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THE PHOTODIMER OF THYMINE: AN NMR STUDY

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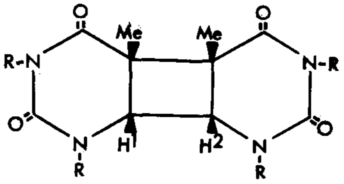
The recent communication of Blackburn and Davies[1] prompts us to report our results on the photodimer of thymine (I), obtained[2] by irradiation of frozen aqueous solutions of thymine. The work described in this paper gives independent confirmation to the head-to-head cis cyclobutane structure for I, predicted[3] from the crystal structure of thymine hydrate[4], and proven by Blackburn and Davies.



I, R=H ; II, R=Me

In the present work, a distinction was made between the four structures[5] for thymine dimer, by determining the coupling constant $J_{H_1H_2}$ between the two cyclobutane protons, from the naturally abundant C^{13}-H satellite proton spectrum[6]. As I is very sparingly soluble in most solvents, the tetramethyl derivative II, prepared by the methylation of I with dimethylsulphate and alkali[5], was used. No change in stereochemistry was likely in the conversion of I to II. The methylated derivative II had the further advantage that the $C^{13}H$ satellites of the two N-Methyl groups formed a convenient internal standard for calibration of the cyclobutane satellite, both for position and intensity. A 25% solution of a highly purified (chromatography, repeated crystallizations) sample of II, m.p. 248°, in deuteriochloroform containing tetramethyl silane was used. The C^{12}-H spectrum was essentially identical with that published[5]. The region, τ 4.34-6.0, was scanned on a Varian A-60 High Resolution NMR spectrometer with a Varian CAT (Computer of Average Transients) attachment, and the sum of 112 scans is shown in Fig. 1.

The cyclobutane satellite was identified as the doublet ($J_{H_1H_2}$, 5 cps) from its position ($J_{C^{13}H}$, 153 ± 2 cps; $J_{NC^{13}-H}$, 139 cps, and 141 cps). The $J_{C^{13}H}$ was expected to be larger than that of cyclobutane[7] (135 cps) due to the electronegativity of the substituent[8]. Only the low-field cyclobutane satellite could be observed, the one at high field was obscured by the $C-CH_3$ group satellite.

The low signal-to-noise ratio made a large number of scans necessary to observe the cyclobutane C^{13}-H satellite, thus causing drift and line-broadening. The limited solubility of II precluded the use of more concentrated solutions. The intensity of each component of the doublet (area under the peak) was approximately one-sixth that of the $N-C^{13}H_3$ satellite, and further confirmed its identity as the C^{13}-H satellite of the cyclobutane proton.

The 5 cps coupling ruled out a 1,3 coupling[9] and showed that I must be a head-to-head dimer. The coupling constant was consistent with a 1,2 *cis* coupling (5 cps) reported for the dimer of N-methylquinolone[10], but a *trans* arrangement could not be ruled out.[11] However, Weinblum and Johns[12], from a study of the photodimers of thymidine, have concluded that thymine dimer I is a meso, and not a *dl* compound. Of the two head-to-head structures, only the *cis* structure is a meso derivative, and hence I must be represented by the head-to-head *cis* structure I.

The stereochemistry of thymine dimer is similar to that of the photodimer of coumarin[13]. The latter has been shown to arise from the excited singlet state of coumarin by Hammond and co-workers[14]. It is tempting to suggest that the photodimer of thymine I formed by U.V. irradiation of DNA in vitro or in vivo[15], arises from the singlet excitation of thymine (cf. Pullman[16]). The triplet signal of U.V. irradiated DNA has been shown by Shulman _et al._[17] to be largely due to thymine anion. The two excited states, singlet thymine, and triplet thymine anion may give rise to different biological effects, namely, repairable thymine dimer formation[18], and a lethal action[19] respectively.

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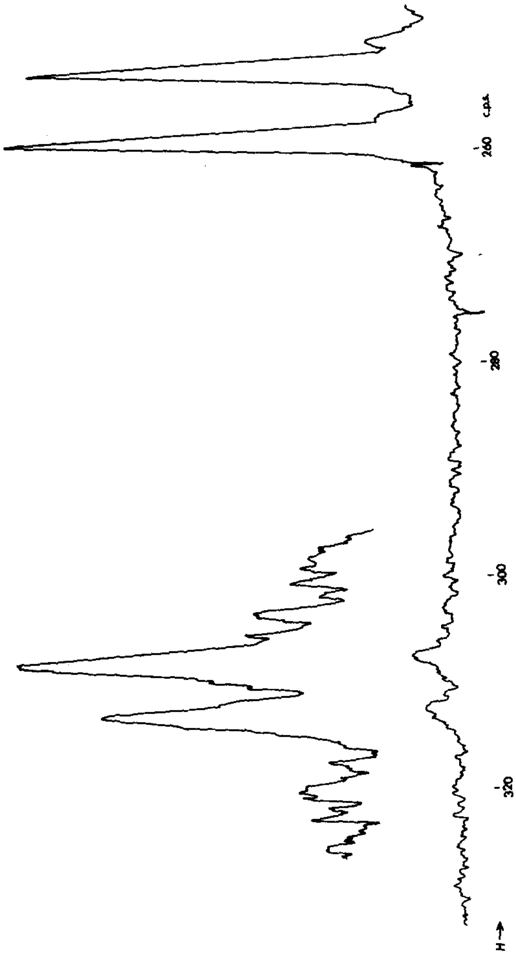

Fig. 1 Low-field C^{13}-H satellite spectrum of the cyclobutane and N-methyl protons of II. C.P.S. are relative to tetramethylsilane. Inset, cyclobutane C^{13}-H satellite.