Tetrahedron. Vol. 26, pp 5923 to 5927 Pergamon Press 1970. Printed in Great Britain

FIELD IONIZATION MASS SPECTRA OF PHOTOPOLYMERS OF THYMINE

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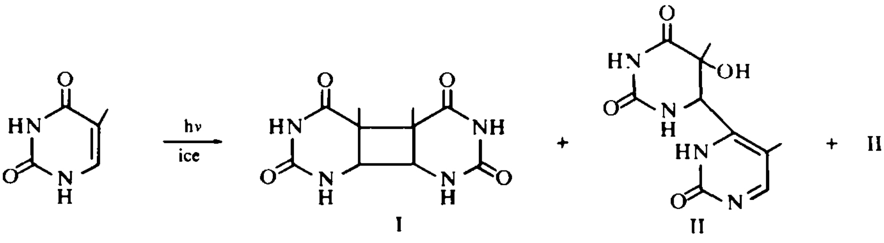
(*Received in the USA* 10 *July* 1970; *Received in the UK for publication* 28 *July* 1970)

Abstract—The field ionization mass spectra of *cis–syn* thymine dimer, thymine-thymine adduct and a new thymine phototrimer have been measured. Results are presented to illustrate that this technique is more efficacious than the electron impact method in structural elucidation of photopolymers of thymine and related compounds.

Spectroscopic studies of the structures of the polymeric photoproducts of DNA,[1] uracil[2] and thymine[3, 4] have been reported with increasing frequency in recent literature. It is hoped that information obtained from such studies will eventually shed light on the chemical basis of UV-induced mutations and also on the molecular mechanism by which irradiation-damaged DNA is enzymatically repaired. Mass spectrometry should be a major technique in such structural elucidations because of its sensitivity and its capability to analyze compounds in non-crystalline form or even in mixtures. However, at least four papers[4–7] report unfruitful attempts to obtain molecular ions of more than 0–5% relative intensity for such photoproducts using electron impact techniques.

Efforts to find suitable conditions for mass spectral analysis of this family of polymers led to the measurement of spectra on several different electron impact instruments[5] and also on an instrument with a field ionization source. This paper describes the successful use of field ionization spectra to identify mol wts and structures of *cis–syn* thymine dimer (T=T) and thymine-thymine adduct (T—T adduct) and also the analysis of a new compound, thymine trimer. These products, as shown in Scheme 1, are formed by the irradiation (254 nm) of thymine in frozen aqueous solution. T=T (I) is the major product, the structural assignment of which was supported by studies of topochemistry,[8] NMR,[9] chemical degradation[6] and X-ray crystallography.[10] T—T adduct (II) had been structurally and stereochemically



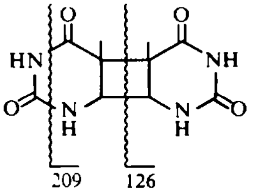
Scheme 1

elucidated by spectroscopic measurements,[11–12] including mass spectrometry and X-ray crystallography. Thymine trimer (III) has recently been isolated in crystalline form[13] and its structural assignment[14] has been made with the aid of mass spectral analysis.

FIG 1. Field Ionization Mass Spectrum of Thymine Dimer I

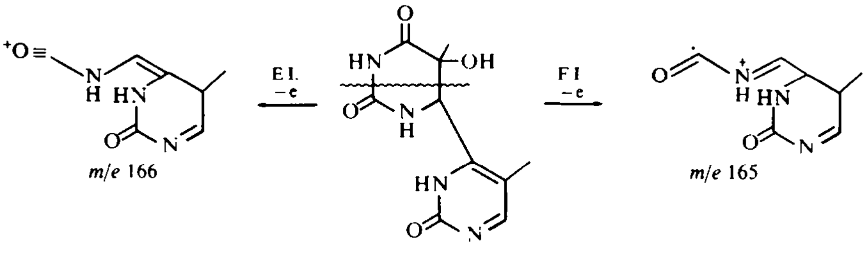
The complete field ionization mass spectrum of T=T is shown in Fig 1. The molecular ion peak at *m/e* 252 is small. However, the peak at *m/e* 253, corresponding to M + 1, has a relative intensity of 15 % and is clearly prominent in the high mass range. The occurrence of large M + 1 peaks in field ionization spectra is common, and is thought to result from intermolecular reactions in material condensed on the blade or filament used to generate the field. The assignment of a peak as M or M + 1 should not be difficult if the class of compounds being analyzed is known, and if the rest of the fragmentation pattern is considered. In the case of a completely unknown sample, a high resolution spectrum or accurate mass measurement will quickly distinguish an odd-electron molecular ion from an even-electron, protonated species.

Peaks at *m/e* 209 and *m/e* 126 correspond to M-43 ions and fragment monomers, respectively, and their presence supports the assigned structure. The latter peak, in particular, is characteristic of cyclobutane linkages and is completely absent in the field ionization spectrum of the T—T adduct. The peak at *m/e* 127 is quite small, although it occurs as the base peak in the electron impact spectrum.[5]



SCHEME 2

Fig 2 is the field ionization spectrum of the T—T adduct, (II). It exhibits the usual simplification, relative to the electron impact spectrum,[5] which reflects the short residence time of ions in the field ionization source and the concomitant reduction in rearrangements and secondary fragmentation processes. Again the M and M + 1 peaks are much more prominent in the field ionization spectrum than in the electron impact spectrum. The distinctive features in both spectra are the peaks at *m/e* 234 and *m/e* 165. The mass 234 ions are formed by loss of water, which may be partially a thermal process. The mass 165 ions are formed by the loss of $C_3H_5NO_2$, 87 mass units, from the molecular ion. In the electron impact fragmentation, formation of the mass 165 ions appears to be a two-step process, involving cleavage of the ring first (Scheme 3) with back transfer of a hydrogen atom to the charge-retaining moiety, and loss in a second step of an H atom from the mass 166 ions. At low electron voltage only the 166 peak remains. The field ionization fragmentation may well be a one-step process, since no peak is observed at *m/e* 166, and H atom transfers are known to be less facile in field ionization fragmentation.



SCHEME 3

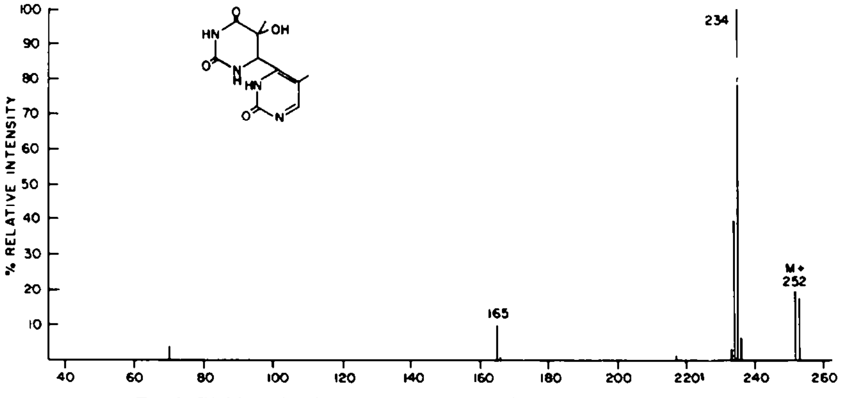


FIG 2. Field Ionization Mass Spectrum of Thymine Adduct II

Both electron impact and field ionization spectra were measured on the new photoproduct (III). The field ionization spectrum proved more valuable for assignment of the structure of this compound for two reasons. First the molecular ion peak is much higher and is unambiguous in the spectrum. Secondly, the suppression of secondary fragmentation greatly reduces not only the number of ions formed, but also the number of modes of formation of a given ion. Thus, more weight can be placed on the structural significance of even the smaller peaks. A pertinent example is the peak at *m/e* 126 whose relative intensity is 90–100% in the electron impact and field ionization spectra of the thymine dimer. This peak is small, but still present in the electron impact spectrum of the adduct.[5] It is completely absent in the field ionization spectrum of the adduct, and thus becomes characteristic of the dimer linkage.

The field ionization spectrum of compound III is shown in Fig 3. The molecular ion peak is judged to be at *m/e* 378, in agreement with the observed occurrence of M + 1 peaks under the conditions required to obtain field ionization spectra of this class of compounds, and the reasonable mass differences between 378 and the next few lower ions. Thus this compound is a trimer of thymine.

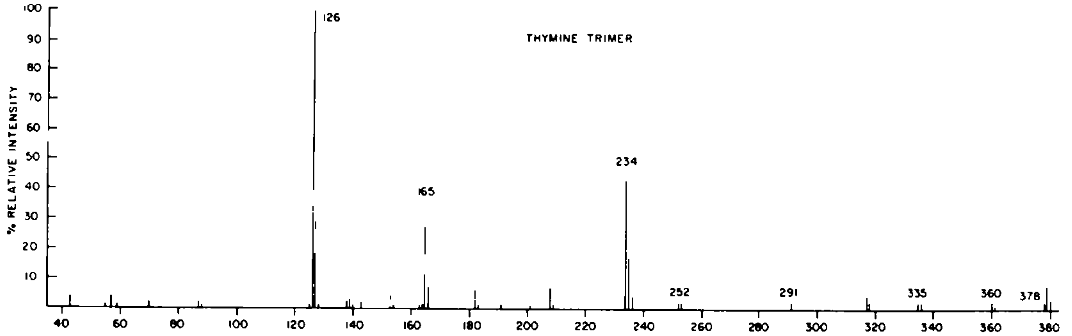
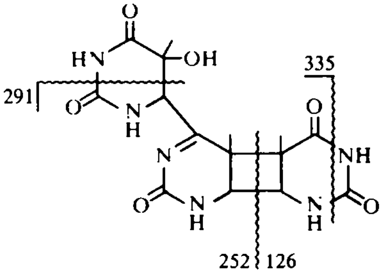


FIG 3. Field Ionization Mass Spectrum of Thymine Trimer III

The base peak in the spectrum at *m/e* 126 corresponds to thymine monomer fragment ions. Such facile formation of the monomer is characteristic of the cyclobutane linkage. The small M-43 peak at *m/e* 335 is also characteristic of this linkage. Scission of the cyclobutane ring with charge retention on the M-126 ion produces species whose subsequent fragmentation is similar to that of the adduct (II). All the peaks in the field ionization spectrum of the adduct are present below *m/e* 253 in the spectrum of the trimer. Fragmentation from the molecular ion of the trimer which is characteristic of the adduct linkage includes the loss of water (producing ions of mass 260) and the loss of 87 mass units (generating ions of mass 291). This latter fragmentation confirms the attachment of three oxygen atoms to one ring, and suggests that this tri-oxygenated ring is not also linked to the third ring.



III

SCHEME 4

This study of the mass spectrum indicates that compound III is a trimer with one ring linked to two others. One of the linkages is like that in the adduct II and the other is a cyclobutane linkage like that of dimer I. Although it is not possible to determine the stereochemistry of the cyclobutane structure by mass spectrometry, this linkage is represented as *cis–syn*. This assignment was made by analogy to that proven in T=T which was isolated when the trimer was treated with base or acid.

To prove a structure, mass spectrometry must be used in conjunction with other techniques. The NMR spectrum of the trimer indicates that an additional molecule of water is present across the C=N bond of the center ring of structure III. It is also known that this saturated compound yields III by dehydration on an ion-exchange column or by boiling in aqueous solution. Thus, vaporization at elevated temperatures in the inlet system of the mass spectrometer also may be expected to effect dehydration to give III. This kind of thermal reaction can be viewed as a limitation of mass spectral analysis; or if fully understood, it can offer further confirmation of a labile structure.

In conclusion, field ionization mass spectrometry appears to be more efficacious than electron impact mass spectrometry in analyzing structures of photopolymers of thymine and related compounds. Molecular ions or M + 1 ions are more abundant, and fragmentation ions are formed more selectively.

EXPERIMENTAL

Mass spectra were obtained on a Varian-MAT SM1-B double focusing mass spectrometer fitted with a Beckey wire field ionization source, using 13 kV accelerating voltage, source temperature at 185°, and probe temps at 220° for I, 195° for II and 240° for III.

Acknowledgement—Financial support was provided in part by AEC contract AT(30-1)-2798, PHS research career development award K3-GM-4134 from NIGMS, USPHS Grant FR-4378, NIH program grant GM-16492, and NSF grant GB-7866 (to Hopkins); and NSF grant GP-6979 (to Arizona State University). This publication is identified as NYO-2798-50.

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