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Note

A differential scanning calorimetric–thin-layer chromatographic study of 5-halo-2′-deoxynucleosides

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Thermal analysis techniques have been used for a number of years in the field of materials characterization. Consequently differential scanning calorimetry (DSC) has provided a universal tool for ascertaining and predicting the effect of preparation and thermal history on the physical properties of polymers. However, when DSC is applied to the study of non-polymeric compounds, the situation may become more complex due to the possible occurrence of chemical as well as physical changes under thermal stress, and require the utilization of auxiliary techniques. Thus our studies involving the thermal stabilities of the components of nucleic acids, necessitated the development of methods for analyzing the products resulting from thermal analysis (DSC) of these compounds.

Precise and accurate methods for the analysis of nucleosides and their thermolytic products are of interest to researchers in the field of prebiotic synthesis as well as many in biology, biochemistry and medicine.

Cleavage of the N-glycosidic bond which binds the bases of deoxyribonucleic acids (DNA) to deoxyribose may cause mutations[1] and inactivation of DNA[2]. Greer and Zamenhof[3] reported that vegetative cells (*Escherichia coli*) and spores (*Bacillus subtilis*) when heated *in vacuo* in the dry state are highly subject to mutations. Recently, the thermolytic cleavage of such bonds in deoxynucleosides has been investigated by DSC[4,5]. These studies indicated that the stability of this bond is proportional to the electron density localized in that region. Our interest in 5-halo-2′-deoxyuridines is not merely due to the influence of the halo substituent on the glycosidic bond but also due to the possibility that, since most of these compounds can be substitutively incorporated for thymidine in DNA[6], thermal depyrimidination may be caused to take preference over the normal course —depurination.

Thermal analysis of a series of 5-halo-2′-deoxyuridines, and 5-halouracils provided thermograms which, while characteristic of a particular halogen substituent, show some surprising differences when compared with one another. It is the objective of this study to identify the processes associated with each of the enthalpic stages along the thermal cycle.

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EXPERIMENTAL

The 5-halo-2'-deoxyuridines, and the corresponding 5-substituted uracils used in this experiment were of the highest purity commercially available from ICN, Nutritional Biochemicals and Calbiochem. All calorimetric measurements were obtained on a Perkin Elmer DSC-1B Differential Scanning Calorimeter. With an instrument of this type, both the transition temperatures and the transition energies are obtained simultaneously with the aid of an Infotronix CRS-110. The calibration of the DSC instrument was carried out in the usual manner. All experiments were performed with samples in the weight range of 0.3–0.6 mg at a scan rate of 20°K/min and range 2.

Thin-layer chromatographic (TLC) separations were carried out on commercially available plates (MN silica gel S-HR/U_{254}) using the solvent system 1: chloroform–methanol–water (4:2:1) and system 2: ethyl acetate–isopropanol–water (75:16:9)[7]. Samples of each of the 5-halo-2'-deoxyuridines, and the corresponding 5-substituted uracils were examined in this manner and exhibited the behavior of a single component. Preparative TLC separations were subsequently made on commercially available plates (silica gel F_{254}, layer thickness 0.25 mm, EM reagents).

Samples of the 5-substituted deoxyuridines and the parent compound, as obtained commercially, were individually encapulated and heated under nitrogen at a scan rate of 20°K/min and a range of 2 mcal/sec (full scale). The heating cycle was stopped immediately after the first peak was recorded, and the sample quickly removed and cooled to its original temperature. Using a fresh sample, the temperature was programmed until the second peak was recorded. Once again the sample was removed. This procedure was repeated until samples were obtained to cover each stage of the progressive thermal cycle.

Each of the aluminum pans was then carefully opened, placed in a small vial and then treated with a drop or two of the same solvent system as would be used in the development of the TLC plates. A small amount of each sample (unheated) was dissolved in the same solvent to serve as a TLC reference.

RESULTS AND DISCUSSION

The thermograms for each of the compounds are shown in Figs. 1 or 2 while the R_F values for the TLC chromatograms covering various stages of the thermal cycle are listed in Table I. The thermogram for the parent structure, 2'-deoxyuridine (Fig. 1) reveals an initial endotherm (438°K), a weak exotherm (506°K) followed by another endotherm (604°K). The first endotherm is associated with fusion of 2'-deoxyuridine. No decomposition accompanies this process as indicated by TLC data (Table I) obtained from a DSC sample heated through this portion of the thermal cycle. However the exotherm at 506°K is associated with cleavage of the glycosidic bond since TLC indicates uracil in samples quenched after heating just through the exotherm. Previous studies[4] have indicated that furfuryl alcohol and water are also products of this thermolysis reaction. The presence of the alcohol is not evident in TLC data however since it is lost through evaporation at the cleavage temperature which lies above the boiling point of furfuryl alcohol (443°K)[8]. The second endothermic peak at 604°K results from the fusion of the thermolysis product uracil as confirmed by the literature value (608°K)[8] and comparative TLC data (Table I).

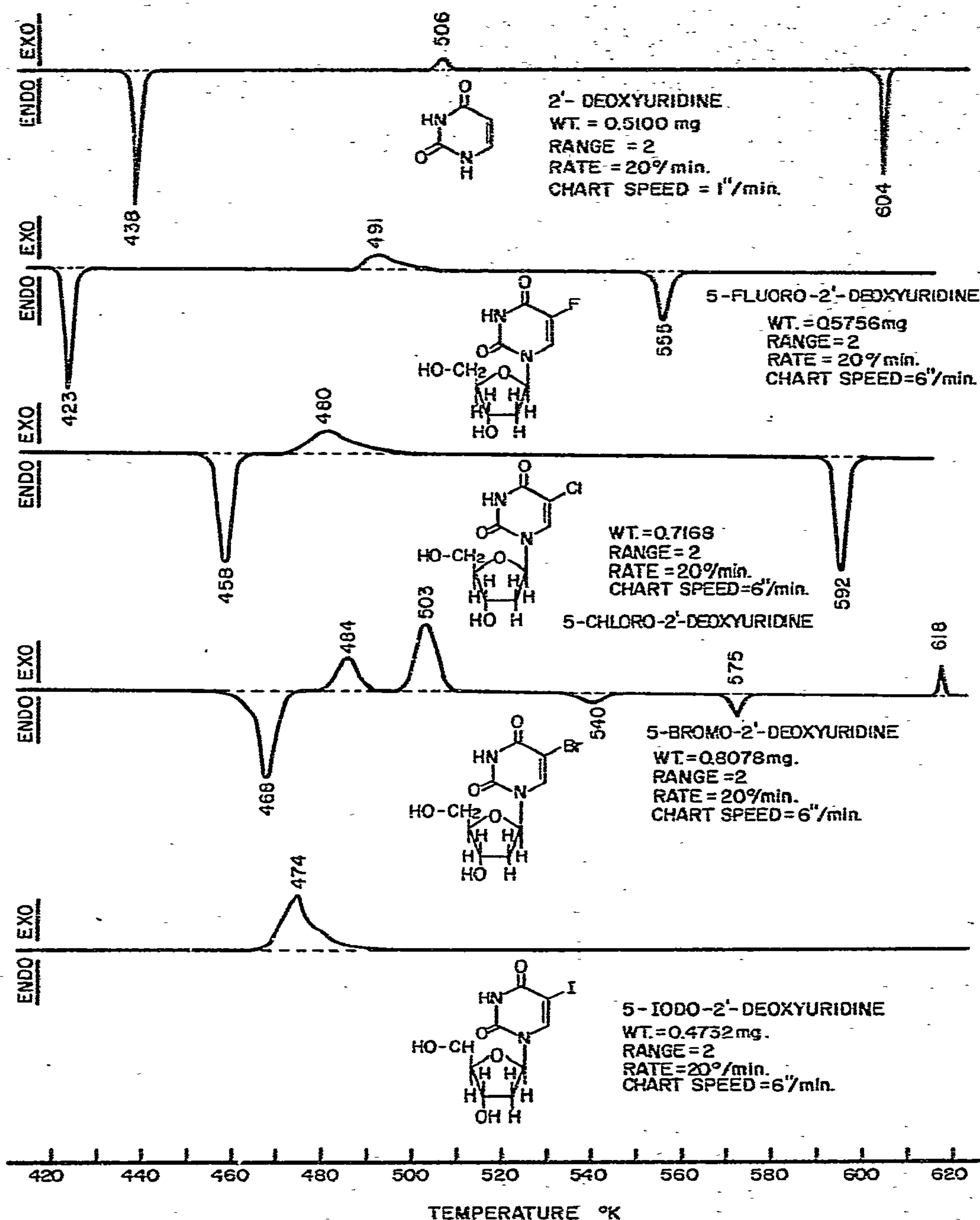

Fig. 1. Thermograms of 5-halo-2'-deoxyuridines over the temperature range 420–620 °K.

5-Fluoro-2'-deoxyuridine, and 5-chloro-2'-deoxyuridine behave in a similar manner to the parent compound under thermal stress (Fig. 1). The thermal product of 5-fluoro-2'-deoxyuridine is 5-fluorouracil as confirmed by TLC and also by com-

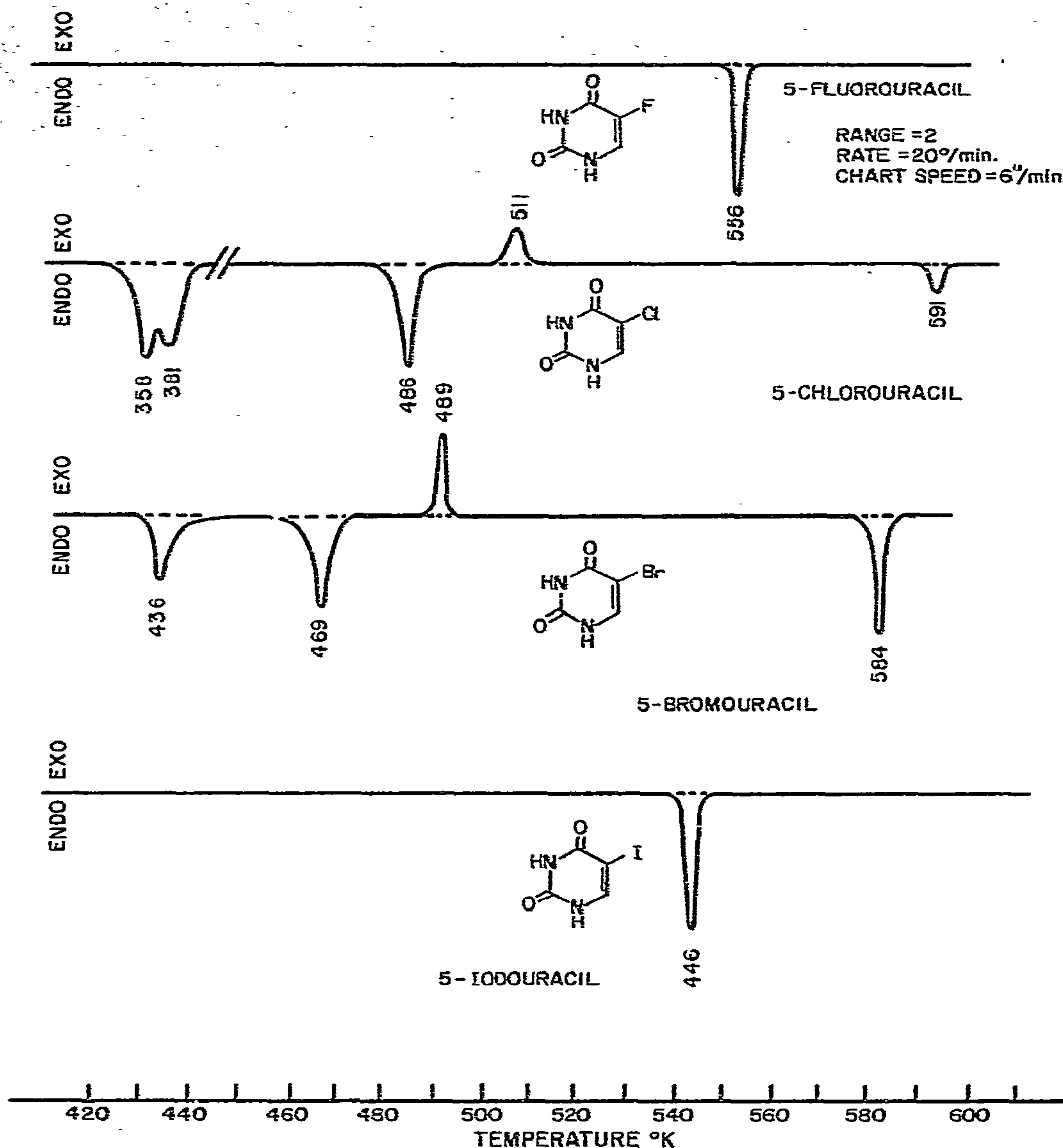

Fig. 2. Thermograms of 5-halouracils over the temperature range 420–600 °K.

parison with the thermogram obtained from a sample of 5-fluorouracil (Fig. 2). Similarly the thermolysis product from 5-chloro-2'-deoxyuridine is 5-chlorouracil as established by TLC (Table I). Comparison of the corresponding DSC data (Fig. 1) with that obtained from a commercial sample of 5-chlorouracil (Fig. 2) did show similarity in the exotherm at 591 °K, the fusion temperature of the base.[8] However it was apparent via DSC (broad endotherm at 486 °K and exotherm at 511 °K) and TLC (Table I) that the commercial sample was highly contaminated with 2'-deoxyuridine.

The thermograms for 5-bromo-2'-deoxyuridine and 5-iodo-2'-deoxyuridine are

TABLE I

R_F VALUES OF 5-HALO-2'-DEOXYNUCLEOSIDES

Compound	DSC temperature (°K)	R_F value	
		System 1	System 2
5-Fluoro-2'-deoxyuridine	300	0.79	0.63
	433	0.79	0.63
	503	0.76	0.71
	563	0.76	0.71
5-Fluorouracil	300	0.76	0.71
5-Chloro-2'-deoxyuridine	300	0.72	0.67
	468	0.70	0.74
	488	0.70	0.74
	603	0.70	0.74
5-Chlorouracil	300	0.70	0.74
			0.50
			0.40
5-Bromo-2'-deoxyuridine	300	0.78	0.76
	473	0.76	0.76
			0.82
	488	0.76	0.82
	513	0.76	0.82
	553	0.76	0.82
			0.50
	583	0.75	0.82
			0.50
5-Bromouracil	300	0.76	0.82
			0.50
Uracil	300	0.73	0.50
5-Iodo-2'-deoxyuridine	300	0.76	0.74
	500	0.73	0.50
5-Iodouracil	300	0.69	0.78
Uracil	300	0.73	0.50
2'-Deoxyuridine	300	0.80	0.40
	440	0.80	0.40
	508	0.75	0.50
	610	0.75	0.50

very different from those obtained for the previous cases. Three endotherms and three exotherms were obtained for 5-bromo-2'-deoxyuridine (Fig. 1). The first broad endotherm was due to 5-bromo-2'-deoxyuridine contaminated with 2'-deoxyuridine. The exotherm at 484°K results from glycosidic cleavage of 5-bromo-2'-deoxyuridine yielding 5-bromouracil as indicated by TLC (Table I). At 503°K glycosidic cleavage of the 2'-deoxyuridine impurity results in the second exotherm and formation of uracil is confirmed by TLC (Table I). The endotherms at 540° and 575°K are fusion endotherms for impure mixtures of 5-bromouracil and uracil, respectively. Data from TLC indicates that debromination of 5-bromouracil is responsible for the exotherm at 618°K since only uracil remains beyond that temperature.

The commercial sample of 5-bromouracil utilized as a reference, though yielding only one spot when subjected to TLC on MN silica gel S-HR/U_{254}, provides a thermogram which indicates the presence of 5-bromo-2'-deoxyuridine as an impurity,

due to the fusion endotherm at 469°K and the exothermic glycosidic cleavage peak at 489°. The peak at 436°K is attributable to release of water of crystallization. Consequently purification of such a commercial sample of 5-bromouracil would only require heating at 489°K at which temperature any 5-bromodeoxyuridine impurity would be converted to 5-bromouracil. Subsequent preparative TLC treatment of the commercially available sample on silica gel F-254 with ethyl acetate–isopropanol–water (75:16:9) did reveal three components.

5-Iodo-2′-deoxyuridine provides a very simple thermal pattern (Fig. 1) exhibiting only one large exotherm at 474°K. The absence of an endotherm prior to this temperature indicates that glycosidic cleavage occurs prior to fusion. In this case however TLC indicates that 5-iodouracil is not liberated as would have been predicted from the thermal reactions of the other 5-halo-2′-deoxyuridines. The liberation of iodine along with other thermal products was apparent by a positive starch iodide test. Furthermore, TLC data indicates an R_F value equivalent to that of uracil. The absence of a fusion endotherm at 604°K provides an anomaly which might better be accounted for in terms of a dimer of uracil having an R_F value equivalent to that of uracil.

CONCLUSIONS

One of the difficulties in examining the influence of thermal stress on biologically active compounds via DSC, has centered on the need for establishing the nature of the physical and/or chemical changes associated with the enthalpimetric transitions. Consequently this has tended to limit the study of thermal reactions via DSC to those substances for which the products have or can be separated on a macro scale. The ability to analyse DSC samples directly via TLC, following various segments of the heating cycle, removes this limitation.

The applicability of this technique has been demonstrated by an examination of the effects of heat on 5-halo-2′-deoxyuridines, and has shown that glycosidic cleavage occurs in each case.

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