SYNTHESIS AND SOME PHYSICAL AND PHOTOCHEMICAL PROPERTIES OF DINUCLEOTIDE ANALOGS.

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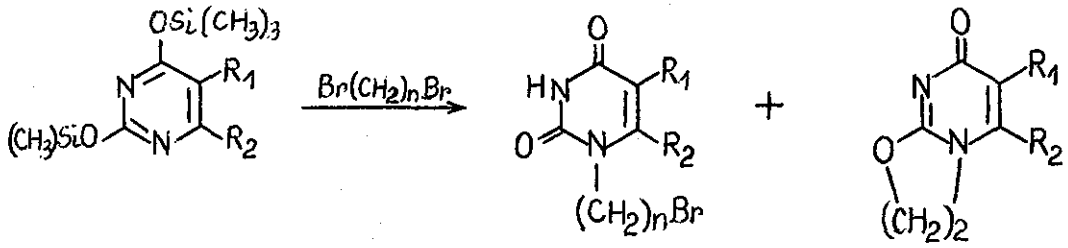
The dinucleotide analogs in which purine or pyrimidine bases are linked together by the polymethylene chains appeared to be excellent models for the studies of unbonded base-base interactions and photodimerization in nucleic acids. This article gives a survey of recent results obtained in this field in our Laboratory.

The investigation of unbonded base-base interaction in nucleic acid strand on natural or synthetical species is highly complicated by the hydrogen bonding or phosphodiester linkages. The importance of this problem to the elucidation of secondary or tertiary structure of nucleic acids has been emphasized in the former literatures[1-3].

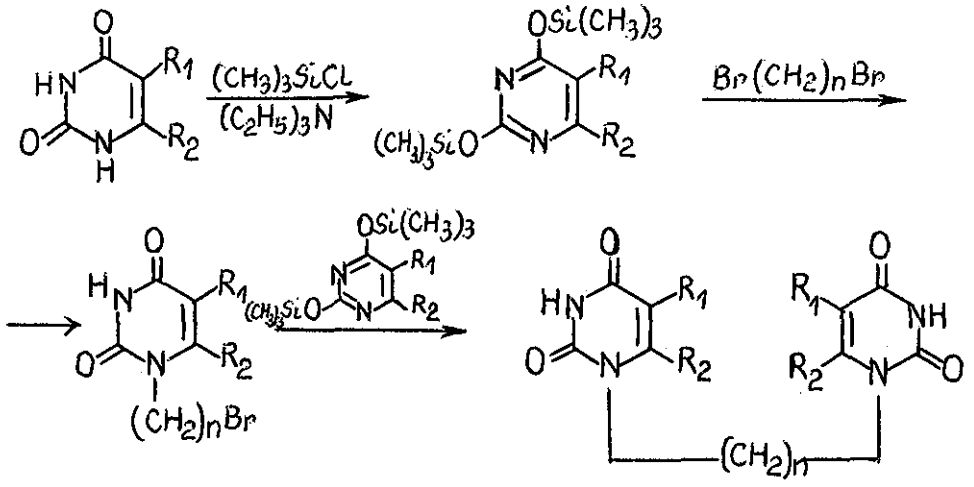
The ingenious solution was found by N.J. Leonard who proposed to investigate the pure base-base interaction on the dinucleotide analogs in which the bases were connected by the polymethylene chains B-$/CH_2/_n$-B' where B and B' were 9-substituted adenine or guanine or 1-substituted uracil, thymine and cytosine[4,5]. It should be noted that polymethylene spacers have been also successfully employed for investigation of photodimerization of such compounds as α , α'-trimethylenebisnaphthalenes[6,7], N,N-polymethylenebis

maleimides[8,9], and 7,7'-polymethylenebisoxycoumarins[10,11]. They also provide the possibility of controlling the inter-ring interactions by attachement of the chain to different positions on the heterocyclic terminus[12,13]. The study of the base-base interaction and photodimerization in 1,1'-poly-methylenebispyrimidine derivatives was in the recent years one of the major problems in our Laboratory. At the beginning we improved the synthesis of these compounds.
According to N.J. Leonard the special care should be taken in the reaction of bistrimethylsilyl derivative of pyrimidine with α , ω -dibromoalkane to avoid the internal cyclization[4]:



To avoid this "theoretical" possibility these reactions were carried out at ambient temperature and lasted about 2 weeks and produced the desired 1-/ω-bromoalkyl/pyrimidine derivatives in a low yield. Our studies showed that an increase of the reaction temperature to 100-110°C not only shortened the reaction to a few hours but also substantialy increased the yield[14]. Also the alkylation of the trimethylsilylpyrimidine derivative with 1-/ω-bromoalkyl/pyrimidine derivative gave better results than the alkylation of the free base in alkaline solution[14]. It allowed to avoid the decrease of the yield on account of the formation of O-alkylated by-products[15,16]. The introduced improvements made easily

accessible several derivatives such as 1,1'-polymethylenebis-pyrimidine derivatives obtained according to the following route:



R_1 = H, CH_3, C_2H_5, C_3H_7, C_4H_9
R_2 = H, CH_3, C_2H_5, C_3H_7, C_4H_9
n = 2, 3, 4, 5.

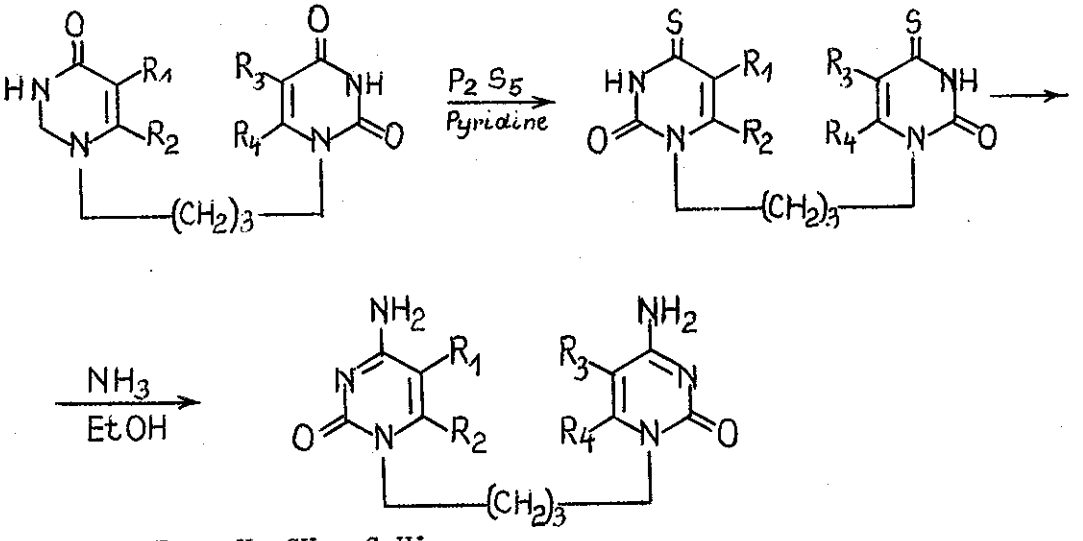
It has been noticed that enlargement of the substituents in 5 positions in these compounds from hydrogen to ethyl effects the chemical ehifts of C^6-H protons as well as the chemical shifts of N^1-CH_2-protons[10,17]. Also the length of the polymethylene chain effects the chemical shifts of the pointed out protons[18]. It can be summarized as follows: The increase of the alkyl substituents in 5 position of the pyrimidine moiety from hydrogen to ethyl shifts the C^6-H signal towards lower magnetic field for about 0.30 ppm.

The further enlargement of the alkyl substituent does not effect chemical shift of C^6-H. The alkyl substituents in 5 position of the pyrimidine moiety have the noticable effect on chemical shifts of N-CH_2 protons that can be seen in the fig. 1.

Fig. 1.

The enlargement of the substituents in 5 position over ethyl also does not effect further the chemical shifts of N-CH_2- protons.

The synthetic procedure for obtaining 1,1'-polymethylenebis-/5-alkyl/uracils was not satisfactory for cytosine derivatives. These compounds were obtained in the following route[20]:



R_1 = H, CH_3, C_2H_5
R_2 = H, CH_3
R_3 = H, CH_3, C_2H_5
R_4 = H, CH_3

The hypochromism may be the measure of the unbonded base-base interaction of the discussed 1,1'-polymethylenebispyrimidine derivatives. To calculate hypochromism the value of oscillator strength is needed. This was obtained by the following procedure: the electronic absorption spectra were digitized at Pach interval of 2 nm width. Oscillator strengths

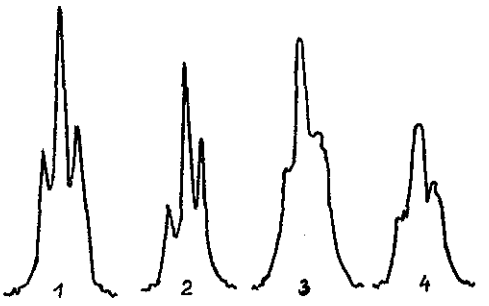


Fig. 1. NMR absorption of N-CH_2-protons in compounds:

1 - 5EtUra /1 /CH_2/$_3$ 1/5EtUra,*) [19]

2 - 5EtUra /1 /CH_2/$_3$ 1/Thy,

3 - 5EtUra /1 /CH_2/$_3$ 1/Ura,

4 - Thy /1 /CH_2/$_3$ 1/Ura.

*) The abbreviations are in accord with the proposition of W.E.Cohn, N.J.Leonard, and S.Y.Wang, Photochem. Photobiol., 1974, 19, 89.

were calculated by an ODRA 1204 computer using a program in an ODRA ALGOL language.

$$f = 4.32 \times 10^{-9} \int \{[\epsilon(\nu)]/(\nu)\}$$

Individual values of oscillator strength were reproducible to within ±0.8%. From thus obtained oscillator strengths of the 1,1'-polymethylenebispyrimidine derivatives J_{AB} and the appropriate 1-alkylpyrimidine derivatives J_A and J_B the hypochromism values were calculated according to equation:

$$\%H = \left\{1 - \left[(J_{AB}) \Big/ (J_A + J_B) \right]\right\} \times 100$$

The obtained results are presented in the table 1.

Table 1

Hypochromism values[18,21] presented in the table 1 show that some regularities may be found only in the series with trimethylene bridges. In this series the order of van der Waals interactions between pyrimidine moieties decreases in the following direction 5EtUra > Thy > Ura.
Some of these compounds in analogy to the dinucleotides[22,23] and polynucleotides[24,25] demonstrated the hypsochromic shift of ν max from the arithmetical means of ν max of the corresponding 1-alkylpyrimidine derivatives. This hypsochromic shift was connected with hypochromism by the following empirical equation:

$$\%H = 0.5(\Delta\nu)^2 + 1.5\,\Delta\nu$$

Table 1. The percent of hypochromism and quantum yield of photodimerization of 1,1'-polymethylenebis/5-alkyl/-uracils.

Compound	%H hypochromism	Quantum yield of photo-dimerization
Thy /1 /CH_2/$_2$ 1/ Ura	2.6	
Thy /1 /CH_2/$_2$ 1/ Thy	7.4	0.0023
5EtUra /1 /CH_2/$_2$ 1/5EtUra	8.2	0.0030
Thy /1 /CH_2/$_3$ 1/Thy	7.0	0.0400
5EtUra /1 /CH_2/$_3$ 1/Ura	4.5	0.0260
5EtUra /1 /CH_2/$_3$ 1/Thy	7.9	0.0350
5EtUra /1 /CH_2/$_3$ 1/5EtUra	8.8	0.0340
Thy /1 /CH_2/$_4$ 1/Thy	5.8	0.0100
5EtUra /1 /CH_2/$_4$ 1/5EtUra	2.6	0.0069
Thy /1 /CH_2/$_5$ 1/Thy	1.0	0.0020
5EtUra /1 /CH_2/$_5$ 1/Thy	1.2	

The graphic representation of this function, which is in accord with the obtained order of interactions, is given in the fig. 2.

Fig. 2.

The similar effect on hypochromism as the increase of alkyl substituents in 5 positions had the alkyl substituents in 3 position that can be seen from the table 2 for the trimethylene bridge series[26,27].

Table 2

Unfortunately the function presented in the fig. 2 was not true for this series[26].
The degree of nonbonding interaction is indicative of the fraction of complexed molecules in which the proximity of the neighboring pyrimidines should facilitate the photodimerization.

Photodimerization of 1,1'-polymethylenebis/5-alkyluracils/.

The compounds presented in the table 1 and 2 were subjected to irradiation with UV light with $\lambda > 290$ nm. The attempts were made to correlate the quantum yield of photodimerization and the structure of photoproducts with the hypochromism values. In all cases when photoproduct could have been isolated it always was cyclobutane-type photodimer of cis-syn configuration. The search for trans-syn isomers,

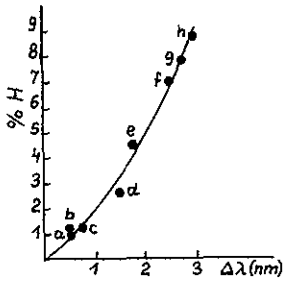


Fig. 2. The dependence of percent hypochromism of 1,1'-polymethylenebis-/5-alkyl/uracils in water solution upon hypsochromic shift of λ_{max} from the arithmetical means of λ_{max} of the corresponding 1-alkylpyrimidine derivatives. The calculated curve of $\%H = 0.5\,/\Delta\lambda/^2 + 1.5\,\Delta\lambda$ and experimentally obtained data for /a/ Thy /1 /CH_2/$_5$ 1/Thy, /b/ Ura /1 /CH_2/$_3$ 1/Ura /c/ 5EtUra /1 /CH_2/$_5$ 1/Thy, /d/ Thy /1 /CH_2/$_3$ 1/Ura, /e/ 5EtUra /1 /CH_2/$_3$ 1/Ura, /f/ Thy /1 /CH_2/$_3$ 1/Thy, /g/ 5EtUra /1 /CH_2/$_3$ 1/Thy, /h/ 5EtUra /1 /CH_2/$_3$ 1/5EtUra.

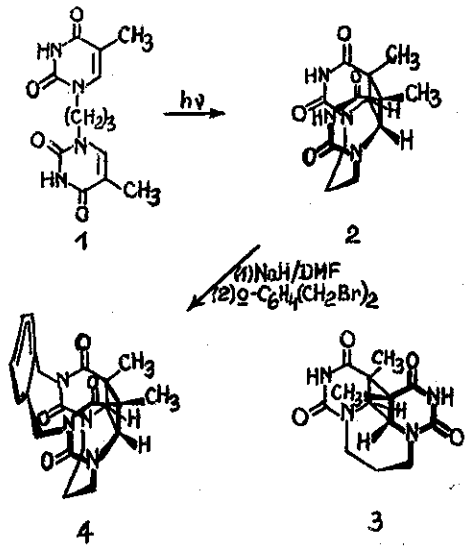
Table 2. The percent of hypochromism and quantum yield of photodimerization of 1,1'-polymethylenebis/5-alkyl/-uracils substituted in 3 position with alkyl groups.

Compound	%H hypochromism	Quantum yield of photodimerization
3MeThy /1 /CH_2/$_3$ 1/3MeThy	11	0.024
3EtThy /1 /CH_2/$_3$ 1/3EtThy	14.3	0.021
3PrThy /1 /CH_2/$_3$ 1/3PrThy	17.7.	0.021
3Me5EtUra /1 /CH_2/$_3$ 1/3Me5EtUra	14.7.	0.012
3Et5EtUra /1 /CH_2/$_3$ 1/3Et5EtUra	15.8	0.009
3Pr5EtUra /1 /CH_2/$_3$ 1/3Pr5EtUra	16.3	0.006
3Me5PrUra /1 /CH_2/$_3$ 1/3Me5PrUra	15.4	0.020
3Et5PrUra /1 /CH_2/$_3$ 1/3Et5PrUra	16.5	0.016
3Pr5PrUra /1 /CH_2/$_3$ 1/3Pr5PrUra	18.3	0.014

was unsuccessful. It is striking analogy to the main photoproducts derived from native DNA which all represented cis--syn configurations[28-31]. The configurations were determined by the spectroscopic, chemical and in some cases X-ray methods.

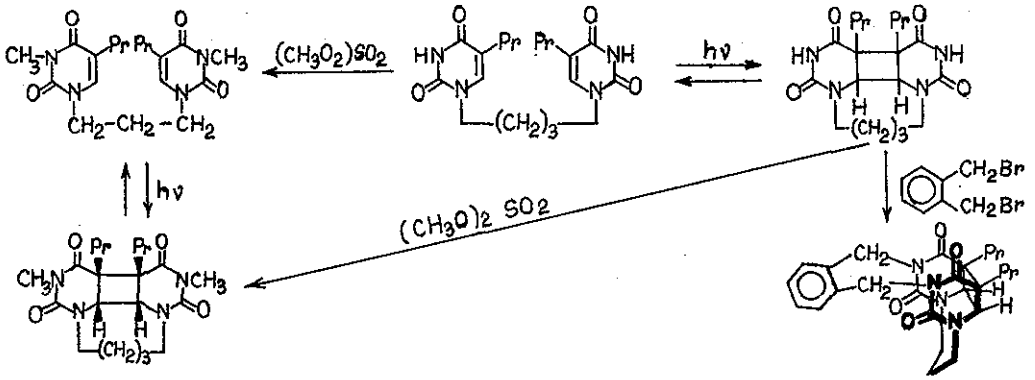
Unfortunately the nmr spectra of photodimers could not be used to make definite assignment of their configuration[32,33]. Although the coupling constants for cyclobutane ring protons, usually being 6 cps are consistent with these being cis oriented[34] it is known that the coupling constants for cis and trans 1,2-cyclobutane protons may lack specifity[35-37]. More convincing is the similarity of UV spectra of the photodimers in 1N NaOH with UV spectrum of Thy [1 /CH_2/$_3$ 1] Thy which structure was established by X-ray method. Also IR spectra of the dipotassium salts of the photodimers in regard to the delocalized bond system /O..C...N...C..O/ were consistent with the data on thymine and uracil dimers reported previously[38-40].

The chemical prove of the cis-syn configuration of the discussed photodimers involved the formation of o-xylylene derivatives which on account of the steric requirements could be obtained only from the cis oriented photodimers[5].

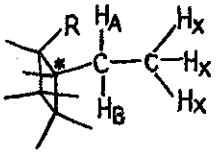


1 2 3 4

The structure of the photodimers of 3,3'-alkylated derivatives of 1,1'-polymethylenebis/5-alkyl/uracils was proved to be cis-syn by the following sequence of the reactions.

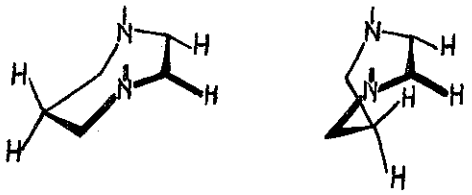


The nmr data yielded valuable informations concerning the alkyl substituents in 5-positions of pyrimidine ring of the photodimers as well as the conformation of the polymethylene chain. The substrates for photodimerization e.q. 1,1'-trimethylenebis/5-alkyl/uracils show uncomplicated nmr spectra due to the flat structure of the pyrimidine moieties and the possibility of the unhindered rotation of alkyl substituents. The A_2X_2 system can be ascribed to the ethyl group in 5 position and $A_2X_2M_2$ /J_{AX} = 0/ system to protons of the trimethylene chain. The formation of the cyclobutane type photodimers abolishes the symmetry plane of pyrimidine rings and brings the asymmetry to 5,5' carbons, which has a significant bearing upon nmr spectra.



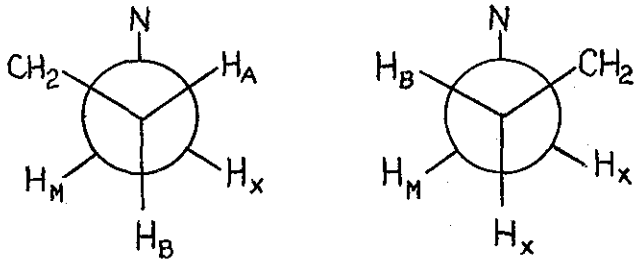
In the photodimer the protons of the ethyl substituent in 5

position have to be described by the ABX_3 system[41]. The methyl protons appear as a triplet centered at $\delta = 0.95$, while the methylene protons of the ethyl group give rise to a complicated multiplet centered at $\delta = 2.1$. By the application of double resonance the following coupling constants were found $J_{AB} = 14 \pm 1$ cps, $J_{AX} = J_{BX} = 7 \pm 1$ cps. The steric arrangement of the trimethylene chain of the photodimers in question and its correlation with the nmr spectra deserve special consideration. It has been unequivocally proved by X-ray analysis that the 1.4-diazacyclobutane ring of the 1,1'-trimethylenebisthymine photodimer exists in a chair conformation in solid state[5].



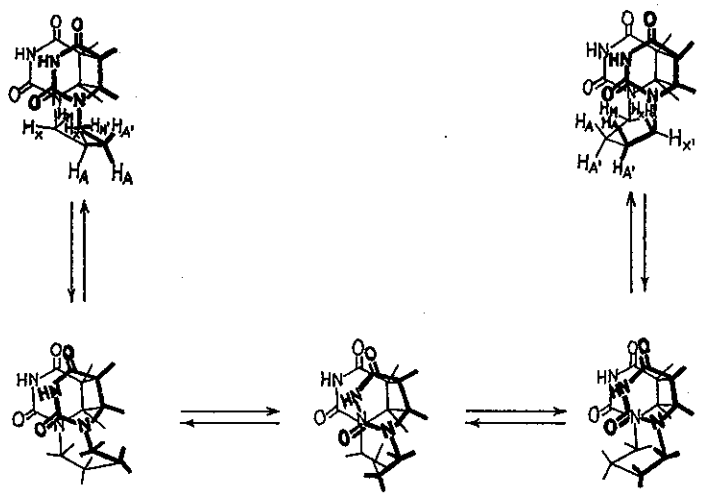
There are good reasons to assume that the chair-like conformation of 1.4-diazacycloheptane ring will also prevail in solution. It can be considered in good approximation that there is an analogy between the 1.4-diazacycloheptane part of the discussed photodimers and cycloheptene for which chair conformation in solution was satisfactorily proved[42,43]. A careful inspection of the steric features of these two compounds revealed that in the case of the 1.4-diazacycloheptane part of photodimers the unbonded interactions in boat-like conformation between protons in 2, 3 and 6 positions are even greater than analogous interactions in boat conformation of cycloheptene which should result in greater stabilization of chair conformation of the 1.4-diazacyclo-

heptane part of the photodimer molecule. The data obtained from precise nmr studies with application of double resonance and higher temperatures strongly suggest that the trimethylene chain of the photodimers exists predominantly in one conformation which in the light of the above discussion has to be chair conformation. On account of anizochromism[44/] the protons of the trimethylene chain of photodimers give rise to the ABMXM'X' system which in some approximation may be treated as AB $[MX]_2$ system in which the protons of two methylenes connected with nitrogen are not coupled. Newman's projections of the trimethylene chain in both conformations may be helpful in further discussion:



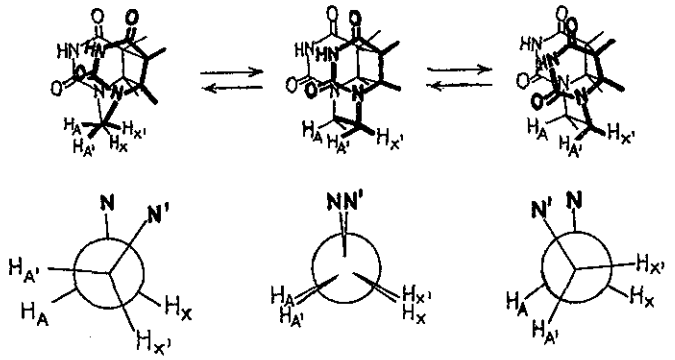
The geminal protons X and M give rise to two multiplets. One is at normal position $\delta = 2.95$ and another at $\delta = 4.55$. The deshielding effect and comparatively big difference in chemical shifts of these protons can, to some extent, be explained by the anisotropic influence of $C^2 = O$ carbonyl groups. A similar diamagnetic effect on the $H^{1'}$ proton and a paramagnetic effect on the $H^{2'}$ and $H^{3'}$ protons have been observed in syn conformations of ribonucleosides[45/]. The AB protons give rise to the broad and poorly separated signals in the region $\delta \approx 1.0\text{-}2.0$. The following coupling constants were found by the double resonance method $J_{MX} = 14.5 \pm 1$ cps, $J_{AM} = 11 \pm 1$ cps, $J_{AX} = J_{MB} = 3.5 \pm 1$ cps. It leaves no doubt that the trimethylene chain has to exist in one rigid conformation. The tendency of the coupling constants of the

vicinal protons to reach the average value of 7 cps, observed usually in aliphatic hydrocarbons could be noticed otherwise. The nmr spectra of the photodimers taken at +120°C demonstrated only better shapes of X protons triplets indicating the decrease of diagonal relaxation constant with the increase of temperature. This is in accord with the former suggestion that the difference in free energies and not too high transition barrier is resposible for one conformation of the 1.4-diazacycloheptane part of photodimer molecule. The nmr features of the photodimers with tetramethylene chain were in accord with theoretical considerations. The tetramethylene chain can exist in different conformation as presented below:



The coupling constants of the geminal protons $J_{MA} = J_{XA} = 9.0 \pm 0.5$ cps are in favour of this proposition[18]. In contrary to the nmr features of 1,1'-tetramethylenebis-/5-alkyl/uracil photodimers, the 1,1'-dimethylenebis/5-alkyl/-uracil photodimers needed special consideration[18]. The protons of ethylene chain have to be treated as AA'XX' system. The chemical shift of these two pairs of protons is effected by anisotropy of $C^2 = O$ and $C^{2'} = O$ carbonyl groups. Two

multiplets separated by about 1.5 ppm can be observed in the nmr spectrum. The coupling constants of geminal protons $J_{AX} = J_{A'X'} = 8.0 \pm 0.5$ cps can be explained only by the deformation of tetrahedral bond angle $H_A - C - H_X$ to about 115° [46]. Also the coupling constants of vicinal protons in ethylene chain $J_{AA'} = J_{XX'} = 12.0 \pm 0.5$ cps are in favour of the rigid structure twisted by about 30° as shown below:



When numbers of the polymethylene chain in 1,1'-polymethylene-bis/5-alkyl/uracil derivatives was 2-4, the photodimerization yielded only one photoproduct e.g. cyclobutane-type photodimer of cis-syn structure. When the chain reached 5 methylene groups the tlc analysis of irradiated solution revealed the presence of at least 5 photoproducts, one of which was isolated in 20% yield and characterized as cyclobutane-type photodimer of cis-syn structure[47]. Introduction of alkyl substituent into 6 position of pyrimidine moiety hindered very strongly the photodimerization although the cis-syn photodimer could have been obtained[48].

<u>X-ray studies of 1,1'-polymethylenebis/5-alkyl/photodimers.</u>

X-ray analysis was made for 1,1'-trimethylenebis-

thymine photodimer[5] and 3,3'-dipropyl-1,1'-trimethylenebis-thymine photodimer[49]. The X-ray analysis confirmed the cis-syn configuration for these two compounds. However, it was found that they exist in different conformations. In contrast to 1,1'-trimethylenebisthymine photodimer the photodimer of 3,3'-substituted derivative has a puckered cyclobutane ring with an average dihedral angle of 164.8°. As a result of the puckering the heterocyclic rings are twisted. The six-membered rings are rotated in respect to each other of about 10.5°. The diazacycloheptane ring has a slightly twisted chair conformation. A similar geometry was found for the cis-syn photodimers of 6-methyluracils[50] and thymine trimer[51]. However, the molecule of the known cis-syn photodimer of 1,3-dimethylthymine with substituted nitrogen atoms is much more distorted[52]. The bond length and angles in the molecules of the discussed photodimers agreed well with those formerly reported[53-55].

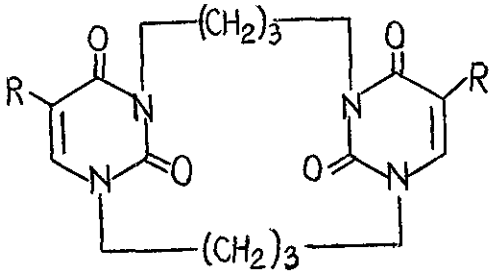
Photochemical studies.

The attempts were made to correlate the quantum yield of photodimerization with the hypochromism which may be the measure of the unbonded base-base interaction in 1,1'--polymethylenebis/5-alkyl/uracils. It can be seen in the tables 1 and 2 that increase of the alkyl substituents in 5 and 3 positions in trimethylene series increase the values of hypochromism and decreases the quantum yields of photodimerization. The oxygen effect in this series was negligible. Moreover photodimerization took place even in the presence of 3M solution of KBr used as an inhibitor of triplet states while the concentration of the substrate was 10^{-4} M. The quantum yields of photodimerization of the trimethylene series did not depend on the excitation energy. In the light of literature

data[56-58] the mechanism similar to the dimerization from excited aggregates can be suggested in this case.
In pentamethylene series in which interactions are smaller the photodimerization quantum yields are at least of one order smaller. Moreover the strong increase of quantum yields in this series with the increase of excitation energy as well as the evident oxygen effect indicates that mechanism of dimerization involves the ground state of one pyrimidine moiety and the excited triplet state of another. The length of polymethylene chains in the compounds discussed precluded the formation of anti-type complexes, permitting only cis-syn and trans-syn conformations. Extremely high stereospecifity of photodimerization, regardless of the duration of photoreaction is indicative of the favoured cis structure in the transition complexes.

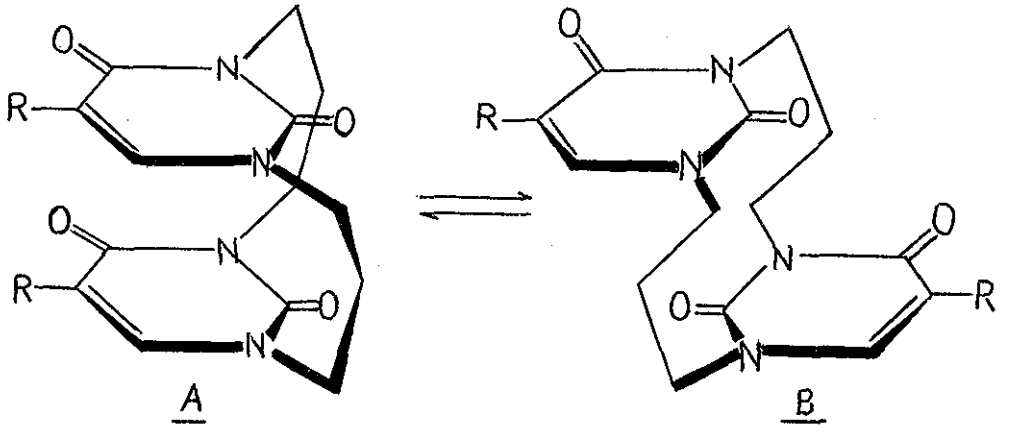
The structural and photochemical phenomena of quasimethacyclophanes derived from 5-alkyluracils[59].

1,1'-Trimethylenebis/5-alkyl/uracils linked in 3,3'-position with trimethylene bridge yielded the compounds which may be regarded as quasimethacyclophanes.



R = CH_3, C_2H_5

It has been proved that such compounds exists in two conformational isomers A and B:



A ⇌ B

Isomer A being more polar than isomer B shows the lower values of Rfs, that is in accord with the former literature data for thymine and uridine derivatives[60,61]. The freshly prepared samples as judged by nmr show the predominance of the conformer A, while in the equilibrium the mixture contains about 65% of the conformer A and 35% of B. It is in contrast with the behaviour of the derivatives of methacyclophanes which exists entirely in trans conformations[62]. It has been found that on irradiation with low pressure mercury vapour UV lamp only isomer A yielded photodimer while conformer B was not effected by irradiation. The rate of photodimerization of conformer A is very high and the photostationary state even for the λ = 254 nm is shifted towards photodimer. The investigation of these very interesting compounds are actually being in progress in our Laboratory.

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