

## MASS SPECTRA OF SOME DIMERIC PHOTOPRODUCTS OF PYRIMIDINES

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**Abstract**—The mass spectra of photo-cycloaddition products of thymines and uracils and a photo-coupled product of 5-bromouracil have been measured. Characteristic fragmentation has been documented for dimers joined through a cyclobutane ring, adducts derived from oxetane and azetidine linkages and a coupled product linked through a 5,5'-bond.

SINCE nucleic acids play an important role in cellular functions, any alterations of nucleic acid structure caused by UV irradiation are known to produce mutagenic and other biological effects.<sup>1</sup> Currently, there is great interest in the study of the photochemical changes related to these biological effects.<sup>2</sup> These changes are now considered to involve intermolecular reactions of pyrimidine bases in nucleic acids. As part of an on-going program of isolation and identification of these photopolymers, the mass spectra of a variety of thymine and uracil dimeric photoproducts of known structure have been measured. The analysis presented here is intended to serve as an aid to the structural characterization of products of further irradiation studies.

The major product,<sup>3</sup> a cyclobutane dimer of thymine, was isolated from the hydrolysates of DNA irradiated with UV doses comparable to biological studies.<sup>4</sup> It has been shown by chemical degradation,<sup>5</sup> by NMR studies<sup>6</sup> and by X-ray crystallography<sup>7</sup> to be *cis-syn* thymine homodimer, compound 1. This is also the major product obtained by irradiation of thymine in ice, a system used as a model for studies of the irradiation of nucleic acids.<sup>8,9</sup> The major product obtained by irradiation of uracil in ice is also a cyclobutane dimer.<sup>8</sup> It was shown by several

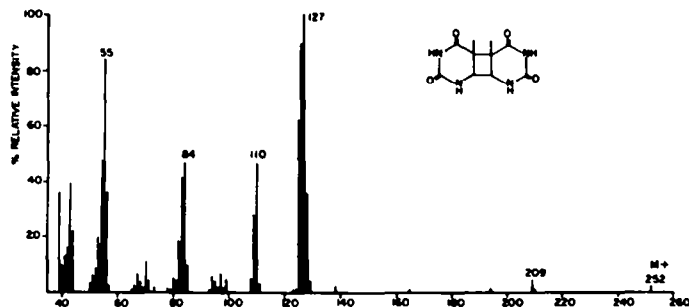
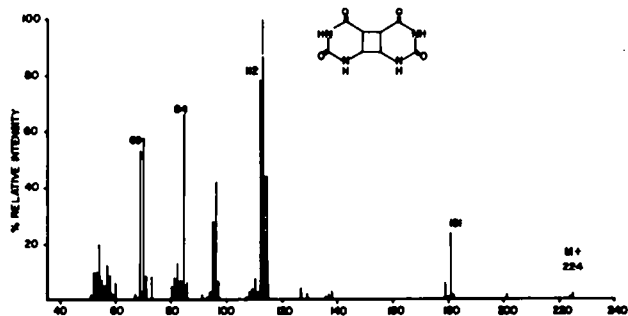
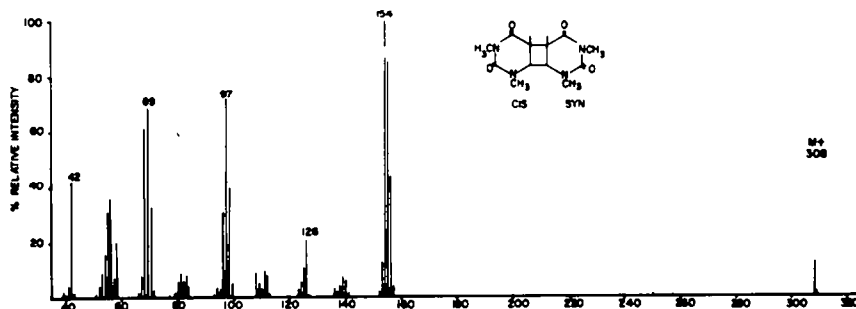
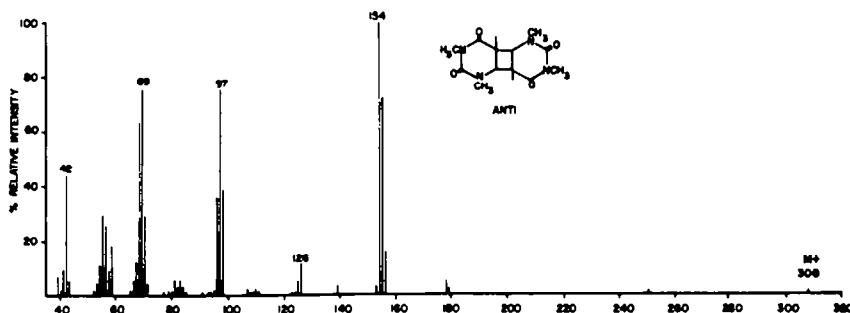
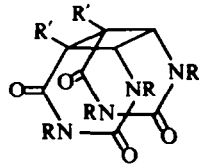


FIG. 1. Mass Spectrum of *cis-syn* Thymine Dimer (1).

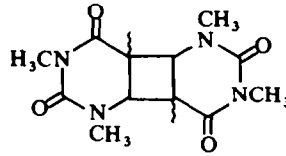
FIG. 2 Mass Spectrum of *cis-syn* Uracil Dimer (2).

lines of evidence<sup>10</sup> including X-ray crystallography also to have the *cis-syn* configuration (compound 2). Uracil dimer has been suggested to be a product in the photochemistry of RNA.<sup>11</sup> The mass spectra of compounds 1 and 2<sup>12</sup> together with two cyclobutyl dimers of 1,3-dimethylthymine, compounds 3 and 4<sup>8,13</sup> have been obtained. Molecular ions were observed under optimal conditions in all the spectra (Figs 1-4) of the dimers 1-4.

FIG. 3 Mass Spectrum of *cis-syn* 1,3-Dimethylthymine Dimer (3).FIG. 4 Mass Spectrum of *anti* 1,3-Dimethylthymine Dimer (4).

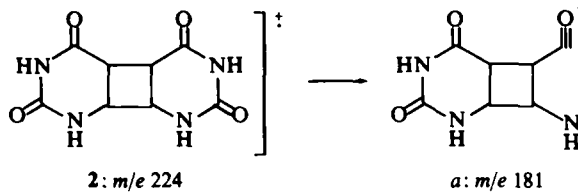


- 1: R = H, R' = CH<sub>3</sub>  
 2: R = R' = H  
 3: R = R' = CH<sub>3</sub>

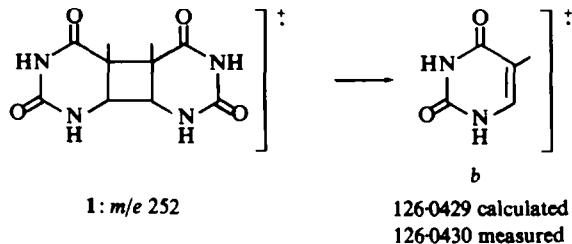


4

Peaks are present in the high mass range of the spectra of the unmethylated dimers 1 and 2 corresponding to  $M-43$  ions. The loss of RNCO is suggested in sequence  $2 \rightarrow a$ , by analogy with the fragmentation<sup>14</sup> of dihydrouracil and dihydrothymine monomers. However, the most characteristic fragmentation in all of these dimers is



scission of the cyclobutane ring, which generates abundant ions of the same composition (confirmed by high resolution mass measurements in compound 1 as the corresponding monomer. This facile halving of the thymine dimer (1) on electron impact is not unexpected, since the same rupture of 1 occurs on excitation at room temperature with  $\beta$ -rays,<sup>15</sup> X-rays<sup>15</sup> or UV light,<sup>8,9</sup> or on heating at 320°. Although the inlet and source temperatures used were below 300°, the possibility of contributions from thermal decomposition to the spectra discussed here cannot be excluded.



The ions of type  $b$  formed by the scission of each of the dimers 1-4 presumably resemble the appropriate ionized monomer, and the low mass half of each dimer spectrum contains all the peaks observed in the spectrum of the corresponding monomer. This is illustrated in the spectra (Fig. 5) of 1,3-dimethylthymine<sup>16</sup> (compound 5) and its *syn* and *anti* dimers (Figs 3 and 4). Our spectra of the other monomers, thymine, uracil and 1,3-dimethyluracil, resemble those already published,<sup>14</sup> except that the molecular ion is the base peak in all of our spectra. These monomers all undergo a fragmentation whereby RNCO is eliminated from the molecular ion.

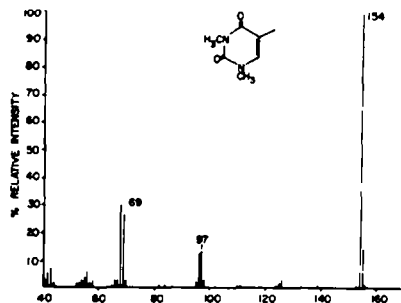
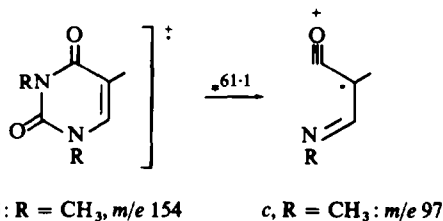
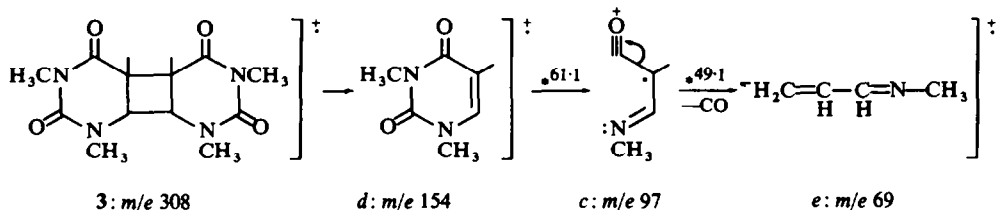


FIG. 5 Mass Spectrum of 1,3-Dimethylthymine (5).

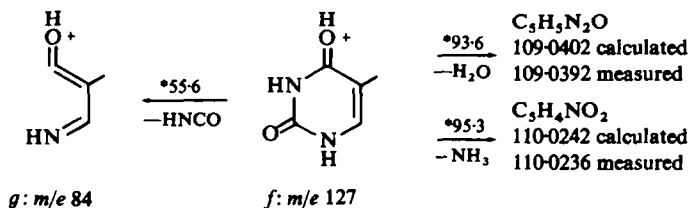
Earlier workers<sup>14</sup> have classed this as a retro Diels-Alder fragmentation. This is open to question since the requisite double bond here is part of an extended system of  $\pi$  and non-bonded electrons. Subsequent elimination of carbon monoxide and also formation of RNCH ions are characteristics of the monomeric decomposition.



Analogous fragmentation paths are followed by the  $\frac{1}{2} M^+$  ions generated by cleavage of the dimers. Metastable ions support the sequence analogous to  $d \rightarrow c \rightarrow e$  in the spectra of all of the dimers 1-4.



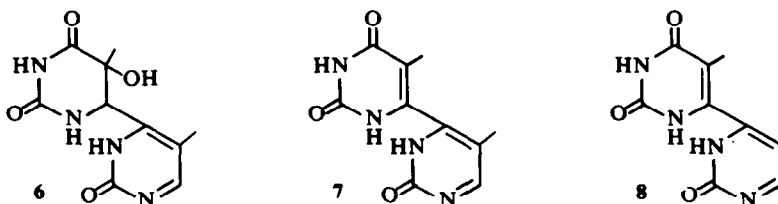
Fragmentation of the dimers 1-4 also leads to sizeable amounts of protonated monomer,  $\frac{1}{2} M + 1$  ions. (See, for example, the peaks at  $m/e$  127 in Fig. 1,  $m/e$  113 in Fig. 2). Metastable ions in the spectrum of thymine dimer confirm the loss of 43 mass units from both the  $\frac{1}{2} M$  and the  $\frac{1}{2} M + 1$  ions (sequence  $f \rightarrow g$ ). Metastable ions in



the same spectrum also confirm the loss of 17 and 18 mass units from the protonated monomer (mass 127) ion. High resolution mass measurements identify the neutral fragments expelled as ammonia and water, respectively. These losses lead to the peaks of moderate size at  $m/e$  110 and  $m/e$  109 in Fig. 1, and to peaks at  $m/e$  96 and  $m/e$  95 in the spectrum of uracil dimer (Fig. 2). The corresponding peaks are quite small in the spectra of the methylated dimers 3 and 4.

The spectra of the pair of *syn* and *anti* isomers 3 and 4 [measured under identical conditions (Figs 3 and 4)] differ only in the intensities of several peaks. The molecular ion in the spectrum of compound 4, the *anti* dimer, has a relative intensity of 13%, while the relative intensity of the molecular ion in the spectrum of the *syn* isomer, compound 3, is about one-tenth as high, 1.3%. Such a distinction requires measurement of both isomers and thus has limited application to characterization of unknown dimers.

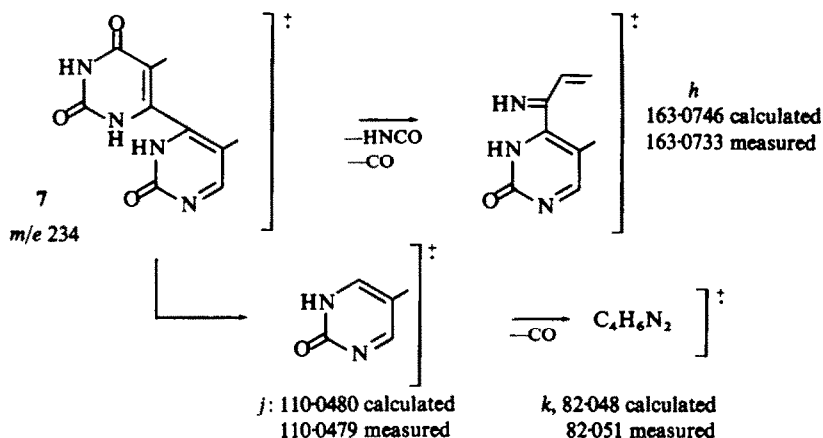
A second dimeric photoproduct isolated from thymine irradiated in ice which has been characterized<sup>17</sup> is 5-hydroxy-6-[4'-(5'-methylpyrimid-2'-one)]-dihydrothymine, compound 6. The original photolytic process is thought to have involved the addition of the 4-CO group of one molecule to the C=C double bond of the second molecule



to form a 4-membered oxetane ring. The oxetane ring opens as part of the photolytic reaction to produce this saturated analog of thymine. Compound 7, 6-[4'-(5'-methylpyrimid-2'-one)]-thymine, is formed<sup>17</sup> by chemical dehydration of adduct 6. Compound 8, 6-[4'-(pyrimid-2'-one)]-thymine, was obtained<sup>18</sup> from the acid hydrolysates of UV irradiated DNA and is thought to be derived from a cycloaddition product of thymine and cytosine which has suffered deamination due to the relatively severe conditions necessary for nucleotide hydrolysis.

The spectra of the homologous pair, 7 and 8, are very similar. In both spectra the molecular ion is also the base peak. Both contain prominent M-17 peaks. The spectrum of methylpyrimidone-thymine (7) contains an M-15 peak, while the spectrum of 8 does not. This suggests that the Me radical eliminated is the Me substituent on the pyrimidone ring. In the spectra of both these compounds, which can be viewed as 6-substituted thymines, the thymine fragmentation pattern is prominent. Thus, the elimination of HNC<sub>2</sub>O from the molecular ion of methylpyrimidone-thymine (7) leads to ions of mass 191 (calculated 191.0650, measured 191.0653) and further elimination of carbon monoxide generates C<sub>8</sub>H<sub>9</sub>N<sub>3</sub>O ions of mass 163 (7 → h). On the other hand, the compound can be viewed as a substituted pyrimidone. This substituent, the thymine ring, may be lost with transfer of hydrogen to form ions of mass 100 with the composition of methyl pyrimidone. The principle peaks in the spectrum of pyrimid-2-one itself are the molecular ion and the ion

resulting from expulsion of carbon monoxide.<sup>19</sup> As indicated in process  $7 \rightarrow k$ , the spectra of compounds **7** and **8** contain peaks corresponding to those expected for elimination of carbon monoxide from the secondary pyrimidone ion. The  $m/e$  82



peak is in fact a quartet, and ions of type  $k$  can best be confirmed by high resolution measurements.

So far, we have viewed these spectra as a combination of thymine-generated and pyrimidone-generated peaks. However, formation of some of the ions identified in the high resolution mass spectrum appears to require the elimination of small neutral molecules from both rings, as for instance,  $\text{C}_7\text{H}_9\text{N}_3$  ions (calculated 135-0796, measured 135-0786) which result from elimination of HNC=O and two carbon monoxide molecules. Perhaps most important for the use of mass spectrometry to identify this structure in unknown polymers is the occurrence of at least one fragmentation which appears to require the interaction of both heterocycles. This fragmentation is the loss of seventeen mass units from the molecular ions of the two pyrimidone-thymines, **7** and **8**. An intense metastable ion at 201.2 in the spectrum of methylpyrimidone-thymine confirms the genesis of M-17 ions from the molecular ion, and the high resolution mass measurements (calculated 217-0487, measured 217-0488) identify the species lost as ammonia.

The elimination of ammonia is not observed in the fragmentation of 6-[4'-(pyrimid-2'-one)]-uracil,<sup>19</sup> a compound isolated from uracil irradiated in ice, which has an analogous ring structure but lacks the 5-Me substituent. This absence suggests that the loss of ammonia from compounds **7** and **8** requires a saturated substituent at C-5 in the thymine ring.

The spectrum of the hydroxy-adduct **6** is shown in Fig. 6. The molecular ion is small. The loss of water, reflected in the large M-18 peak at  $m/e$  234, probably generates ions similar to the molecular ions of the methylpyrimidone-thymine, compound **7**. The same series of fragmentations ( $6 \rightarrow k$ ) can be discerned in this spectra as in that of the chemically dehydrated species. Thus, the large peak at  $m/e$  163 corresponds to ions of the formula  $(\text{C}_8\text{H}_9\text{N}_3\text{O})$  whose formation can be visualized ( $6 \rightarrow h$ ) as involving loss of water, followed by the loss of HNC=O and carbon monoxide characteristic of thymine. Other primary processes, besides the loss of water,

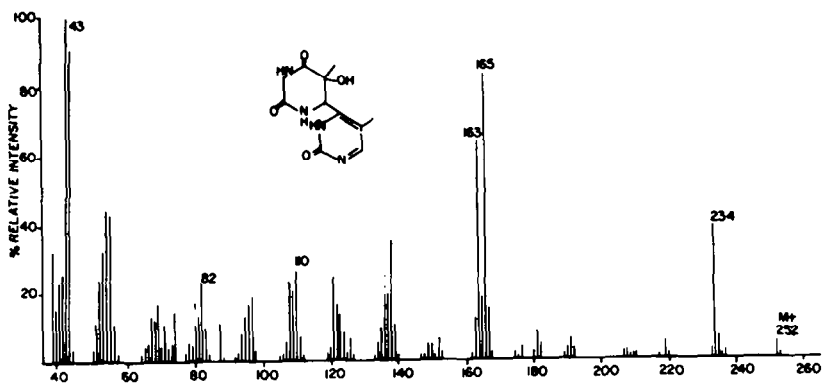


FIG. 6 Mass Spectrum of 5-Hydroxy-6-[4'-(5'-methylpyrimid-2'-one)]-dihydrothymine (6).

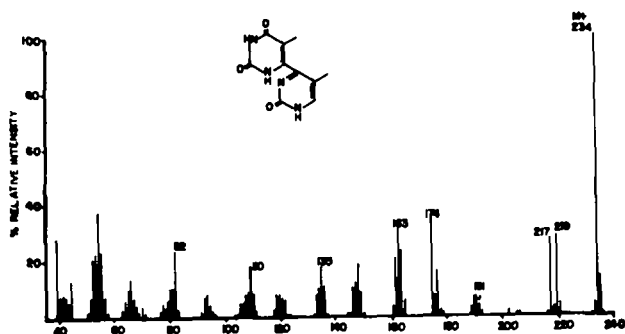


FIG. 7 Mass Spectrum of 6-[4'-(5'-Methylpyrimid-2'-one)]-thymine (7).

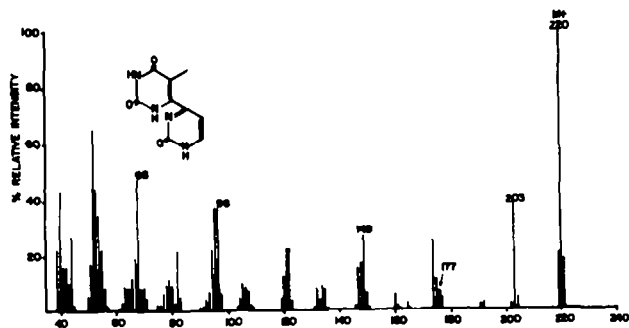
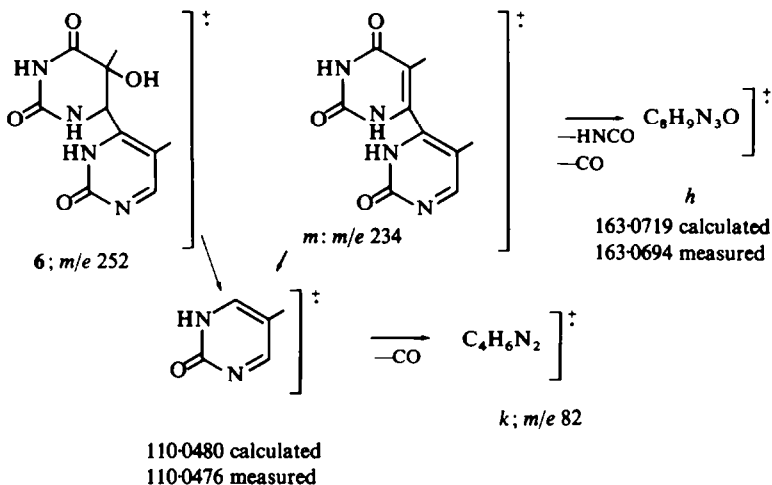


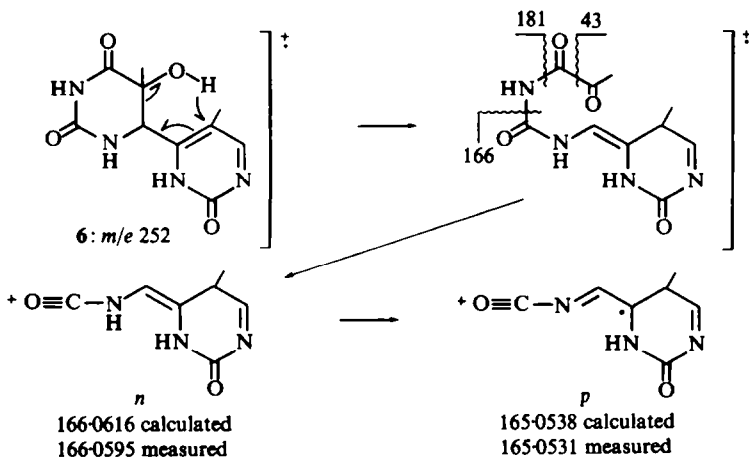
FIG. 8 Mass Spectrum of 6-[4'-(Pyrimid-2'-one)]-thymine (8).

include the loss of Me radical and the elimination of carbon dioxide (calculated 208-0960, measured 208-0984). This latter elimination of one carbon bound to two O atoms suggests that some rearrangement of oxygen occurs in the molecular ion prior to fragmentation.



The scheme  $6 \rightarrow k$  illustrates the pyrimidone-characteristic fragmentation which is inferred from the presence of the appropriate mass 110 ions, and of ions corresponding to those expected from subsequent loss of carbon monoxide.

Once again, the presence of a number of prominent peaks in the spectrum cannot be explained by the pyrimidone fragmentation pattern; nor are they part of the dehydrated adduct's fragmentation pattern. Notable among these are the series of ions of masses 166, 165 and 164, of compositions  $\text{C}_7\text{N}_3\text{O}_2\text{H}_8$ ,  $\text{C}_7\text{N}_3\text{O}_2\text{H}_7$  and  $\text{C}_7\text{N}_3\text{O}_2\text{H}_6$ , respectively. The fragmentation leading to these ions must reflect the





hydroxy dihydrothymine structure, and thus is important to efforts to establish criteria for the identification of various polymer linkages.

From current understanding of electron impact processes one would predict that the most labile bond in this compound would be that between C-5 and C-6. Scission here is  $\alpha$  to an amino group,  $\alpha$  to a hydroxy group and allylic to a double bond. It is well documented<sup>20</sup> that when an OH group is located  $\beta$  to a double bond, as the OH group is here, one highly favored fragmentation involves scission of the bond between, with transfer of the OH hydrogen across the broken bond. This possibility has been indicated as a 6-membered cyclic hydrogen transfer in  $6 \rightarrow n$ . Thus, the adduct might be envisioned to rearrange to an  $\alpha$ -diketo ion, whose fragmentation can then account for a number of otherwise unexplained ions in the spectrum, M-71 ions of the formula  $C_7H_9N_4O_2$ , mass 43 ions, the mass 166 ion which heads the prominent  $C_7H_7N_3O_2$  series. Two sequential hydrogen losses will extend the conjugation and account for the lighter members of the series ( $n \rightarrow p$ ).

The incorporation of 5-bromouracil or 5-iodouracil into DNA in place of thymine results in increased radiation sensitivity of biological systems. In the search for a chemical mechanism for this increased sensitivity, 5,5'-di-1,3-dimethyluracil (compound 9) and 5,5'-diuracil were isolated as photoproducts from the UV irradiation of aqueous solutions of 5-bromo-1,3-dimethyluracil and 5-bromouracil, respectively.<sup>21</sup> The mass spectrum of compound 9 contains the fragmentation pattern characteristic of compounds of the thymine/uracil class. The molecular ion peak is the base peak in the spectrum (Fig. 9). The loss of  $CH_3NCO$  and carbon monoxide

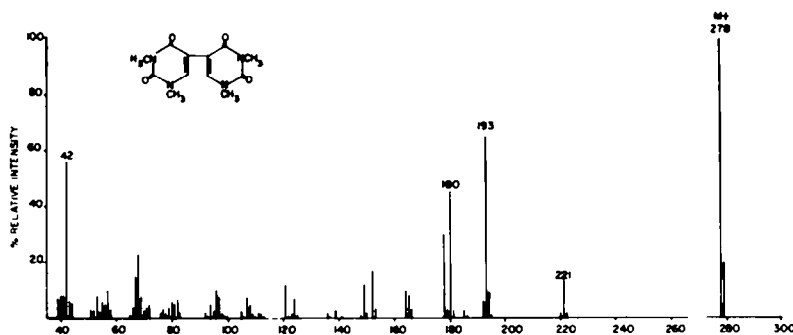
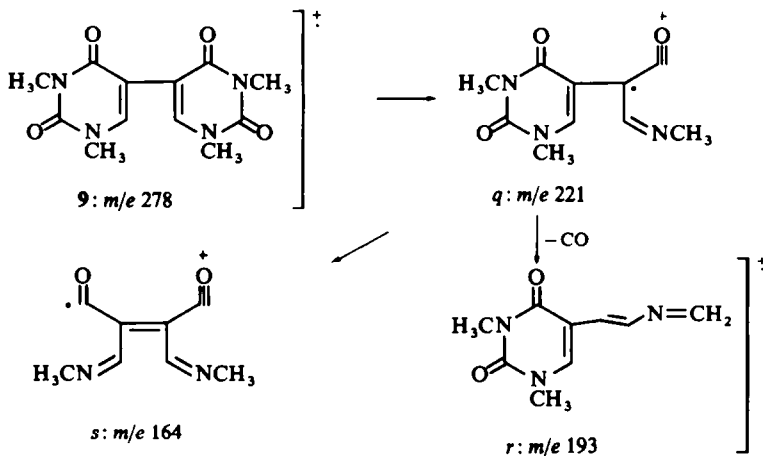


Fig. 9 Mass Spectrum of 5,5'-Di-1,3-Dimethyluracil (9).

( $9 \rightarrow r$ ) lead to abundant ions of mass 193. The  $CH_3NCH$  ion of mass 42 is prominent. Variations of this pattern include the loss of two  $CH_3NCO$  fragments, presumably one from each ring (formally, s). The generation of monocyclic ions is minimal compared to the fragmentation of the cyclobutyl dimers.

Abundant ions formed by the loss of 98 a.m.u. from the molecular ion and by the subsequent loss of an additional 28 mass units (M-98-28) reflect fragmentation characteristic of this kind of coupled product.



The considerations outlined here are intended to assist in distinguishing thymine dimers joined through a cyclobutane ring from bimolecular adducts derived from either an oxetane or an azetidine precursor. They should facilitate identification by fragmentation pattern of substituted thymine compounds and thymine-substituted pyrimidone rings. These correlations are presently being applied to analysis of several unknown photopolymers which appear to incorporate some of these features.

### EXPERIMENTAL

Low resolution mass spectra reported for compounds 1-5 were measured on a Varian Atlas CH-4 instrument with source temp at 250° and probe temp between 40° and 280° as required. The spectra reported for compounds 6-9 were measured on Hitachi RMU 6 instruments, with inlet and source temp between 190° and 250°, at the lowest temp at which the sample is sufficiently volatile. Complete high resolution mass spectra of compound 6 and compound 7 were obtained on the CEC 110 mass spectrometer at the Purdue Mass Spectrometry Center, and individual exact mass measurements on other compounds were made on a Varian Atlas SM1-B mass spectrometer.

The mass spectra of this family of compounds are quite sensitive to temp effects, pyrolysis and/or enhanced decomposition due to additional thermal energy. This is primarily reflected in the size of the molecular ion. The spectra of compounds 1-9 have been measured at minimum temp on several instruments and, with the notable exception of the molecular ion, the peaks discussed herein as characteristic of a given dimer remain prominent in the various spectra of that dimer. Molecular ions for compounds 1-5 were greatly reduced or absent<sup>12</sup> at the temp required to obtain spectra on RMU 6 instruments in two different laboratories and occurred more favorably in spectra obtained on the CH-4 instrument. If the application of mass spectrometry to structure elucidation of pyrimidine polymers is contemplated, the optimal instrumental conditions might be established using the commonly occurring thymine dimer 1. This compound is extremely sensitive to variations in the extent of heating required by different inlet systems, and has been reported<sup>5, 12</sup> twice previously to produce spectra with no peaks above  $m/e$  127.

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