Tetrahedron Letters No.37, pp. 4471–4475, 1966. Pergamon Press Ltd.
Printed in Great Britain.

THE STRUCTURE OF URACIL PHOTO-DIMER

G.M. Blackburn and R.J.H. Davies
Department of Chemistry, University of Sheffield
and University Chemical Laboratory, Cambridge.

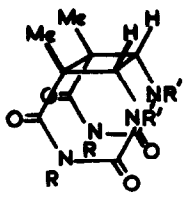
(Received 8 July 1966)

The dimerisation of pyrimidines plays a major role in the ultraviolet (UV) photochemistry of the nucleic acids.[1] In 1960, Beukers and Berends[2] reported the photodimerisation of thymine by UV irradiation of a frozen aqueous solution, and since then many other pyrimidines have been shown to behave similarly.[3] Dimerisation can lead in theory to any of four stereoisomers having the cyclobutane structure although the product from intrastrand dimerisation in nucleic acids should have cis-syn stereochemistry.[4] This prediction has been verified[5] for the thymine dimer obtained from irradiated DNA. While the structures of a number of dimers have been postulated from the crystal structure of the monomer,[3] an unambiguous chemical assignment has been achieved[6] only for thymine photo-dimer (1a).

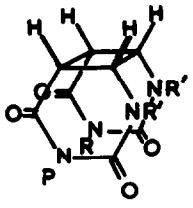
Dimers of uracil are formed naturally in UV-irradiated RNA[7] and are also produced by deamination of cytosine dimers in irradiated DNA.[1] Wang[3,8] has shown that uracil and a number of its derivatives display photodimerisation similar to that of thymine on irradiation in ice. The uracil dimer so produced has usually been treated as being homogeneous[8-10] and shows no change in infrared absorption on repeated crystallisation. The apparent inhomogeneity of this material

as deduced[11] from its paper chromatographic properties is readily demonstrated to result from overloading of chromatograms. Also, although the dimer is partially degraded by formic acid treatment,[12] we have found that undegraded material is identical with the starting material. There is, accordingly, no evidence for inhomogeneity in uracil ice-dimer.

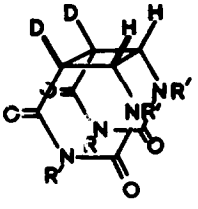
The dimer forms a disodium salt with 4N sodium hydroxide but does not undergo a reaction with bromine and alkali parallel to the rearrangement shown by thymine dimer.[6] An alternative approach to the elucidation of its stereochemistry is necessary.



1a R=R'=H
1b R=R'=Me



2a R=R'=H
2b R=Me, R'=H
2c R=R'=Me



3 R=Me, R'=H

Uracil dimer is readily methylated in N alkali to give 3,3'-dimethyl uracil-dimer (2b) in 65% yield, which on photoreversion gives only 3-methyluracil. The n.m.r. spectrum of this dimer, in CF_3COOD solution, shows the N-Me protons as a sharp singlet at 6.70 τ with the cyclobutane protons at ca. 5.6 τ comprising an A_2B_2 pattern too

complex for interpretation. However, Anet[13] was able to determine the coupling constant between the identical cyclobutane protons in tetramethyl thymine-dimer (1b) by making use of the ^{13}C-H satellite n.m.r., and it appeared that a similar result could be achieved if two of the cyclobutane protons in uracil dimer were to be isotopically substituted by deuterium.

Decarboxylation of 5-carboxyuracil[14] on heating in deuterophosphoric acid[15] gave 5-deuterouracil. This was characterised by conversion into 5-deutero-1,3-dimethyluracil, shown by mass spectrometry to contain 95% deuterium in the 5-position. Its n.m.r. spectrum, in D_2O solution, showed a singlet at 2.33τ and there was no evidence for any deuterium in the 6-position.[16]

Dimerisation of this 5-deuterouracil in the usual way gave a product which upon methylation afforded 5,5'-dideutero-3,3'-dimethyl uracil-dimer (3). The n.m.r. spectrum of this dimer, in CF_3COOD, showed a broad singlet at 5.60τ replacing the A_2B_2 system seen in the protium compound (2b). The low-field ^{13}C-H satellite was observed using a Varian Computer of Average Transients to effect spectrum accumulation. It appeared as a doublet showing a coupling constant $J_{H_6H_{6'}}$ of 5 c/sec, some fine structure being attributable to coupling with neighbouring deuterons. The ^{13}C-H coupling constant was 166 c/sec.

The magnitude of the $H_6H_{6'}$ coupling constant is consistent only with these hydrogens being vicinal. Moreover, it is the same as that observed[13] in the case of tetramethyl thymine-dimer (1b), which is known to have cis-syn stereochemistry.[6] It is clear, therefore, that uracil

ice-dimer (2a) has the same stereochemistry.* This isomer is that most likely to arise from solid state dimerisation of crystalline uracil.[3,17]

Ultraviolet irradiation of 1-methyluracil[18] or of 3-methyluracil[19] in an ice matrix leads to less than 3% dimerisation under conditions which give 71% dimer from thymine.

It is not at present possible to correlate uracil dimer with either of the two dimers obtained[3] from dimethyluracil since attempts to prepare 1,1',3,3'-tetramethyl uracil-dimer (2c) by alkylation of uracil ice-dimer have been unsuccessful.

Acknowledgements: We gratefully record the assistance of Dr. J. Feeney of Varian Associates in determining the CAT spectrum and thank S.R.C. for the award to one of us (R.J.H.D.) of a Research Studentship.

---

* During the course of this work, Dönges and Fahr[10] communicated results of a chemical degradation of uracil dimer which also proves its cis-syn stereochemistry.

REFERENCES

1. R.B. Setlow and W.L. Carrier; J. Mol. Biol., 17, 237 (1966).
2. R. Beukers and W. Berends; Biochim. Biophys. Acta, 41, 550 (1960).
3. S.Y. Wang; Fed. Proc., 24, S-71 (1965)
4. D.L. Wulff and G. Fraenkel; Biochim. Biophys. Acta, 51, 332 (1961).
5. G.M. Blackburn and R.J.H. Davies; Biochem. Biophys. Res. Comm., 22, 704 (1966)
6. G.M. Blackburn and R.J.H. Davies; Chem. Comm., 215 (1965)
7. P.D. Harriman and H.G. Zachau; J. Mol. Biol., 16, 387 (1966).
8. S.Y. Wang; Nature, 190, 690 (1961).
9. A. Smietanowska and D. Shugar; Bull. Acad. Polon. Sci. (Biol. Series) 9, 375 (1961).
10. K. H. Dönges and E. Fahr; Z. Naturforsch., 21b, 87 (1966).
11. K.C. Smith; Photochem. Photobiol., 2, 503 (1963).
12. R.B. Setlow, W.L. Carrier, and F.J. Bollum; Proc. Nat. Acad. Sci. (U.S.), 53, 1111 (1965).
13. R. Anet; Tet. Letters, 3713 (1965).
14. E. Ballard and T.B. Johnson; J. Amer. Chem. Soc., 64, 794 (1942).
15. Supplied by CIBA; greater than 98% atom D.
16. cf. J.M. Rice, G.O. Dudek, and M. Barber; J. Amer. Chem. Soc., 87, 4569, (1965).
17. G.S. Parry; Acta Cryst., 7, 313 (1954).
18. Sample provided by Dr. D.M. Brown.
19. C.W. Whitehead; J. Amer. Chem. Soc., 74, 4267 (1952).