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Study on Fragmentation Process of Thymidine by Mattauch-Herzog Geometry High Resolution Mass Spectrograph

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High resolution mass spectra of thymidine were studied by Mattauch-Herzog geometry mass spectrograph. The element map of it was presented and the fragmentation processes were shown by solid or dotted lines on the map. The spectra were measured with higher accelerating voltages (about 0.1%) in order to obtain many metastable peaks on the photographic plate. By a single focusing mass spectrometer ten metastable peaks were measured and "intentionally defocused" high resolution spectra gave twenty five diffused peaks with slight shifts to the high mass region though no metastable peaks were observed under normal operating conditions.

Recently many remarkable improvements have been made on organic chemical applications of high resolution mass spectrometry.[1,2] It is also a well-developed technique at the present time to use metastable peaks as keys to the determination of the fragmentation process. Various reports have been published on their measurements and assignments.[3-8] In this paper, the author studied on slightly "defocused" high resolution mass spectrum of thymidine with Mattauch-Herzog geometry mass spectrograph so that the "metastable peaks" could be observed on the photographic plate at the same time with the "normal peaks." Barber and his coworkers[8] proposed a method to observe metastable transitions which occur in the field free region between the source and the electrostatic analyser by increasing the accelerating voltage, V, so that the daughter ions are permitted to pass through the analyser. In the studies of thymidine described here, the increment of V was restricted to only about 0.1%. This low increment permits all of the normal ions to be still in focus on the photographic plate together with metastable peaks.

Experimental

Reagent grade thymidine, $C_{10}H_{14}N_2O_5$, was used without further purification. Measurements were carried out with Hitachi RMU-6D 90° magnetic sector type single focusing mass spectrometer and CEC 110 Mattauch-Herzog geometry double focusing mass spectrograph. Sample introduction were made through direct inlet systems. The single focusing type was used in order to compare the results with those obtained double focusing. Ion source temperature was set at 230°C and ionization voltage at 70 eV in both cases.

By the single focusing measurement, a mass spectrum from 1 to 250 *m/e* was recorded within 1 min using an electron multiplier and a Honeywell visicorder.

In double focusing measurement, about 50 μg sample was introduced with the marker (PFK) according to the normal operating condition (accelerating voltage 8 kV), another two exposures were made successively on the same photographic plate with slight increases of accelerating voltage, +9.6 V (condition I), and +16.8 V (condition II) respectively. Accurate masses were determined by Datex comparator connected to the densitometer, and the center of the diffused peak was measured on the paper recorded spectra. Whole results were presented in Biemann's element map system.[9-11]

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TABLE 1. METASTABLE PEAKS AND THEIR ORIGINS

CEC	Hitachi		Corresponding fissions
	Obsd	Cald	
24.3		24.1	$C_3H_5O_2^+ \xrightarrow{} C_2H_2O^+$
25.3		25.1	$C_2H_5^+ \xrightarrow{-H_2} C_2H_3^+$
26.3*	26.1	26.0	$C_4H_7O \xrightarrow{-CO} C_3H_7$
27.3		27.0	$C_2H_5^+ \xrightarrow{-H} C_2H_4^+$
28.1*	27.8	27.7	$C_3H_5O_2^+ \xrightarrow{-CO} C_2H_5O^+$
	32.9	32.8	$C_5H_7O_2^+ \xrightarrow{-C_2H_2O} C_3H_5O^+$
34.3		34.0	$C_5H_7O_2^+ \xrightarrow{-C_2HO} C_3H_6O^+$
35.4		25.1	$C_4H_5NO^+ \xrightarrow{-CHO} C_3H_4N^+$
36.3		36.0	$C_5H_7N^+ \xrightarrow{-C_2H_3} C_3H_4N^+$
36.7*	36.5	36.5	$C_4H_5NO^+ \xrightarrow{-CO} C_3H_5N^+$
37.4		37.4	$C_4H_6NO^+ \xrightarrow{-CO} C_3H_6N^+$
38.4		38.0	$C_3H_4^+ \xrightarrow{-H} C_3H_3^+$
39.4		39.1	$C_3H_7^+ \xrightarrow{-H_2} C_3H_5^+$
40.4		40.0	$C_3H_6^+ \xrightarrow{-H} C_3H_5^+$
41 1		41 1	$C_2H_5O^+ \xrightarrow{-H_2} C_2H_3O^+$
42.4		42.1	$C_2H_4O \xrightarrow{-H} C_2H_3O^+$
	45.5	45.6	$C_5H_9O_3^+ \xrightarrow{C_2H_4O} C_3H_5O_2^+$
48.4*	48.2	48.2	$C_5H_7O_2^+ \xrightarrow{-CH_2O} C_4H_5O^+$
49.4		49.1	$C_5H_5O_2^+ \xrightarrow{-CO} C_4H_5O^+$
50.4		50.0	$C_5H_6O_2 \xrightarrow{-CO} C_4H_6O^+$
51.4*	51.0	50.9	$C_5H_7O_2^+ \xrightarrow{-CO} C_4H_7O^+$
53.4		53.4	$C_5H_6N_2O_2^+ \xrightarrow{-CH_2NO} C_4H_4NO^+$
54.4		53.8	$C_5H_7O_2^+ \xrightarrow{-C_2H_2} C_3H_5O_2^+$
	54.8	54.6	$C_5H_6N_2O_2^+ \xrightarrow{-HNCO} C_4H_5NO^+$
56.5		56.3	$C_4H_7O_2^+ \xrightarrow{-OH} C_4H_6O^+$
	66.3	66.3	$C_5H_7O_2^+ \xrightarrow{-H_2O} C_5H_5O^+$
79.5		79.1	$C_7H_9N_2O_2^+ \xrightarrow{-HNCO} C_6H_8NO^+$
84.6*	83.8	83.9	$C_3H_9O_3^+ \xrightarrow{-H_2O} C_5H_7O_2^+$
96.6		96.0	$C_5H_6O_2^+ \xrightarrow{-H} C_5H_5O_2^+$

Results and Discussion

Low Resolution Spectra. Ten metastable peaks were obtained and completely assigned by use of N. Mancuso's computer program by considerations of the products of the intensities of the parent and daughter peaks and their elemental compostions.7) Fragmentation processes are shown in Table 1 and also in Fig. 1 by solid lines. Assuming that neutral fragments are expelled as one piece, the daughter ions are derived easily without any drastic rearrangement from the thymidine structure, so the investigation of a metastable peak gives very important informations on the structure

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12) J. A. McClosky, Ph. D. Thesis, Massachusetts Institute of Technology, Cambridge (1963).

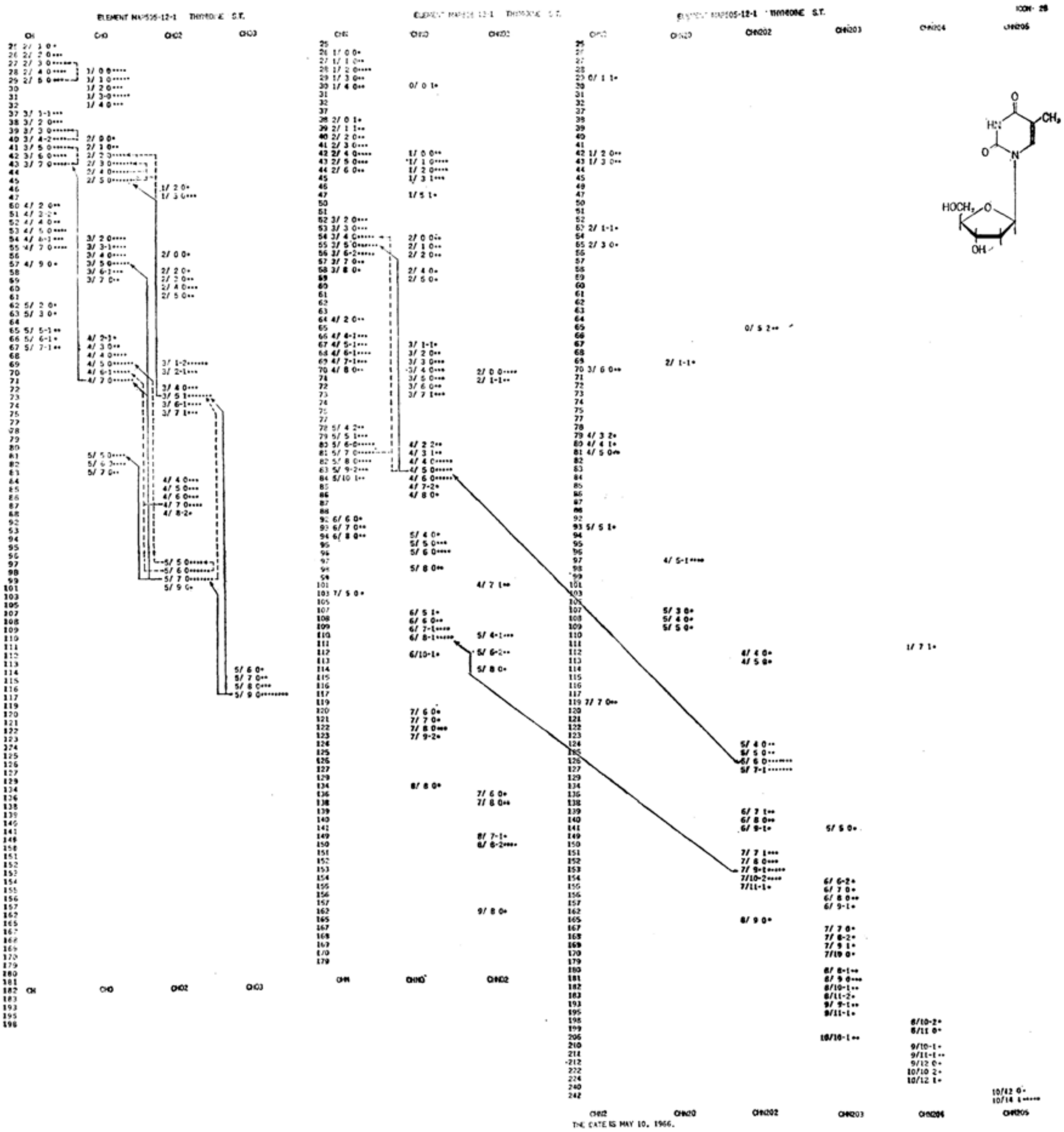

Fig. 1. Element map of thymidine.

of the sample.

Some of these fragmentation processes, for example, from $C_5H_6N_2O_2^+$ to $C_4H_5NO^+$, are well agreed with the previous reports[12,13] on some thymidine type compounds and make it clear that thymidine decomposes in the same manner as thymine.

Mattauch-Herzog Geometry High Resolution Spectra. No metastable peaks were observed under normal measuring conditions, indicating that the thymidine fragmentation is in a sense different from 1-*n*-butylthymine which shows many metastable peaks under usual operation.[7] In the case of thymidine no decomposition occurs in the field free region between electric and magnetic sector. If the fissions are supposed to proceed near the ion source, the daughter ions should be able to reach the detector by increasing the accelerating voltage to $V=m_1 \cdot V_0/m_2$,[4,5] where m_1 and m_2 are the masses of the parent and daughter ions respectively and V_0 is the usual accelerating voltage. Many diffused peaks were observed by "intentionally defocused" measurements (condition I and II). In the case of thymidine some fissions are thought to occur not only in the ionization chamber but also in the electric sector, probably

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[13] J. M. Rice, G. O. Dudek and M. Barber, *J. Am. Chem. Soc.*, **87**, 4569 (1965).

near the exit slit, because the increase of accelerating voltage, 9.6 or 16.8 V, is too small to carry the daughter ions through the electric sector to the detector. In Table 1, fissions of thymidine are listed in terms of the metastable peaks under condition I with corresponding reaction formulas. Under condition II, normal ion path is deflected more than under condition I, and then relative intensities of metastable peaks increase, accompanied with larger shifts to the high mass direction. It has been known that the diffused peaks of some hydrocarbons shift to the high mass region when the accelerating voltage is increased.[14,15] Then it follows that the relative positions of the metastable peaks on the photographic plate must depend upon the regions where the fissions occur. The amount of the displacement of the m^* position differs from peak to peak. This is reasonable bacause it is considered that the shift depends upon the position where the decomposition occurs, the energy loss at the moment of the fission and also upon the mass to charge ratios of the parent and daughter ion. Portions of the spectra measured under condition I and II are shown in Fig. 2 (a) and (b) respectively.

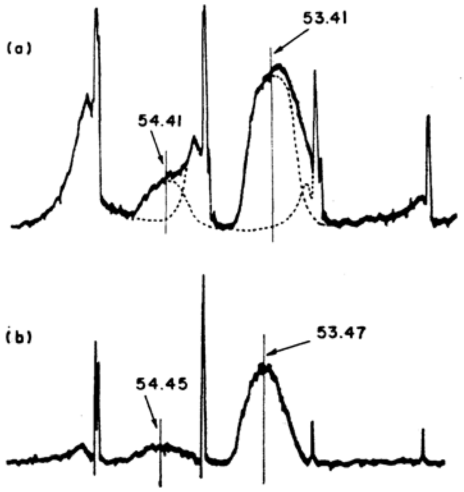

Fig. 2. Metastable peaks in the intentionally defocused thymidine spectra; (a) condition I, 8 kV 9.6 V. (b) condition II, 8 kV 16.8 V. The peaks in (a) show "tailing" on the high mas sides by the "defocusing."

Under the considerations of these facts, the metastable peak assignment was carried out assuming that the theoretical value must have smaller mass to charge ratio, and the limit of the range is 0.7 mass unit in high and 0.4 mass unit in low mass region. The metastable peaks marked with $*$ are those which are measured by single focusing instrument. Among ten reactions measured by single focusing, six are observed also by double focusing. It is worth to notice there are many metastable transitions which are observed only under these "intentionally defocused" conditions and not by the single focusing instrument. Besides the structural data, this method would supply valuable informations on chemical kinetics.

Element Map of Thymidine and Its Fragmentation Process. Figure 1 shows the element map of thymidine. A solid line shows the fission determined by the metastable peak measurement with Hitachi instrument and a dotted line that by intensionally defocused Mattauch-Herzog experiment under condition I.

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14) Unpublished results by N. Mancuso and the author.

15) Private communications from JEOL mass spectroscopy reaserch group.