very recently determined experimental ones [18]. The agreement between the two sets of values is very satisfactory.

In a previous study on highly strained hydrocarbons [2] there was a fairly good correlation found between the %-s character of the C–H bond (defined in the extended Hückel scheme as ($C_{2s} - H_{1s}$ overlap population)/(C–H total overlap population)) and the NMR C^{13}–H satellite coupling constant. This

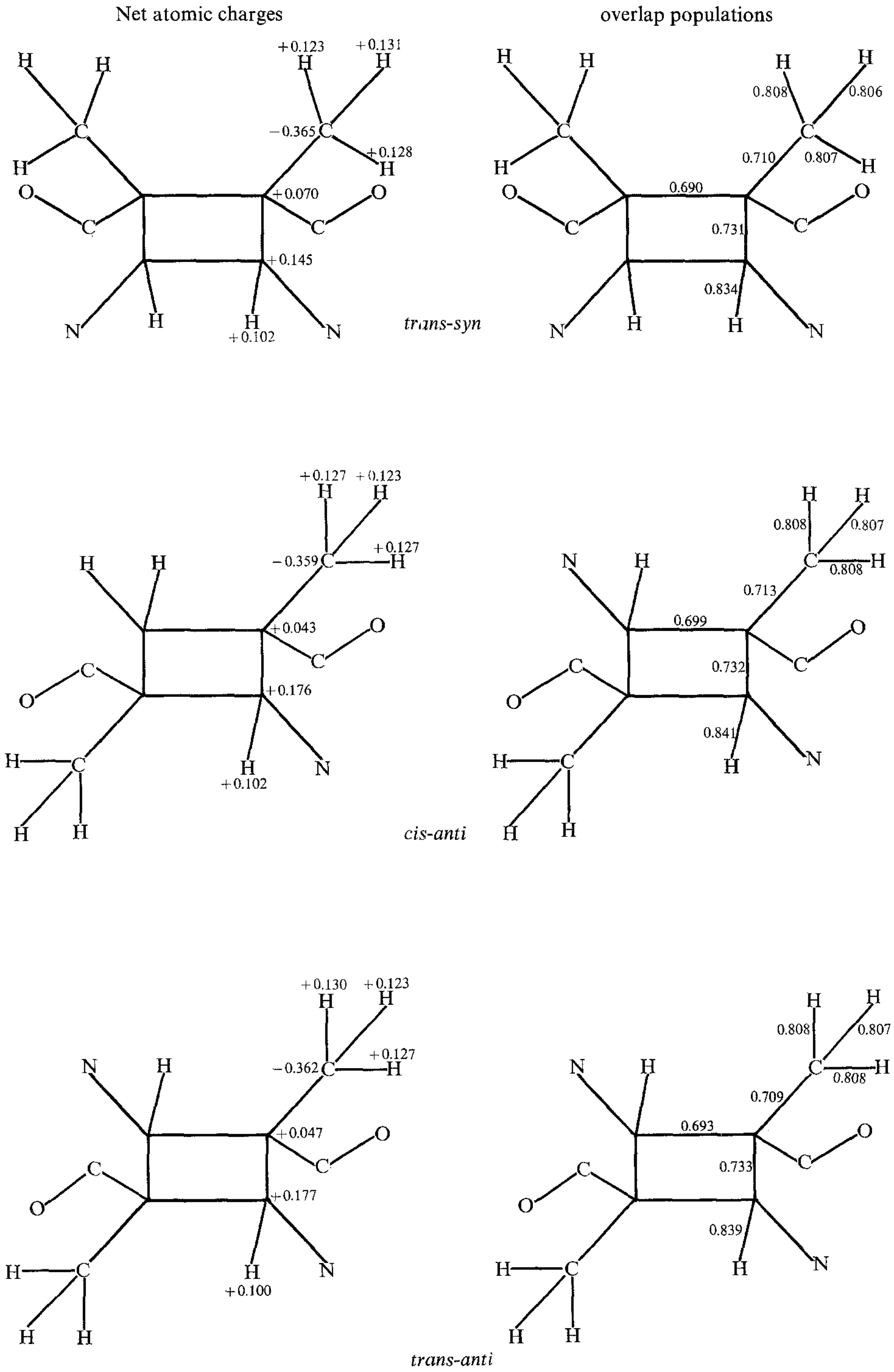


Fig. 7. Net charges and overlap populations in the cyclobutane environment of the thymine dimers

Table 4. *Computed and experimental dipole moments (Debye units)*

Molecule	EHT theoretical moment	"Corrected" moment	Experimental
Uracil	12.27	3.90	3.90[a]
DHT	11.42	3.62	3.61[b]
cis-syn thymine dimer	19.99	6.34	6.04
trans-syn thymine dimer	10.27	3.25	2.79
cis-anti thymine dimer	16.86	5.33	5.75
trans-anti thymine dimer	0.00	0.00	—

[a] N_1–N_3-dimethyluracil [17].

[b] Dihydrodimethylthymine [18].

correlation of %-s-character $=0.20\,J_{C^{13}-H}$ was first suggested by Muller and Pritchard [19]. The number obtained in the thymine dimers is 35.5% for the C–H bond on cyclobutane. This can be compared with the 154 cps. experimentally measured value [20] which would imply ~31%. Both these values (theoretical and experimental) are higher than the values in unsubstituted cyclobutane (32.2% theoretical and 27% experimental). Although the theoretical values are always higher than the experimental ones, they reflect the increase in strain around the ring as well as the differences in electronegativity in the surrounding atoms.

References

1. Hoffmann, R.: J. chem. Physics **39**, 1397 (1963).
2. Jordan, F.: Ph. D. Thesis, University of Pennsylvania (1967).
3. —, and B. Pullman: Theoret. chim. Acta (Berl.) **9**, 242 (1968).
4. Nofre, C., M. Murat, and A. Cier: Bull. Soc. Chim. (France) **1965**, 1749; Rouillier, P., J. Delmau, and C. Nofre: Bull. Soc. Chim. (France) **1966**, 3515.
5. Furberg, S., and L. H. Jensen: J. amer. chem. Soc. **90**, 470 (1968).
6. Beukers, R., and W. Berends: Biochim. Biophys. Acta **41**, 550 (1960).
7. Setlow, R. B.: Science **153**, 379 (1966).
8. Mantione, M. J., and B. Pullman: Biochim. Biophys. Acta **95**, 568 (1965).
9. Wulff, D. L., and G. Fraenkel: Biochim. Biophys. Acta **51**, 332 (1961).
10. Weinblum, D., and H. E. Johns: Biochim. Biophys. Acta **114**, 450 (1966).
11. Smith, K. C.: Biochem. Biophys. Res. Comm. **25**, 426 (1966).
12. Lamola, A. A., and J. P. Mittal: Science **154**, 1560 (1966).
13. Ben Hur, E., D. Elad, and R. Ben Ishai: Biochim. Biophys. Acta **149**, 355 (1966).
14. Blackburn, G. M., and R. J. H. Davies: J. amer. chem. Soc. **89**, 5041 (1967).
15. Pullman, B.: International Symposium on "Basic Mechanisms in Photochemistry and Photobiology", Caracas, Dec. 1967, in press.
16. Nagata, C., A. Imamura, Y. Tagashira, M. Kodama, and N. Fukuda: J. theoret. Biol. **9**, 357 (1965).
17. Pullman, A.: International Symposium on "Electronic Aspects of Biochemistry". New York: Academy of Sciences, Dec. 1967, in press.
18. Weinblum, D., F. P. Ottensmeyer, and Q. F. Wright: Biochim. Biophys. Acta **155**, 24 (1968).
19. Muller, N., and D. E. Pritchard: J. chem. Physics **31**, 768, 1471 (1959).
20. Anet, R.: Tetrahedron Letters **1965**, 3713.

Professor Dr. B. Pullman
Institut de Biologie Physico-Chimique
13, rue Pierre Curie,
F 75 Paris V/France