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THERMAL ANALYSIS OF 5-HALO-2'-DEOXYNUCLEOSIDES

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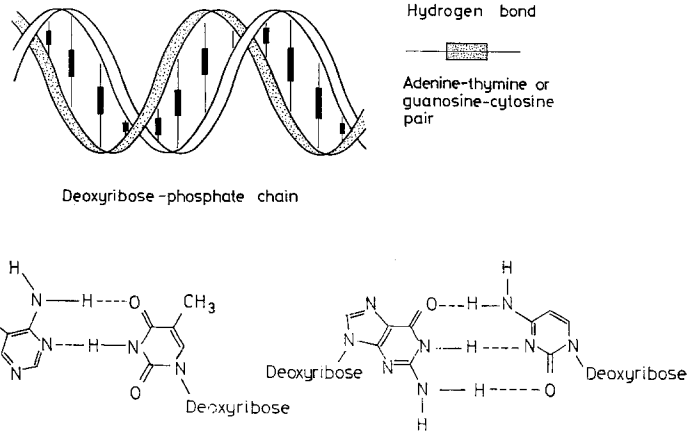
Heat may be a mutagenic agent which could be more important to evolution than are the ordinary errors in DNA replication. Therefore, it is necessary to know the stability of heredity determinants to thermal energy. Consequently, a thermal analysis, via differential scanning calorimetry (DSC), has been undertaken, using model compounds (crystalline 2'-deoxyribonucleosides and their 5-halo derivatives) to provide information regarding: 1. bond free energies maintaining the fibrous structure in nucleic acids e.g. H-bonding, π-complexing and dipole induced dipole interaction. 2. susceptibility to thermal degradation e.g. thermolytic cleavage of the glycosidic bond and deamination of the bases.

Interpretation of the thermal curves has been facilitated and enhanced by utilization of a DSC-TLC analytical technique. Based on these data, a mechanism for the thermolysis of the glycosidic bond in the melt has been considered.

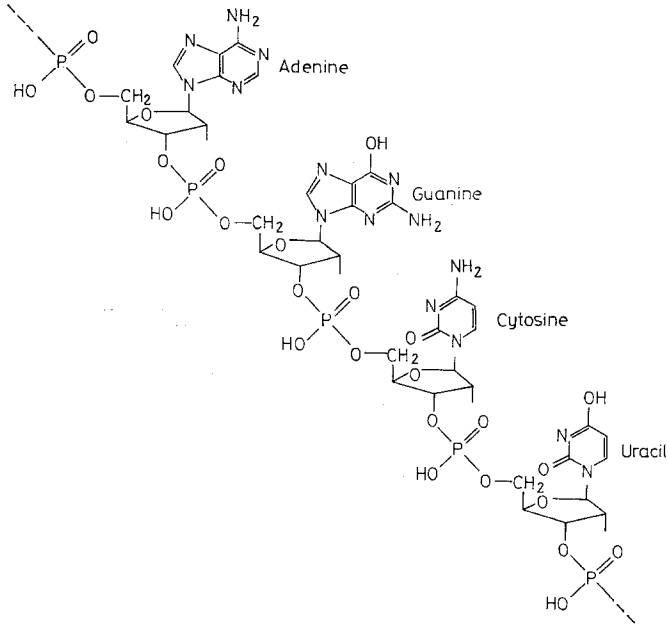
Life must involve the ability of living things to reproduce themselves. According to the Watson–Crick hypothesis, the required genetic code is contained in DNA (deoxyribonucleic acid) located in the cell nucleus. The DNA code molecule is a long chain defined by the sequence in which four nucleotide bases appear along a backbone of ribose and phosphate. The key to both the use and duplica-



tion of the code contained in the sequence of a DNA molecule, lie in the concept of complementary pairing of nucleotide bases by hydrogen bonding. Among the four DNA bases, the only pairs that form strong hydrogen bonds are adenine-thymine and guanine-cytosine, one pyrimidine and one purine base in each pair.

In view of the importance of base pairing to the functioning of DNA, the integrity of the *N*-glycosyl bonds that bind the individual bases to the DNA polymer chain, as well as the maintenance of the character of the base itself, are essential to continued identity of the code.

The individual heredity determinants of the same cell have, however, different stabilities to heat. Some are modified by prolonged heating, resulting in mutagenesis. Thus heat may be a mutagenic agent which is more important to evolution than are the ordinary errors in DNA replication. Consequently, it is important to measure the relative stability of heredity determinants to thermal energy. The present study was therefore designed to provide information regarding:



A section of deoxyribonucleic acid (DNA)

1. bond free energies maintaining the fibrous structure in nucleic acids,
2. thermolysis of the glycosidic bond,
3. substituent effects on glycosidic cleavage,
4. identification of impurities in nucleosides and the corresponding bases,
5. a mechanism for thermolyzed depurination,
6. thermolyzed deamination.

2'-Deoxynucleosides, the sub-components of DNA were used as models in determining the above characteristics.

Differential enthalpic analysis provides an especially suitable method for directly measuring the magnitude of forces maintaining the crystal lattice and also for monitoring the onset of chemical changes associated with the thermal process. Accordingly, the selected crytalline 2'-deoxynucleosides were subjected to examination by DSC and the magnitude of the energies associated with physical and chemical changes was determined when these models were subjected to a programmed input of thermal energy.

Bond-free energies maintaining crystalline nucleosides. Interaction between bases, both in crystals and in fibers, of nucleic acids is divided into two main types [1,2]: horizontal (hydrogen bonds) and vertical (stacking interactions). These attractive forces play an important role in the architecture of nucleic acids and determine many physical and chemical properties of these macromolecules. Thus the biological activity of nucleic acids is closely dependent on characteristic short range forces, hydrogen bonding, π-complexing and dipole induced dipole-dipole interactions which stabilize the helical structure of DNA. These forces are reflected in the stacking and bond-free energies between various purine and pyrimidine base pairs in DNA and determine in part the melting behavior of that polymer [3,4]. Therefore a quantitative and qualitative understanding of the melting behavior and thermal properties of the constituent nucleosides will provide a measure of the interaction present in DNA. In the case of crystalline 2'-deoxynucleosides, chosen as models for the evaluation of intermolecular forces in DNA, the following values were obtained when these molecules were subjected to differential scanning calorimetric analysis (Table 1) [5].

The low enthalpy value obtained for 2-deoxyguanosine dihydrate (5.8 kcal/mole) may be rationalized in terms of available crystal data [6] which indicate that this compound has the unique characteristic of existing in the syn form. This conformation can only be stabilized by a possible intramolecular hydrogen bond between the $N_{(3)}$ of the guanine moiety and the 0(5') of the sugar. None of the other possible hydrogen bond donors or acceptors appear to contribute to the overall stabilization of the lattice. Thus it appears that most of the hydrogen bonding sites are involved in complexation of water molecules within the lattice, necessitating that the lattice be held together by different weak forces (e.g. dipole-induced dipole and π complexation), a factor which accounts for the low enthalpic value obtained.

A low enthalpic value cannot however, be categorically attributed to all hydrated 2'-deoxynucleosides, since 2'-deoxyadenosine monohydrate shows the highest value (8.3 kcal/mole) indicating an alteration in intermolecular forces. An interesting feature of the latter system is that packing of the molecules in the crystal is determined by hydrogen bonds, with all available groups participating and the resulting chains of NH—N bonds between the bases being related by a screw axis and a distorted trigonal arrangement of HO---H bonds with water molecules [7].

Table 1

Heats of fusion and corresponding enthalpies obtained for nucleosides at a heating rate of 20/min

Nucleoside	Fusion endotherm T_m (maxima)	ΔH, kcal/mole
2'-deoxyuridine	438	6.7
2'-deoxycytidine · HCl	457	5.6
2'-deoxyadenosine · H_2O	462	8.3
2'-deoxyguanosine · $2H_2O$	469	5.8
Thymidine	464	7.0
2'-deoxyinosine	483	7.6
2'-deoxycytidine	486	5.0

The enthalpic values obtained for 2'-deoxyinosine, thymidine and 2'-deoxyuridine (7.6, 7.0 and 6.7 kcal/mole respectively) indicate that the base stacking forces decrease in order:

2'-deoxyinosine > thymidine > 2'-deoxyuridine [8]

The greater stacking force of the purine, 2'-deoxyinosine is attributable to increased π delocalization of the purines in comparison with pyrimidines and hence to the greater availability of electrons. In the case of thymidine, the introduction of a methyl group to the pyrimidine ring increases the electron density of the ring due to a positive inductive effect, thereby increasing the π bonding between infinite stacks of partially overlapping base.

Thermolysis of the glycosidic bond. Two types of structural changes take place when neutral DNA is subjected to increasing temperature. In the region of the helix coil transition temperature denaturation of the macromolecular structure of DNA results in separation of the two strands [9]. This occurs when the input of thermal energy is sufficient to overcome the weak intermolecular forces. However, a slower heat induced degradation of the primary structure also occurs. This is probably due in large extent to depurination of DNA [10, 11] and/or

Table 2

Temperature maxima and minima values associated with differential enthalpic analysis of 2'-deoxynucleosides

2'-Deoxynucleosides	Fusion, K	Glycosidic cleavage	Fusion of base
2'-deoxyguanosine	469	483	513 (sublimes)
2'-deoxyadenosine	462	496	618 (sublimes)
2'-deoxycytidine	486	499	—
2'-deoxyuridine	438	506	604
thymidine	464	528	588

formation of chain breaks [12] and crosslinks [13] that result subsequent to glycosidic cleavage.

The curves obtained from differential scanning calorimetric analysis of 2'-dexynucleosides exhibit in addition to the fusion endotherm, a second endotherm (Fig. 1) which lies approximately at the fusion temperature of the corresponding base (Fig. 2). Furthermore a weak exotherm or endotherm is generally noted between these two fusion endotherms and is attributed to glycosidic cleavage of the base (Table 2) [14]. The relative ease of thermolytic cleavage of 2'-deoxynucleosides and the identity of the resulting degradation products

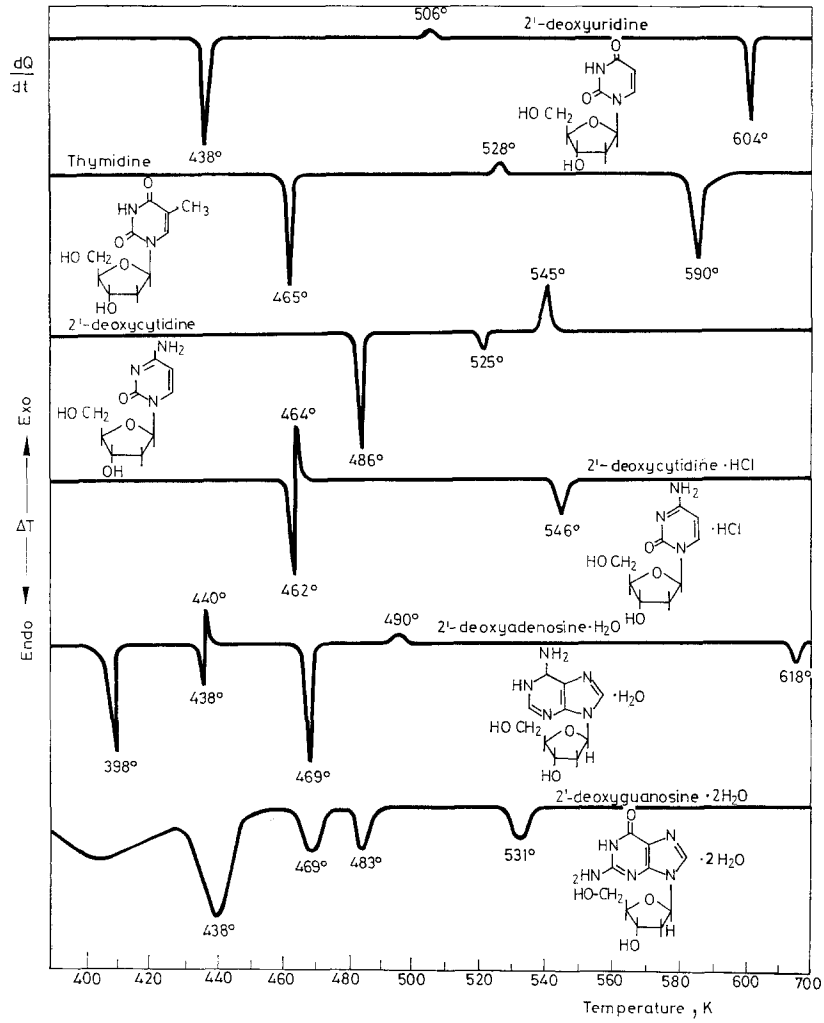


Fig. 1. DSC curves for 2'-deoxyribonucleosides over the temperature range 390–620 K

were determined by heating the nucleoside at its melting point in a sublimator under reduced pressure. The crystalline sublimate, identified by Mass, UV, IR and PMR spectra as well as DSC, was the corresponding base from the 2'-deoxynucleoside, indicating that thermolytic cleavage had occurred at the glycosidic bond.

The other products of the reaction were obtained by heating the nucleoside above its melting point at atmospheric pressure. A liquid distillate and solid residue were obtained. The PMR spectrum and the VPC retention time of the distillate were found identical to a known mixture of furfurylalcohol and water.

It may therefore be concluded that deoxynucleosides exhibit a weak exothermic or endothermic process following fusion, indicative of base cleavage. The tem-

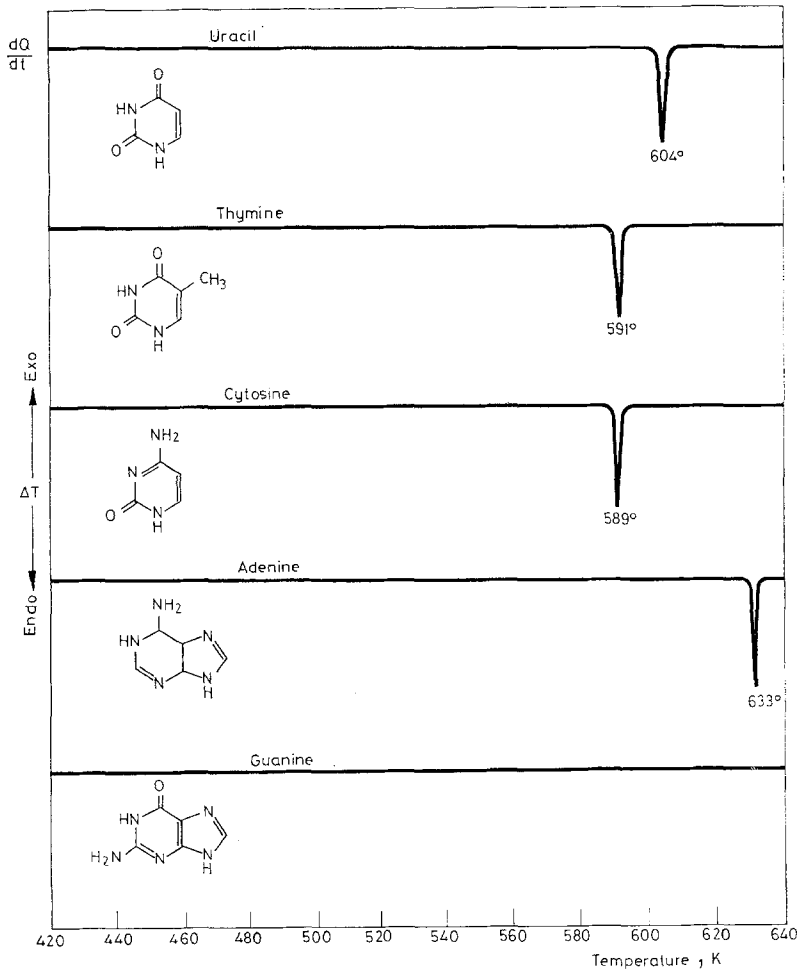


Fig. 2. DSC curves for nucleic acid bases over the temperature range 420—640 K

perature associated with these peaks reflects the relative ease with which glycosidic cleavage may be accomplished and provides the following order of susceptibility to thermolysis:

2'-deoxyguanosine > 2'-deoxyadenosine > 2'-deoxycytidine > 2'-deoxyuridine thymidine

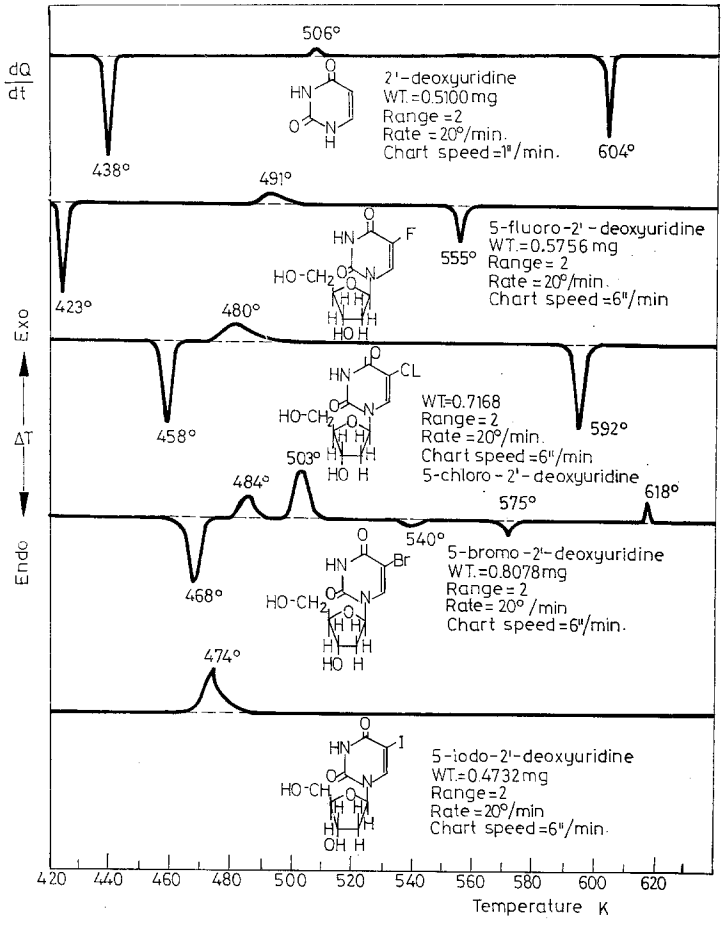


Fig. 3. DSC curves for 5-halo-2'-deoxyuridines over the temperature range 420—620 K

Thus the data from DSC studies indicate that pyrimidines as well as purines can be released from deoxynucleosides. The relative ease of depurination is in agreement with biological findings.

Zamenhof [15] reported that vegetative cells (Escherichia coli) and spores (Bacillus subtilis) when heated *in vacuo* (155° for 15 min) in the dry state, were highly subject to mutations. Under these conditions of mutagenesis, DNA

was found to liberate 1.5 molecules of purine within an area of 500 nucleotide pairs (possible size of a functional unit). High thermal mutability was attributed to the number of possibilities for biological base replacement following removal of a purine. Depurination, which appeared to occur throughout the entire DNA

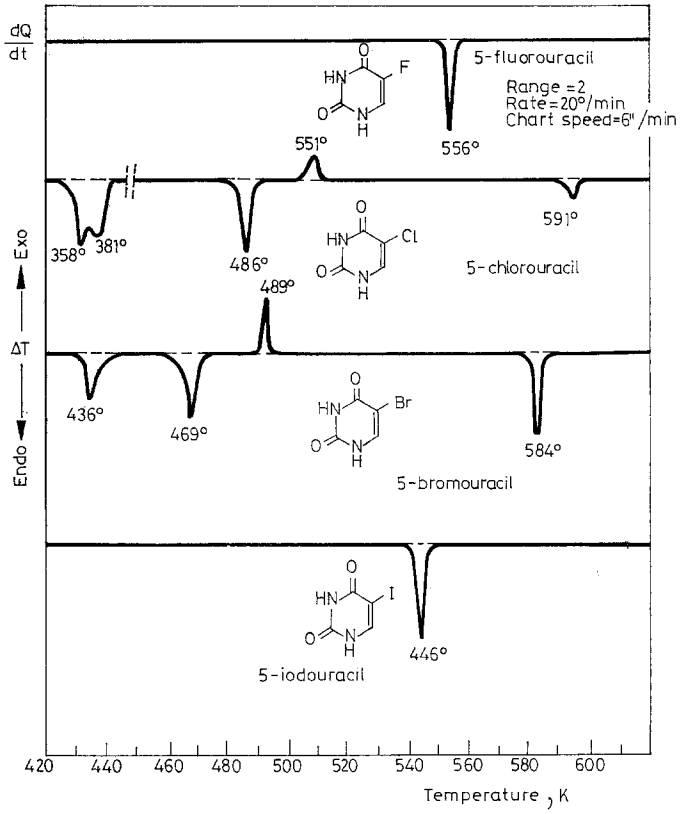


Fig. 4. DSC curves for 5-halouracils over the temperature range 420—620 K

molecule under most conditions, resulted in a slightly greater liberation of guanine than adenine. Furthermore, the specificity for liberation of purines but not pyrimidines indicated that the N-glycosidic bonds in polynucleotides, as in deoxynucleosides, are weaker when they involve purines. Consequently it is apparent that pyrimidine glycosidic bonds in DNA are too strong to be released under the conditions (155° for 15 min) utilized to induce mutations in vegetative cells *in vacuo* in the dry state. Therefore, if pyrimidines are to be removed competitively with purines, it would be necessary to reduce the strength of the glycosidic bond associated with pyrimidines by the presence of an appropriate substituent on the base.

Substituent effects on glycosidic cleavage. Our previous studies on the thermolytic cleavage of 2'-deoxynucleosides (Table 2) showed that 2'-deoxyuridine is more susceptible to this reaction than is thymidine, [14] indicating that the positive inductive effect of the 5-methyl substituent has a stabilizing influence on the glycosidic bond. Thus it would be expected that electron withdrawing groups such as the halogens would probably provide the desired effect.

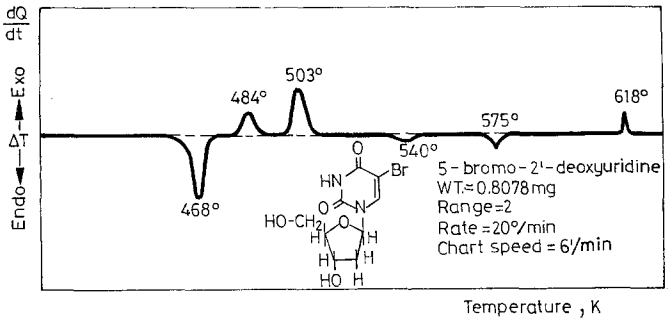


Fig. 5. DSC curve for 5-bromo-2'-deoxyuridine over the temperature range 390—620 K

In this study, 5-halodeoxyuridines were chosen for examination, not merely because of the presence of an electron withdrawing group on the pyrimidine ring but also because 5-bromo-2'-deoxyuridine can be biologically incorporated for thymidine in DNA [16]. This series was therefore investigated to determine the relative ease of glycosidic cleavage with the ultimate aim of providing a means for removing pyrimidines from DNA in preference to purines, and at a sufficiently low temperature to minimize killing effects in the process. Thermal analysis of a series of 5-halo-2'-deoxyuridines and 5-halouracils provided curves which, while characteristic of a particular halo substituent, show some surprising differences when compared with each other e.g. 5-bromo-2'-deoxyuridine and 5-iodo-2'-deoxyuridine *vs.* 2'-deoxyuridine and 5-chloro-2'-deoxyuridine (Fig. 3) or 5-chlorouracil and 5-bromouracil *vs.* 5-fluorouracil and 5-iodouracil (Fig. 4). These comparisons stress one of the difficulties in examining the influence of thermal stress on biologically active compounds via DSC, namely 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. To remedy this situation we resorted to the use of a DSC-TLC combination.

Samples of 5-halo-2'-deoxyuridines and the parent compound, 2'-deoxyuridine, were individually encapsulated and heated under nitrogen at a scan rate of 20°/min and a range of 2 mcal/sec full scale. The heating cycle was stopped immediately after the first cycle 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 thermal cycle [17]. The DSC-TLC analysis of 5-bromo-2'-deoxyuridine and 5-iodo-2'-deoxyuridine exemplify the effectiveness of this technique.

In the curve of 5-bromo-2'-deoxyuridine (Fig. 5) the first broad endotherm was due to the fusion of 5-bromo-2'-deoxyuridine contaminated with 2'-deoxyuridine. The exotherm at 484 K resulted from glycosidic cleavage of 5-bromo-2'-deoxyuridine yielding 5-bromouracil as indicated by TLC. At 503 K glycosidic cleavage of the 2'-deoxyuridine impurity results in the second exotherm and formation of uracil as confirmed by TLC. The endotherms at 540 and 575 K resulted from fusion endotherms for impure mixtures of 5-bromouracil and uracil respectively. Data from TLC (Table 3) indicate that debromination of 5-bromouracil was responsible for the exotherm at 618 K since only uracil remains beyond that temperature.

Table 3

R_F values for samples of 5-bromo-2'-deoxyuridine following programmed heating to various temperatures

Compound	Temperature, K	System 1, R_F	System 2, R_F
5-Bromo-2'-deoxyuridine	300	0.78	0.76
5-Bromo-2'-deoxyuridine	473	0.76	0.76
5-Bromo-2'-deoxyuridine			0.82
5-Bromo-2'-deoxyuridine	488	0.76	0.82
5-Bromo-2'-deoxyuridine	513	0.76	0.82
			0.50
5-Bromo-2'-deoxyuridine	553	0.76	0.82
			0.50
5-Bromo-2'-deoxyuridine	583	0.75	0.82
			0.50
5-Bromouracil	300	0.76	0.82
			0.50
Uracil	300	0.73	0.50

System 1—chloroform—methanol—water V/V 4 : 2 : 1
System 2—ethyl acetate—isopropanol—water V/V 75 : 16 : 9
Commercially available plates — MN Silica Gel S-HR/U_{254}

5-Iodo-2'-deoxyuridine provided a very simple thermal pattern (Fig. 6) 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 data (Table 4) indicated that 5-iodouracil was not liberated as would have been predicted from the thermal reactions of other 5-halo-2'-deoxyuridines. The liberation of iodine along with other products was apparent

by a positive starch-iodide test. Furthermore, TLC data indicated an R_F value equivalent to that of uracil. Absence of a fusion endotherm at 604 K (Fig. 2) yielded 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. This possibility is being subjected to further investigation.

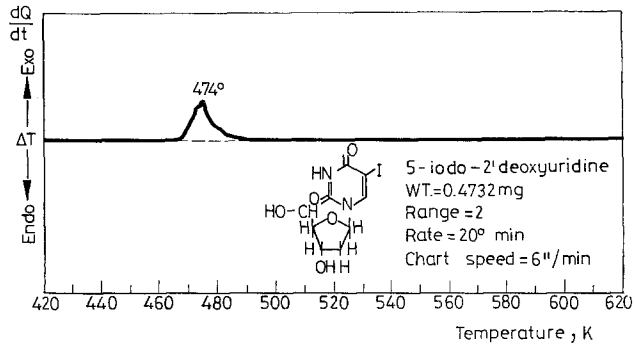


Fig. 6. DSC curve for 5-iodo-2-deoxyuridine over the temperature range 420—620 K

Table 4

R_F values for samples of 5-iodo-2'-deoxyuridine following programmed heating to various temperatures

Compound	Temperature, K	System 1, R_F	System 2, R_F
5-Iodo-2'-deoxyuridine	300	0.76	0.74
5-Iodo-2'-deoxyuridine	500	0.73	0.50
5-Iodouracil	300	0.69	0.78
Uracil	300	0.73	0.50

System 1—chloroform—methanol—water V/V 4 : 2 : 1
System 2—ethyl acetate—isopropanol—water V/V 75 : 16 : 9
Commercially available plates — MN Silica Gel S-HR/U_{254}

Identification of impurities in nucleosides and the corresponding bases. The need for purity of compounds used in biochemical studies is well recognized and it is for this reason that commercially available deoxynucleosides have been used in the present study. Investigations utilizing the DSC-TLC combination prove their value in rapid identification of contaminants. Thus an examination of the curve (Fig. 5) and TLC data (Table 3) for commercial 5-bromo-2'-deoxyuridine readily reveals the presence and nature of impurities whereas in the NMR spectrum the presence of these impurities is not as obvious. Similarly the curve of 5-bromouracil (Fig. 7) reveals 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 K. The peak at 436 K is attributable to release of water of crystallization. Consequently, purification of such a sample of 5-bromouracil would require heating at 489 K at which temperature any 5-bromo-2'-deoxyuridine impurity would be converted to 5-bromouracil.

A mechanism for thermolyzed depurination. Discussion to this point has shown that in pure crystalline deoxynucleosides, a weak exothermic or endothermic process following fusion is indicative of base cleavage. The temperature associated with these peaks reflects the relative ease with which glycosidic cleavage may be accomplished and provides the following order of susceptibility of thermolysis:

2'-deoxyguanosine > 2'-deoxyadenosine > 2'-deoxycytidine >
2'-deoxyuridine > thymidine

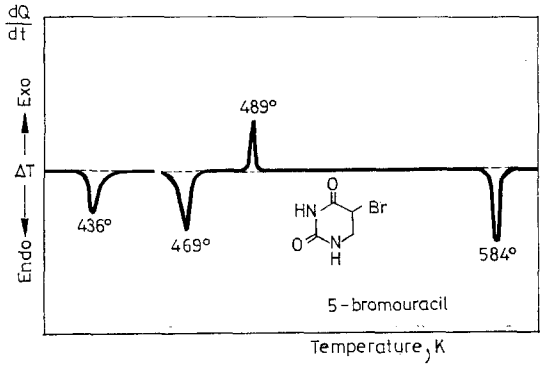
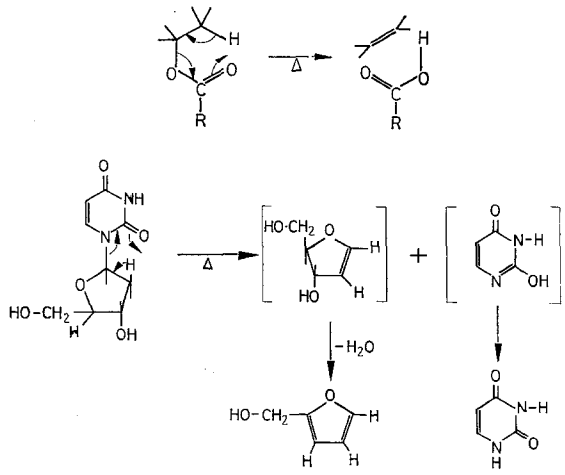


Fig. 7. DSC curve for 5-bromouracil over the temperature range 400 – 600 K

The simple mechanistic concept of thermolytic cleavage would involve homolytic cleavage followed by abstraction of a hydrogen from the 2' position of the furanose ring. Such a process would be independent of the relative position of the hydrogen atom with respect to the original position of the base. Therefore such an abstraction from 2'-deoxyuridine would occur twice as readily as from uridine. However experimental data involving sublimation of the resulting base indicated that thermolysis of uridine occurred much slower than would be accounted for on the basis of a free radical mechanism, and suggested that the stereochemistry of the nucleoside must be involved.

Thermal depyrimidination and depurination, as previously discussed, is accompanied by formation of furfuryl alcohol and water. The presence of these products suggests the possibility that in the fused state and in the absence of specific reagents, glycosidic cleavage is occurring via a pyrolytic E_i elimination resembling that of the ester pyrolysis or Chugaev reactions [18]. A comparable E_i elimination involving 2'-deoxyuridine might proceed as follows:



It is important to note at this point that strong base is not required in the Chugaev or ester pyrolysis reactions and similarly would not be required for the proposed (vide infra) mechanism for deoxyribonucleosides. Since the above mechanism involves a cis elimination it was anticipated that thermolytic cleavage of ribonucleosides would occur in a similar manner to that of the 2'-deoxynucleosides.

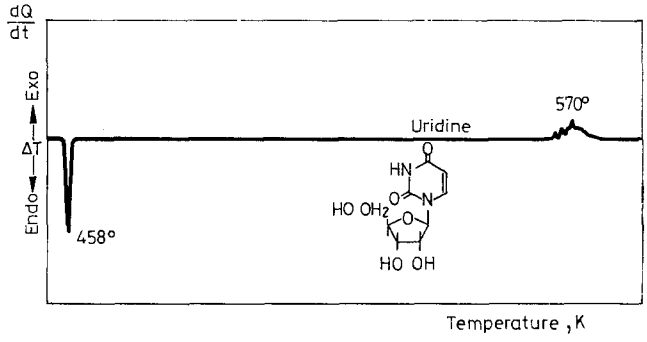
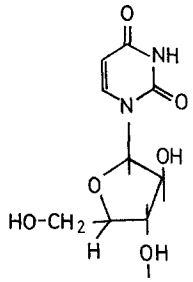


Fig. 8. DSC curve for uridine over the temperature range 440 – 600 K

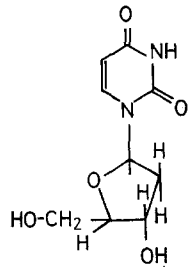
DSC analysis of the ribonucleoside, uridine (Fig. 8) indicated that glycosidic cleavage occurred at 570 K as compared with 506 K for deoxyuridine (Fig. 3). It may be concluded that the presence of the 2'-hydroxyl group decreases the ease of thermal cleavage of the glycosidic bond.

A comparison of thermal data for uridine (Fig. 8) and 2'-deoxyuridine (Fig.1) suggested the possibility that the elimination process favored a trans rather than cis removal of hydrogen. To test this alternative, uracil-β-D-arabinofuranoside was subjected to thermal analysis. This nucleoside, having the base and

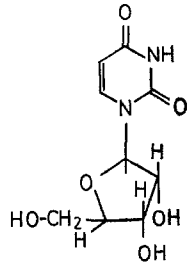
the 2'-hydroxyl group cis to one another, was thermally stable to glycosidic cleavage. Consequently, it may be concluded that cis elimination is essential if thermolytic cleavage is to occur but is rendered more difficult by the presence of a hydroxyl group on the 2'-position bearing the hydrogen. It is improbable that thermolysis involves intermediate formation of a saturated O^6,5-cyclo-2'-deoxyuridine via intramolecular addition of the 5'-hydroxyl to the 5,6 double bond of the deoxynucleoside [19] since it is difficult to account for the associated thermal products – water and furfuryl alcohol. A mechanism proceeding via formation of a pyrimydine intermediate [20] is equally improbable in the absence of a strong base in the fused mixture. Likewise the absence of an acid renders mechanisms involving the opening of the furanose ring unlikely [21].



Uracil - β-D-arabinofuranoside



2'-deoxyuridine



Uridine

If an E_i pyrolytic elimination is involved, it is necessary to account for the retarding influence of a trans hydroxyl group when present on the 2'-position of the furanose ring. Like other elimination mechanisms, the E_i may tend to proceed to an ion pair (bond breaking preceding bond making on the one hand or bond making preceding bond breaking) (Fig. 9). It is probable in the present case that the influence of the hydroxyl group is electronic in nature rather than steric. If this elimination process is proceeding by ion pair formation then the rate would be influenced by electronic factors at other locations on the molecule. Calorimetric data have indicated that 5-halo substituents promote this reaction. To determine if a direct relationship exists between the inductive effect of a meta halo substituent and the ease of glycosidic cleavage, the meta Hammett substituent constant was plotted against the glycosidic cleavage temperature (T_M) for a series of 5-halo-2'-deoxyuridines. A linear relationship was obtained (Fig. 10) [22] indicating the importance of withdrawal of electrons from the N(1) atom. Thus if the bond breaking step involving the glycosidic bond precedes bond formation with the development of an ion pair, then the bonding electrons must move towards the N(1) nitrogen and away from the C(1') carbon of the furanose ring. Consequently, the 2'-hydroxy group would retard the reaction

by inhibiting formation of a positive charge on C(1') and would account for thermal analysis observations (Table 5).

Table 5

T_M temperatures for exotherms associated with glycosidic cleavage in nucleosides

Nucleoside	T_M for glycoside cleavage, K
uridine	570
5-chlorouridine	545
2'-deoxyuridine	506
5-chloro-2'-deoxyuridine	480

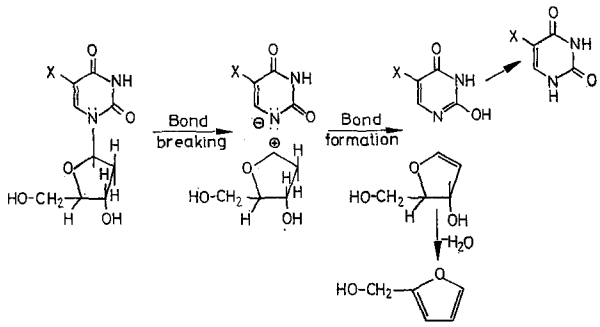


Fig. 9. Possible mechanistic route for thermal glycosidic cleavage of 5-halo-2'-deoxyuridines

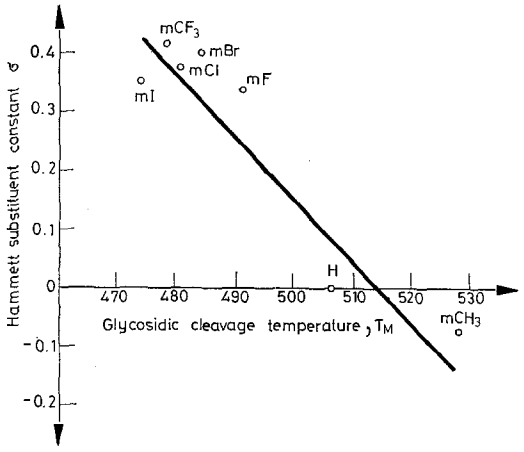


Fig. 10. Relationship between temperature associated with glycosidic cleavage and the Hammett substituent constant

It is important to reiterate at this point that discussion herein has been limited to thermal reactions occurring in melts of nucleosides. Consequently, these processes need not bear any direct relationship to the usually postulated acid and base catalyzed hydrolysis mechanisms. Presently, the conditions, under which a thermal glycosidic cleavage reaction may be duplicated in solution, are under investigation in these laboratories.

Thermolyzed deamination of cytosine

Structural modification, other than, glycosidic cleavage, may occur when melts of naturally occurring 2'-deoxynucleosides are subjected to a programmed heating cycle. Thus the curve for 2'-deoxycytidine (Fig. 1) is very different from that of 2'-deoxyuridine. The first endotherm is associated with fusion at 486 K. The second weak endotherm is followed by an exotherm at 545 K, but no endotherm is obtained at 589 K as noted in the curve for cytosine. The TLC data indicate that fusion occurs at 486 K and is followed by glycosidic cleavage at a second weak endotherm at 526 K with liberation of cytosine. The exotherm at 545 K is associated with partial deamination of cytosine as indicated by the presence of two spots on the TLC plate for sample heated 10 K above the exotherm (Table 6). One of these spots is indistinguishable from the R_F of commercial cytosine and the other from that of uracil.

Table 6

R_F values for samples of 2'-deoxycytidine following programmed heating to various temperatures

	Temperature, K	System 1, R_F	System 2, R_F
2'-Deoxycytidine	300	0.68	0.06
	495	0.68	0.06
	535	0.59	0.09
	555	0.59	0.09
		0.80	0.50
Uracil	300	0.81	0.50

It is interesting to note in connection with this reaction that deamination of cytosine residues rather than depurination has been suggested as the cause of heat mutagenesis, at least in T4 bacteriophage [23]. Calorimetric data indicate however, that in the fused melt cytidine is not converted to uridine since glycosidic cleavage occurs prior to deamination.

Conclusions

The nature of physical and chemical changes associated with enthalpimetric transitions, resulting from the application of thermal stress on deoxynucleosides has been established by the combined utilization of DSC and TLC. This study has been particularly effective in demonstrating the power of this system to differentiate thermal transitions associated with physical processes from those of chemical reactions, such as glycosidic cleavage and the conversion of cytosine to uracil, processes which play an important role in thermal mutagenesis.

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ZUSAMMENFASSUNG — Die Wärme als mutagener Faktor könnte in der Entwicklung eine wichtigere Rolle spielen als die gewöhnlichen Fehler in der DNS-Replikation. Deshalb ist es erforderlich die Stabilität der Erbdeterminanten gegenüber thermischer Energie zu kennen. Von der Differential—Scanning—Kalorimetrie (DSC) an Modellverbindung (kristalline 2'-Desoxyribonukleoside und ihre 5-Haloderivate) wurden Informationen zu folgenden Problemen erwartet:

1. Bindungsfreie Energien, welche die Faserstruktur in Nukleinsären aufrecht erhalten, z. B. H-Bindungen, π-Komplexierung und durch Dipole angeregte Dipol-Dipol Wechselwirkungen.

2. Empfindlichkeit gegenüber thermischem Abbau, z. B. thermolytische Spaltung der Glycosidbindung und Desaminierung der Basen.

Die Deutung der Thermogramme wurde durch Einsatz einer analytischen Technik, bestehend aus DSC und TLC, erleichtert und gefördert. An Hand dieser Daten wurde ein Mechanismus für die Thermolyse der Glycosidbindung in der Schmelze vorgeschlagen.

RÉSUMÉ — La chaleur pourrait être un agent mutagène plus important dans l'évolution que ne le sont les erreurs ordinaires de la réplique à l'ADN. Pour cela, il est nécessaire de connaître la stabilité des déterminants héréditaires vis-à-vis de l'énergie thermique. C'est pourquoi l'étude du traitement thermique a été entreprise par analyse calorimétrique différentielle (DSC) sur des composés modèles (désoxy-2'-ribonucléosides cristallins et leurs dérivés halo-5) afin d'obtenir des renseignements sur:

1. les énergies libres de liaisons qui maintiennent la structure fibreuse dans les acides nucléiques, p. ex. les liaisons hydrogènes, la formation de complexes π et les interactions dipôle-dipôle induites par des dipôles,

2. la susceptibilité vis-à-vis de la dégradation thermique, p. ex. le clivage thermolytique de la liaison glycosidique et la désamination des bases.

L'interprétation des enregistrements DSC a été facilitée et appuyée par l'utilisation combinée de la technique TLC. A partir de ces données, un mécanisme est proposé pour la thermolyse de la liaison glycosidique pendant la fusion.

Резюме — Теплота может быть мутагенным показателем, который должен быть более важным для выделения, чем обычные ошибки в репликации ДНК. Поэтому необходимо знать стабильность наследственных детерминантов к термической энергии. В связи с этим был предпринят термический анализ посредством дифференциальной сканирующей калориметрии (ДСК), используя модельные соединения (кристаллические 2'-дезоксирибонуклеозиды и их 5-галогенпроизводные), чтобы выделить информацию, касающуюся:

1. свободных энергий связи, поддерживающих волокнистую структуру в нуклеиновых кислотах, например: водородной связи, π-комплексообразования и наведенного диполь-дипольного взаимодействия,

2. восприимчивость к термической деградации, как например, термолитическое расщепление гликозидной связи и деаминирование этих оснований.

Интерпретация термограмм была облегчена и усилена использованием комбинированной ДСК—ТСХ аналитической техники. На основе этих данных был рассмотрен механизм темолиза гликозидной связи в расплаве.