The Structure of Thymine Photo-dimer

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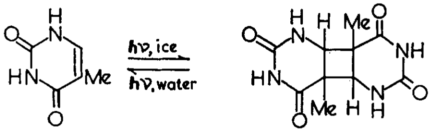
WHEN a frozen aqueous solution of thymine (I) is irradiated with ultraviolet light ($254\ m\mu$) a major[1] and a minor[2] photo-product are produced. The major product is also obtained from irradiated thymine as a thin film,[3] thymidylyl-(3′-5′)-thymidine,[4] DNA,[5] and bacteria.[6] Its homogeneity has been established by chromatographic,[2] spectroscopic,[7] and chemical[7] data and by a study of the kinetics of its photoreversion to thymine on irradiation in aqueous solution.[8]

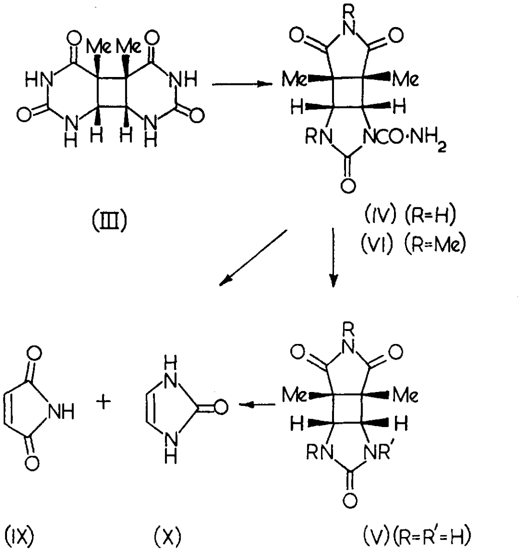
This photo-product is a dimer of thymine for which spectroscopic data support a structure containing a cyclobutane ring (II) formed by cycloaddition across the 5,6-double bond.[9] It has been pointed out, however, that spectroscopic methods cannot distinguish between four possible isomers which contain this structural unit.[7]



(I) (II)

The work described in this communication leads to the assignment of structure (III) for the dimer obtained from frozen aqueous solutions of thymine, hereafter referred to as thymine dimer. This is the isomer predicted[9,10] for dimer produced from adjacent thymine residues in DNA and in oligonucleotides.

Thymine dimer (III) with 10N-sodium hydroxide gives a solid disodium salt which reverts to dimer on solution in dilute acid. (This product can be formulated as the salt of a bis-ureidocarboxylic acid by analogy with the alkaline hydrolysis products of 5,6-dihydrouracils).[11] When a fine suspension of this salt in 40% sodium hydroxide solution is treated with bromine a non-oxidative rearrangement occurs to give a product, isomeric with (III), formulated as (IV) [n.m.r. spectrum in CF_3CO_2H, chemical shifts in τ units (followed by intensity and multiplicity): 8·45 (3;1), 8·38 (3;1), 5·55 (1;2, J = 9 c./sec.), 4·96 (1;2, J, = 9 c./sec.), 2·55 (1;1), 0·02 (1;1)]. An additional weak splitting of τ 5·55 (J = 2 c./sec.) must be due to a vicinal NH since it is absent from the spectrum of (IV) in CF_3CO_2D.



(III) (IV) (R=H) (VI) (R=Me)

(IX) (X) (V) (R=R′=H) (VII) (R=Me; R′=H) (VIII) (R=Me; R′=NO)

The dimer (IV) loses a carbamoyl group on treatment with nitrous acid to give (V). The dimethyl derivative (VI) formed by methylation of (IV), reacts with nitrous acid similarly with the production of (VII) and, additionally, the *N*-nitroso-derivative (VIII). The n.m.r. spectra of (VI), (VII), and (VIII) all show an AB-system near τ 5·5, whereas the spectrum of (V) has an A_2 peak

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reflecting its symmetry. On pyrolysis at 240° *in vacuo* both (IV) and (V) afford maleimide (IX) and imidazol-2-one (X).

The formation of dimethylmaleimide and the n.m.r. spin–spin coupling of the cyclobutane protons in (IV) show that thymine dimer must have the methyl groups vicinal as in (III) and eliminates structures of type (II).

Since *cis*-1,2-diaminocyclobutane readily forms a cyclic urea with phosgene but the *trans*-isomer does not,[12] the cyclobutane hydrogens in (IV) must be *cis*-oriented; and the properties of *cis*-1,2-dimethyl-1,2-cyclobutanedicarboxylic acid[13] requires that the methyl groups in (IV) also must be *cis* to one another. These relationships are supported by the pyrolytic fragmentations of (IV) and (V) which are characteristic of compounds having *cis*-fused ring junctions.[14] Clearly, then, thymine dimer has *syn*-linked six-membered rings as in (III).

The remaining stereochemical feature of the dimer structure to be considered is that of the ring junctions. Although *trans*-6:4 ring systems can be reproduced by photochemical addition,[15] the formation of 5:4:6 and 6:4:6 systems by the irradiation of two cyclic compounds results in predominant *cis*-fusion both in solution[15] and in the solid state.[16] Moreover, dimerisation in the solid phase is known to be dependent on orientation of the crystal lattice[17] and proceeds by *cis*-addition. Additional support for *cis*-junctions in (III) is provided by the photolytic[9] and pyrolytic[9] reversion of dimer to thymine.

Thymine dimer can thus be assigned the *cis-syn*-structure (III).

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