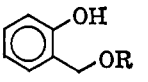
(b) 1,1-d_2-Propanol (0.30 g., 4.8 mmoles) and paraformaldehyde (0.07 g., 2.3 mmoles) were heated under reflux in ethanol-free chloroform (10 ml.) overnight, together with a few small crystals of *p*-toluenesulfonic acid. No attempt was made to azeotropically remove any water formed. The reaction was cooled and washed with sodium carbonate and then sodium chloride solutions. The solvent was removed under reduced pressure, and the crude product bulb-distilled at 50° (25 mm.), furnishing 0.39 g. (59%) of impure acetal. Gas chromatography was used to quantitatively separate acetal XXI (retention time 2.7 min. on 6-ft. 20% SE-30 on Chromosorb P column at 90°) from residual 1,1-d_2-propanol (retention time 1.0 min.). Essentially this same procedure, commencing from purified reactants, was used for all other acetals prepared in this work. Purity was established for each compound by gas chromatographic analysis, the mass spectrum, and especially the highly characteristic n.m.r. spectrum coupled with integrated proton count.



XXIX

As an example of n.m.r. assay, di-*n*-propoxymethane (VIII) had the following spectral data (all chemical shifts are quoted in δ values, TMS = 0): 4.53 (2-proton singlet), 3.40 (4-proton triplet), 1.48 (4-proton multiplet), 0.92 (6-proton triplet). The d_4-analog XXI had resonances at 4.53 (2-proton singlet), 1.50 (4-proton quartet), 0.92 (6-proton triplet). No other absorption was detected. The aromatic acetal XII exhibited peaks at 7.00 (5-proton multiplet), 5.14 (2-proton singlet), 3.95 (1-proton multiplet), 1.15 (6-proton doublet). The corresponding d_6-compound XXV absorbed at 7.00 (5-proton multiplet), 5.15 (2-proton singlet), 3.93 (broad 1-proton singlet). These data, together with infrared spectral evidence, exclude the alternative formula XXIX for the aromatic acetals.

Mass Spectra of Nucleic Acid Derivatives. Pyrimidines

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The mass spectra of pyrimidine, 2-aminopyrimidine, uracil, 6-methyluracil, thymine, 1,3-dimethyluracil, dihydrouracil, dihydrothymine, cytosine, 5-methylcytosine, 5-hydroxymethyluracil, and the corresponding deuterated compounds have been obtained. Molecular ions were observed for all compounds. Fragmentation patterns characteristic of the position and nature of substituents and of the extent of unsaturation of the pyrimidine ring were interpreted in each case with the aid of metastable peaks and deuterium labeling. Interpretations were often facilitated by recording spectra at low electron beam energies in addition to the standard 70 e.v. The mass spectra of these compounds can serve as useful models for determination of the structures of chemically or biologically modified pyrimidines or their nucleosides.

Introduction

Mass spectra of compounds containing the pyrimidine ring were first obtained by Biemann and McCloskey,[3] who studied the naturally occurring nucleosides by time-of-flight mass spectrometry. They observed molecular ion peaks and fragmentation corresponding principally to cleavage of the purine– or pyrimidine ribose bond, with the production of both ribose and purine or pyrimidine ions, and to fragmentation of the ribose moiety of each compound. The potential utility of the double-focusing mass spectrometer for structural studies on chemically modified pyrimidines, including the products of photochemical addition of aromatic hydrocarbons to these compounds,[4] has prompted us to study the mass spectra of a series of substituted pyrimidines, chiefly those of the nucleic acids. The small amount of sample required, the ease with which the spectra are obtained, and the specificity of the fragmentation patterns observed all suggest that the mass spectra of these compounds can serve as useful models with which the spectra of chemically or biologically altered pyrimidines could be compared to assist in structure elucidation.

The observation of peaks due to metastable ion transitions provided proof of the origins of ions produced in many fragmentation processes.[5] These transitions involve the decomposition of an ion of mass m_1 to form another ion of mass m_2 plus a neutral fragment in the field-free region of the spectrometer, and are recorded as broad peaks of low intensity at m/e values m^* given by the relation[6] $m^* = (m_2)^2/m_1$.

The high ionization voltage of 70 e.v. which is commonly used in mass spectrometry is sometimes necessary to obtain reproducible spectra, but this voltage often results in high-energy fragmentation processes which may involve extensive rearrangements. It is generally difficult to write mechanisms for such processes, and they are usually of less interest for structure

(1) Predoctoral Fellow of the National Science Foundation.
(2) Associated Electrical Industries, Ltd., Manchester, England.
(3) K. Biemann and J. A. McCloskey, *J. Am. Chem. Soc.*, **84**, 2005 (1962); K. Biemann, "Mass Spectrometry: Organic Chemical Applications," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, pp. 351–354.
(4) J. M. Rice, *J. Am. Chem. Soc.*, **86**, 1444 (1964).
(5) Ionic fragmentation processes do not always give rise to metastable peaks, however, and failure to observe these peaks does not prove that such reactions do not occur.
(6) J. H. Beynon, "Mass Spectrometry and its Applications to Organic Chemistry," Elsevier Publishing Co., Amsterdam, 1960, p. 252.

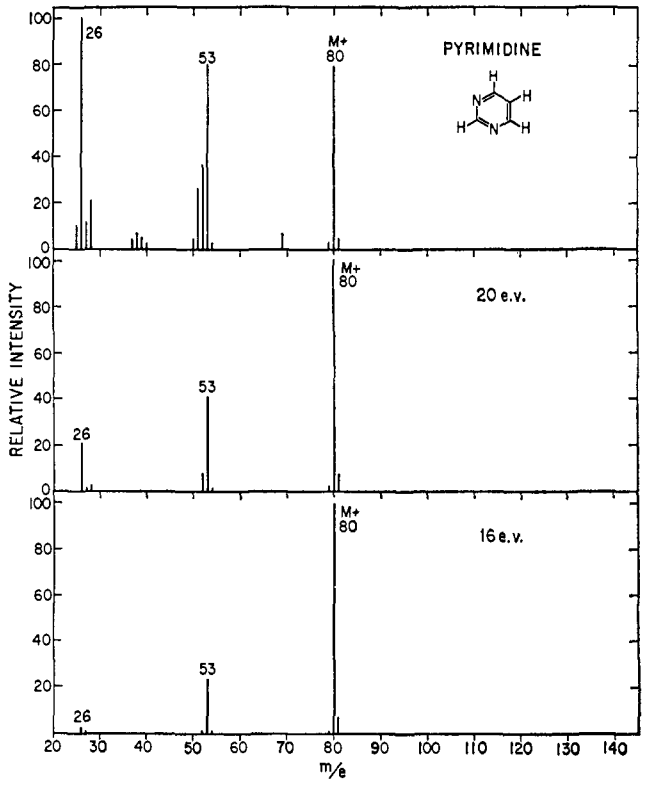

Figure 1. Mass spectra of pyrimidine at 70, 20, and 16 e.v. M^+ denotes the molecular ion peak.[10]

determination than the more easily interpreted low-energy paths, which often (but not always) involve the cleavage of fewer bonds. A substantial simplification of the spectrum can frequently be achieved by lowering the energy of the electron beam from 70 to 20 e.v. or less, thus eliminating the high-energy fragmentation paths.[7] The spectra of conjugated molecules are often perfectly reproducible under these conditions, and we have found this technique quite useful in the present work.

We have chosen to represent the sequence of decomposition reactions which follow ionization by mechanistic schemes intended to illustrate the origins of fragment ions and to rationalize their formation. Since both nitrogen and oxygen have the capacity for increased valency in the ionized state, and since the positive charge is considered to be delocalized over the entire π-bonded framework of an organic ion, the single valence-bond structures with localized charge used in the present study to represent these ions are admittedly distortions of reality. In spite of deficiencies in representation, these mechanisms provide plausible explanations of the data and are generally considered preferable to alternative methods of presentation.[8]

Experimental Section

All compounds were obtained from the California Corporation for Biochemical Research in the highest purity commercially available. Compounds labeled A grade were used as received. 1,3-Dimethyluracil was checked for chromatographic homogeneity; 2-aminopyrimidine, 6-methyluracil, and dihydrouracil were recrystallized from water. Dihydrouracil, which is very susceptible to hydrolysis, was found by infrared spectroscopy (KBr pellet) to be contaminated with β-ureidopropionic acid before and after recrystallization, but no indication of volatilization of this zwitterionic contaminant was observed. Deuteration was achieved by dissolving the compounds in hot D_2O, followed by freezing, lyophilization, and storage in a desiccator over P_2O_5 prior to use. A single treatment yielded a product which contained a substantial amount of partially deuterated compound, but was adequate for our purposes. The spectrum (Figure 7) of deuterated 6-methyluracil is included here as an illustrative example. Replacement of hydrogen by deuterium is presumed to occur exclusively at amino and ring nitrogen atoms, and (with less facility) at the oxygen atom of the hydroxymethyl group in 5-hydroxymethyluracil.

Solid compounds were introduced directly into the ion source of an Associated Electrical Industries MS-9 double-focusing mass spectrometer. Approximately 50–100 μg. of sample was used; heating to the vicinity of 200° was often necessary to generate a sufficiently high vapor pressure. The volatility of these compounds closely parallels their solubility in water; 1,3-dimethyluracil, which is incapable of intermolecular bonding, is both the most soluble and the most volatile compound in this series. It was necessary to warm dihydropyrimidines cautiously, since the relative intensities of mass peaks in the spectra of these compounds are markedly dependent on the temperature of the ion source. Spectra were recorded at ionization voltages of 70, 20, 16, and 12 e.v., and the elemental composition of some ions was determined by high-resolution mass measurement relative to argon, nitrogen, or fragments of perfluorotri-*n*-butylamine.

Results

The pyrimidine ring undergoes fragmentation after ionization even when fully aromatic.[9] Pyrimidine itself (the only liquid compound studied) yields a molecular ion (M^+, Figure 1[10]; I, Figure 2) at *m/e* 80, which loses HCN to produce a fragment at *m/e* 53, probably II. The ion radicals I and II can lose a hydrogen atom to give III, *m/e* 79, and IV, *m/e* 52; IV can also arise by loss of HCN from III. Metastable peaks were observed for each of these transitions. Probably HCN is lost from II to give ionized acetylene (V), but no metastable peak was observed for this process, and the mass of the ion at *m/e* 26 was not measured. Ion II would be expected to decompose preferentially to neutral HCN and acetylene, because of the significantly lower ionization potential of the latter.[11] An alternative mechanism can be written in which the initial loss of HCN involves C-6 rather than C-2. Without a deuterium label at one of these posi-

(7) J. H. Beynon, ref. 6, p. 105.

(8) *Cf.* H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, Inc., San Francisco, Calif., 1964, p. iv.

(9) Heterocyclic aromatic compounds generally tend to undergo more extensive fragmentation than their carbocyclic analogs. This tendency has been discussed by S. Meyerson, *Appl. Spectry.*, **9**, 120 (1955).

(10) Throughout this paper the letter M is used to designate the mass number of a molecular ion, and the notation (M − 43), etc., to denote the mass of an ion produced by loss of 43 mass units from the molecular ion.

(11) *Cf.* the discussion by J. H. Beynon, G. R. Lester, and A. E. Williams, *J. Phys. Chem.*, **63**, 1861 (1959).

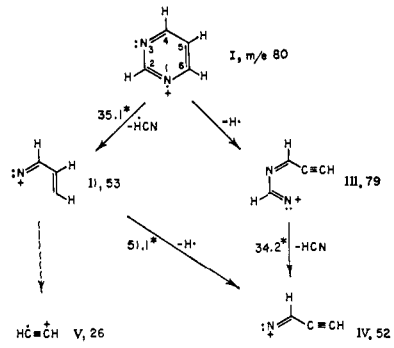

Figure 2. Proposed principal fragmentation paths in pyrimidine. A number followed by an asterisk indicates the *m/e* value of the metastable peak observed for the indicated transition. A dashed arrow indicates that no metastable peak was observed for that transition.

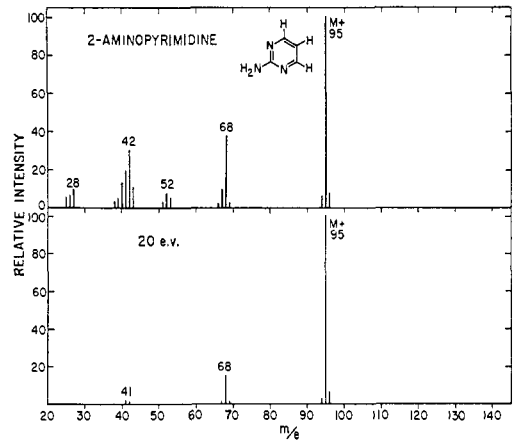

Figure 3. Mass spectra of 2-aminopyrimidine at 70 and 20 e.v.

tions we cannot decide this point; the mechanism given seems the more plausible.[12] At 70 e.v. the ring can also split in several ways to give minor fragments of *m/e* 37–40. Comparison of spectra recorded at 70, 20, and 16 e.v. (Figure 1) also suggests that sequential loss of two HCN molecules from the pyrimidine molecular ion is the major fragmentation path. Molecular ions generated at 70 e.v. are often sufficiently energetic to eject two successive neutral fragments of mass 27; the base peak in the spectrum occurs at *m/e* 26. At 20 e.v., however, the less energetic molecular ion becomes the base peak, and at 16 e.v., where the molecular ion peak is four times as intense as the (M − 27) peak, the peak at *m/e* 26 is quite small. The minor fragments disappear at 20 e.v.

The molecular ion of 2-aminopyrimidine can undergo fragmentation by several paths at 70 e.v. These differ in several respects from the fragmentation paths of pyrimidine, as a result of the directing influence of the amino group.[13] The more important processes (those which still occur at 20 e.v.; Figure 3) involve loss of HCN, followed either by loss of C_2H_2 to give a peak at *m/e* 42 ($CH_2N_2^+$, mass measured relative to argon) or by loss of a second molecule of HCN to give a peak at *m/e* 41 ($C_2H_3N^+$). The dideuterated molecular ion can eject either HCN or DCN, as shown by the presence of metastable peaks at *m/e* 50.5 (97 → 70) and 49.1 (97 → 69). The second molecule of HCN can not be shown to contain deuterium; metastable peaks are observed at *m/e* 25.6 (69 → 42) and 24.7 (68 → 41), but not at 24.3 (69 → 41). The molecular ion can also lose a hydrogen (deuterium) atom followed by HCN. The ion which would result, VII, might eject acetylene, but no metastable peak is observed for this decomposition and the peak at *m/e* 41 is found to consist entirely of $C_2H_3N^+$, rather than the ion CHN_2^+ which would result from this process. If VII decomposes, the charge may remain on the acetylene fragment, producing the minor peak at *m/e* 26. A minor fragmentation path consists of initial loss of cyanamide, followed by ejection of a hydrogen atom to produce the ions of *m/e* 53 and 52. No metastable peaks were observed for this sequence, which is observed only at 70 e.v. and is unaffected by deuteration.

One of several ways in which these processes can be represented mechanistically is by successive fragmentation of the imino tautomer (Figure 4). This does not imply that the imino structure is the prevalent tautomeric form of this molecule in the gas phase; in fact, the amino tautomer predominates in the solid phase and in aqueous solution.[14] The facile and frequent occurrence of hydrogen rearrangement during fragmentation is well established,[15] and we could write mechanisms for each of the initial fragmentation steps involving loss of HCN which would involve simultaneous transfer of an amino deuterium atom to the ring. The data do not dictate a choice between these mechanisms. The point of importance for the present study is the substantial effect of the amino group on the fragmentation patterns of the pyrimidine ring.

The 2,4-dioxypyrimidines undergo fragmentation as diagrammed in Figure 5, provided that alkyl substituents contain no heteroatoms. Uracil at 70 e.v. gives a molecular ion at *m/e* 112 (Figure 6) which expels HNCO (43 mass units) by path A to produce a peak at *m/e* 69 ($C_3H_3NO^+$), with a metastable peak at *m/e* 42.5 (112 → 69). The $C_3H_3NO^+$ peak appears at *m/e* 70 in the deuterated compound and is still prominent at 20 e.v. The ion it represents (VIII) can lose CO by path B, producing an ion of *m/e* 41 whose mass, measured relative to argon, corresponds to $C_2H_3N^+$. This process is confirmed by a metastable peak at *m/e* 24.3 (69 → 41), and is followed by loss of the hydrogen atom at C-6, accompanied by a metastable peak at *m/e* 39.1 (41 → 40). The reverse of this process, loss of a hydrogen atom followed by decarbonylation, can also occur and is designated path D; it is characterized by metastable peaks at *m/e* 67.0 (69 → 68) and at 23.5 (68 → 40). The only ion observed at *m/e* 40 in the uracil spectrum is $C_2H_2N^+$. VIII may also disintegrate by path E to give the ion $HC{=}NH^+$ at *m/e* 28, but no metastable peak was observed for this process. Finally, VIII can lose HCN by path C, which is analogous to E but involves transfer of a hydrogen atom (which is subsequently lost) from the

(12) An attempt to prepare pyrimidine-2-*d* from 2-aminopyrimidine by diazotization followed by H_3PO_2 reduction failed. Only 2-chloropyrimidine was isolated from ether extracts of the reaction mixture.

(13) *Cf.* the directive influence of the amino group on the fragmentation of aniline, discussed by P. N. Rylander, S. Meyerson, E. L. Eliel, and J. D. McCollum, *J. Am. Chem. Soc.*, **85**, 2723 (1963).

(14) D. J. Brown, E. Hoerger, and S. F. Mason, *J. Chem. Soc.*, 4035 (1955).

(15) J. H. Beynon, ref. 6, p. 264.

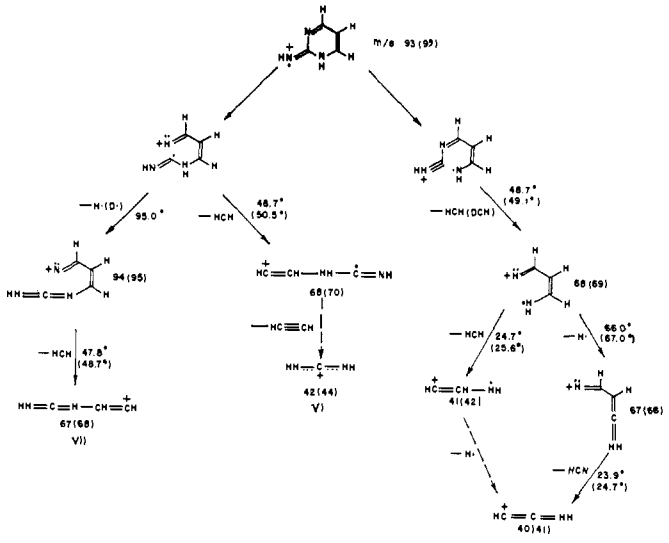

Figure 4. Electron-impact fragmentation of the imino tautomer of 2-aminopyrimidine. Numbers in parentheses refer to the dideuterated compound.

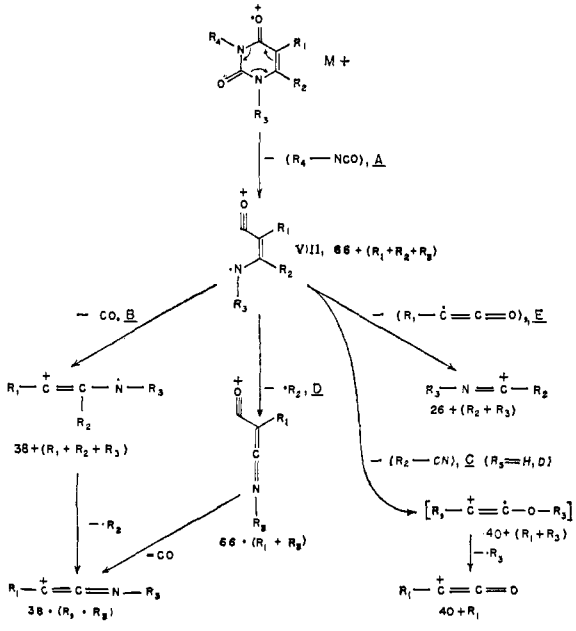

Figure 5. Fragmentation patterns of 2,4-dioxypyrimidines: uracil, $R_1 = R_2 = R_3 = R_4 = H$; thymine, $R_1 = CH_3$; 6-methyluracil, $R_2 = CH_3$; 1,3-dimethyluracil, $R_3 = R_4 = CH_3$.

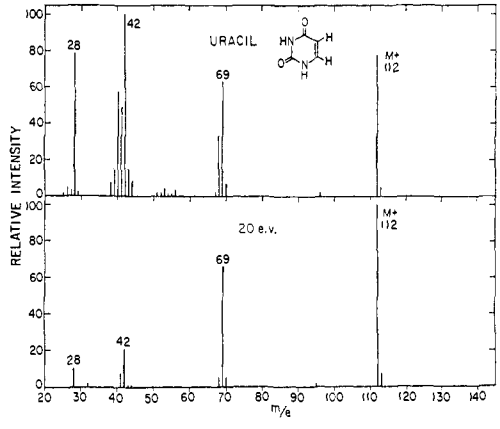

Figure 6. Mass spectra of uracil at 70 and 20 e.v.

nitrogen atom to the remaining ketene fragment. The resulting ion at *m/e* 42, $C_2H_2O^+$, should produce a peak one mass unit higher in the spectrum of the deuterated compound. This does not stand out clearly in the spectrum of deuterated uracil, but is apparent in the spectra of related compounds.

6-Methyluracil (Figure 7) also loses HNCO from its molecular ion as the initial step in fragmentation, followed by loss of the methyl group. This loss of 15 mass units from VIII, independent of deuteration, is characterized by a metastable peak at *m/e* 55.7 (83 → 68) and is followed by decarbonylation, as shown by another metastable peak at *m/e* 23.5 (68 → 40). Since VIII at *m/e* 83 does not eject a hydrogen atom, path D as represented in Figure 5 is unequivocally established. There is no peak at *m/e* 56, and consequently no loss of HCN from VIII, and the peaks at *m/e* 55 and 54 corresponding to successive loss of CO and a hydrogen atom by path B are very small because formation of the resulting vinyl carbonium ion is less favorable energetically than the other paths available. The metastable peak at *m/e* 53.1 (55 → 54) for the loss of this hydrogen atom disappears from the spectrum of the deuterated compound and is replaced by another at *m/e* 52.2 (56 → 54). Loss of the deuterium atom probably follows nucleophilic attack of the lone-pair electrons of the nitrogen atom on the positively charged carbon atom at position 5, resulting in closure of an aziridine ring (*cf.* Figure 9). This process may occur in uracil, but could not be confirmed by deuterium labeling. The methyl group can be lost instead of the hydrogen (deuterium) atom, giving rise to the ion $C_2H_2N^+$ at *m/e* 40 by path B, but neither parent nor daughter ion for this process is abundant and the accompanying metastable peak at *m/e* 29.1 (55 → 40) is barely detectable. A metastable peak was observed for loss of CH_3CN by path C and HC_2O (ketene radical) by path E in the spectra of both 6-methyluracil and its deuterated derivative; in this case paths C and E both lead to ions at *m/e* 42, each of which should shift to *m/e* 43 on deuteration. The peak at *m/e* 42 in the spectrum of the deuterated compound is partly due to decomposition of the monodeuterated

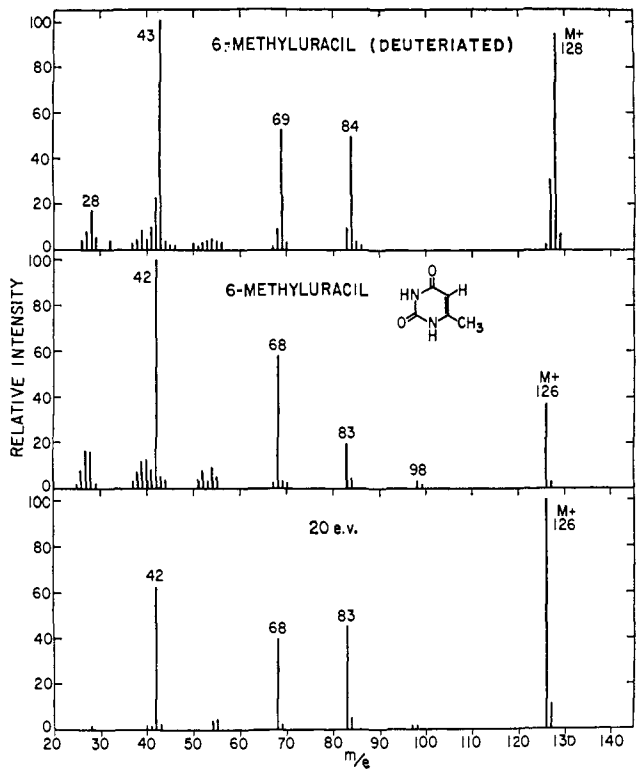

Figure 7. Mass spectra of deuterated 6-methyluracil at 70 e.v. and of 6-methyluracil at 70 and 20 e.v.

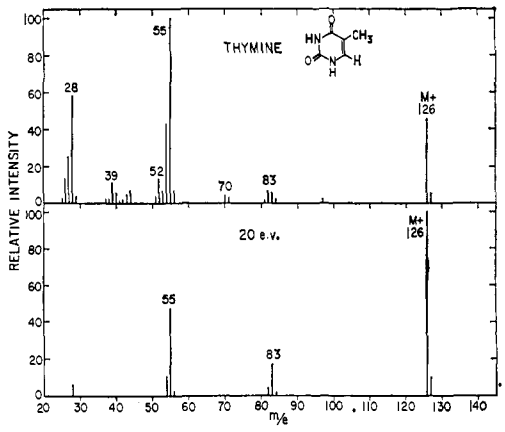

Figure 8. Mass spectra of thymine at 70 and 20 e.v.

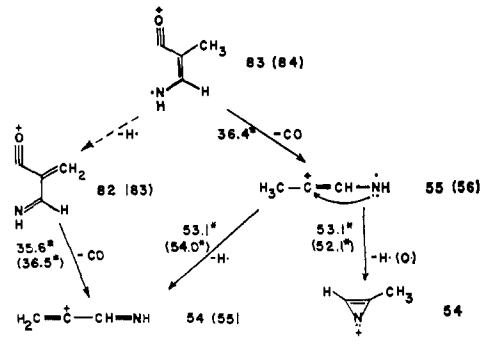

Figure 9. Fragmentation paths specific for thymine.

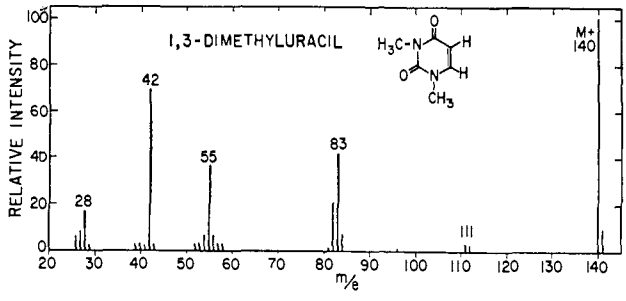

Figure 10. Mass spectrum of 1,3-dimethyluracil at 70 e.v.

contaminant, only a fraction of which loses its deuterium with the HNCO fragment. The masses of the ions at *m/e* 42 were measured and found to correspond to $C_2H_4N^+$ from path E and $C_2H_2O^+$ from path C in the ratio 3:1.

The fragmentation of thymine (Figure 8) is perfectly analogous to that of uracil with respect to paths A and C. The methyl group is not expelled from ion VIII, and its presence at position 5 favors decarbonylation of ion VIII (83 → 55) over loss of HCN by path C (83 → 56), in marked contrast to the behavior of 6-methyluracil. Successive loss of CO and a hydrogen atom in either order occurs readily from ion VIII in thymine, but the presence of the methyl group at C-5, from which a hydrogen atom could be lost, allows an alternative mechanism (Figure 9) to paths B and D. A decision between these mechanisms cannot be made from the data available. Decarbonylation of deuterated VIII is followed by loss of either a hydrogen or a deuterium atom, as shown by the metastable peaks indicated in Figure 9. Loss of the deuterium atom can be explained by the same mechanism advanced previously for a similar process in 6-methyluracil.

The spectrum of the final member of this series, 1,3-dimethyluracil, is characterized by a molecular ion peak at *m/e* 140 and intense fragment ion peaks at *m/e* 83, 55, and 42 corresponding to loss of HNCO by path A and subsequent ejection of CO and ketene radical (HC_2O) by paths B and E, respectively (Figure 10). Path C would involve transfer of a methyl group, which does not occur to any significant extent. The ion of *m/e* 56 which would result is not prominent at 70 e.v., and at 20 e.v. this peak is no more intense than would be expected from the C^{13} content of the ion at *m/e* 55. There is also an intense peak at *m/e* 82, corresponding to loss of a hydrogen atom from ion VIII. It is likely that in this case the hydrogen atom is ejected from the methyl group, thus lengthening the chain of conjugation. The resulting ion, which is highly stabilized by resonance, does not eject CO; no metastable peak for decarbonylation is observed, and the peak at *m/e* 54 which would result is quite small. This peak can be accounted for by ejection of a hydrogen atom following decarbonylation of ion VIII, for which the expected metastable peak is observed at *m/e* 53.1 (55 → 54). Probably the hydrogen atom which is lost in this case also originates from the methyl group, rather than from C-6 (as in path B, Figure 5).

The fragmentation scheme outlined in Figure 5 accounts for the presence and, often, the relative intensities of the major peaks common to 2,4-dioxypyrimidine spectra. It also resolves ambiguities in interpretation arising from the redundancy of structural features characteristic of these compounds. The amide groups (HNCO), which involve three distinct combinations of

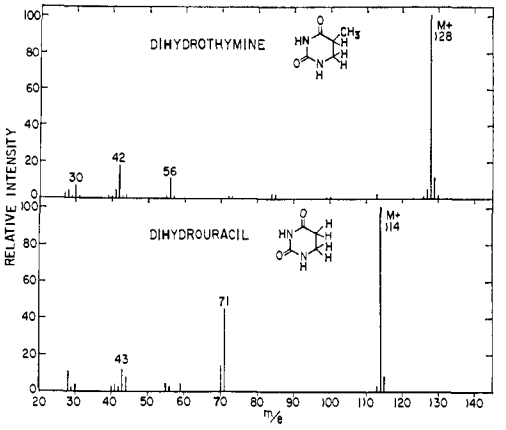

Figure 11. Mass spectra of dihydrothymine and dihydrouracil at 70 e.v. The temperature of the ion source was kept as low as possible during the recording of these spectra.

C and N, are the primary redundant features. We believe that initial loss of HNCO always involves C-2 and N-3: this leads to the most highly conjugated, and hence presumably the most stable intermediate (ion VIII), which alone can undergo all the subsequent fragmentation steps without extensive and improbable rearrangements. We represent this process by a concerted "retro Diels–Alder" mechanism,[16] in the absence of any evidence that the molecular ion ring opens prior to ejection of HNCO. The loss of CO from an open-chain molecular ion would be expected to be a major fragmentation path, but a peak at (M − 28) is barely detectable in 6-methyluracil and 1,3-dimethyluracil and is nonexistent in uracil and in thymine, where the presence of a methyl group at C-5 would be expected to facilitate expulsion of the adjacent carbonyl group.

A similar fragmentation pattern occurs in the mass spectra of the purine alkaloids theobromine, theophylline, and caffeine, which contain a 2,4-dioxypyrimidine structure fused at positions 5 and 6 to an imidazole ring. The initial fragmentation step involves the loss of CH_3NCO, which has been shown by Spiteller[17] to result in the formation of an ion analogous in structure to VIII.

The spectra of 2,4-dioxy-5,6-dihydropyrimidines differ from those of the unsaturated parent compounds in several respects. Molecular ion peaks strongly predominate over fragment peaks at 70 e.v. (Figure 11) in the spectra of dihydrouracil and dihydrothymine recorded *at low ion source temperatures*. Under these conditions the spectra of both compounds show broad, intense metastable peaks for the loss of 43 mass units (HNCO) from the molecular ion, but the (M − 43) peaks are very small. The prominence of the metastable peaks and the small size of the fragment ion peaks suggest that these processes are very slow and hence have a large activation energy. Saturation of the 5,6-bond blocks the retro Diels–Alder mechanism and makes the loss of HNCO much more difficult. While simple 2,4-dioxypyrimidines invariably undergo the facile retro Diels–Alder reaction as the initial fragmentation process, their ring-saturated derivatives easily lose

(16) K. Biemann, ref. 3, p. 102.
(17) G. S. Spiteller and M. Spiteller-Friedmann, *Monatsh.*, **93**, 634 (1962).

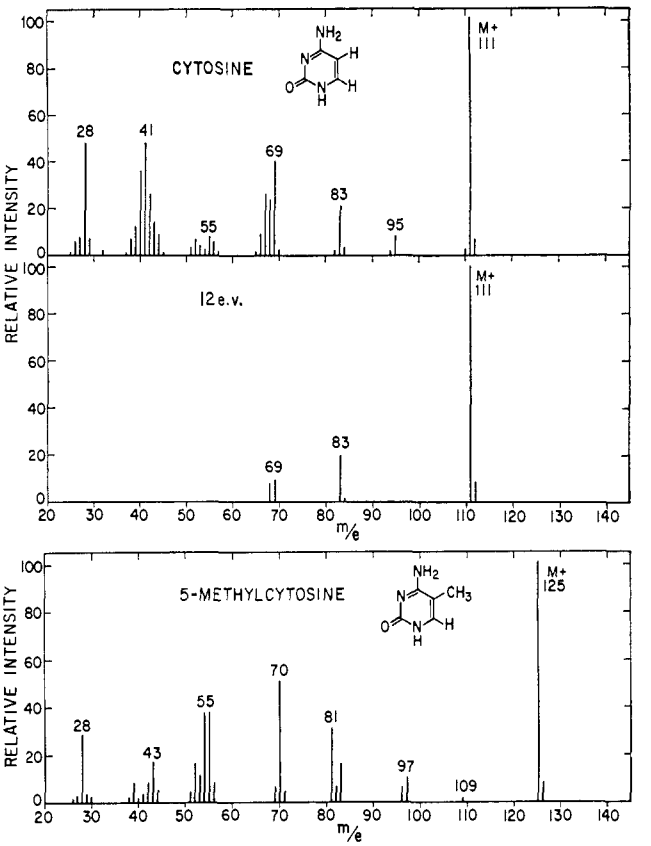

Figure 12. Mass spectra of cytosine at 70 and 12 e.v. (upper). Mass spectrum of 5-methylcytosine at 70 e.v. (lower).

neutral fragments other than HNCO from the molecular ion. Loss of a hydrogen atom from dihydrouracil and of either a hydrogen atom or the methyl group from dihydrothymine results in the formation of an even-electron daughter ion in which the π-bonded framework over which the positive charge is delocalized has been extended to C-5 or alternatively, perhaps, to C-6. The spectra of both compounds also show intense metastable peaks for the loss of 28 mass units (CO) from the molecular ion, but the peak at (M − 28) is visible only in the spectrum of dihydrothymine.

The intensities of the propene and methylketene peaks (*m/e* 42, $C_3H_6^+$, and *m/e* 56, $C_3H_4O^+$, mass numbers unchanged by deuteration of the sample) in the spectrum of dihydrothymine increase very rapidly with increasing ion source temperature. The ethylene and ketene peaks (*m/e* 28, $C_2H_2^+$, and *m/e* 42, $C_2H_2O^+$) in the spectrum of dihydrouracil behave similarly. This behavior is suggestive of thermal decomposition of the sample, but metastable peaks do occur in the spectrum at *m/e* 6.9 (114 → 28) and 15.5 (114 → 42), proving that ions with mass numbers 28 and 42 are formed in one step by fragmentation of the dihydrouracil molecular ion. The peak in the dihydrouracil spectrum at *m/e* 28 has two minor components, CO^+ and CH_2N^+, so the metastable peak at *m/e* 6.9 may not characterize the formation of $C_2H_2^+$. However, the dihydrothymine spectrum contains a metastable peak at *m/e* 13.8 (128 → 42) for the formation of $C_3H_6^+$ from the molecular ion which is unambiguous. Investigation of the complex spectra of these compounds is continuing.

Cytosine (Figure 12, upper) and its 5-methyl derivative (Figure 12, lower) undergo fragmentation by three dis-

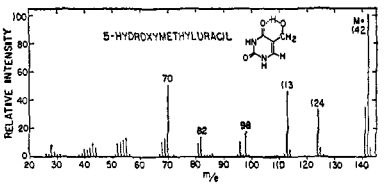

Figure 13. Mass spectrum of 5-hydroxymethyluracil at 70 e.v.

units (H_2NCO) from the molecular ion, but no evidence for operation of the two-stage mechanism.

Our results conclusively define the modes of decomposition of the cytosine and 5-methylcytosine molecular ions, but do not unambiguously demonstrate the structures of ions produced in secondary fragmentation steps. Complete mechanistic schemes for the fragmentation of these compounds must therefore await further data.

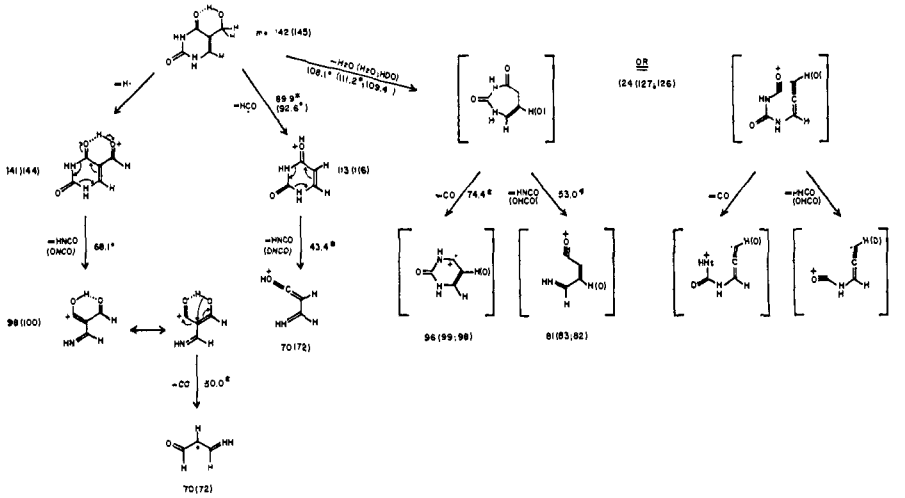

Figure 14. Proposed fragmentation patterns of 5-hydroxymethyluracil. The structures of the fragments resulting from the loss of H_2O from the molecular ion are largely conjectural, as indicated by their enclosure in brackets.

tinct paths. A high-energy process involves expulsion of the amino group (loss of 16 mass units from the molecular ion and 18 from its deuterated derivative) followed by loss of HCN. The ions produced by this path are not observed at 16 e.v. The second path is characterized by loss of CO from the molecular ion, followed by expulsion of HCN. Metastable peaks show that in this step deuterated 5-methylcytosine loses HCN and that deuterated cytosine loses DCN. The metastable peaks are small and the failure to detect similar peaks for the loss of DCN from 5-methylcytosine and expulsion of HCN from cytosine does not rule out the occurrence of these processes.

The third principal fragmentation path observed in cytosine spectra, the retro Diels–Alder reaction, is the most complicated of the three. The cytosine molecular ion can expel NCO radical, HNCO, or a hydrogen (deuterium) atom followed by HNCO, after which HCN is lost. The result is a series of three peaks at *m/e* 67, 68, and 69 in Figure 12, upper (*m/e* 81, 82, and 83 in Figure 12, lower) accompanied by another triplet at *m/e* 40, 41, and 42 (*m/e* 54, 55, and 56 in Figure 12, lower) and by characteristic metastable peaks; high-resolution mass measurement confirmed the identities of these ions. Metastable peaks in the spectrum of deuterated cytosine show unambiguously that in each case either HCN or DCN can be lost. Expulsion of DCN could conceivably occur at either end of the intermediate ion, and our data leave this ambiguity unresolved. Of the three retro Diels–Alder paths, the sequence involving successive loss of a hydrogen atom and HNCO requires the most energy; it is not observed at 12 e.v. In the spectrum of 5-methylcytosine, the two stages of this path occur in a single step: there is a metastable peak at *m/e* 52.5 (125 → 81) for the loss of 44 mass

The last compound studied, 5-hydroxymethyluracil, is postulated to contain an intramolecular hydrogen bond as shown in Figure 13. It is the only compound in which total replacement of exchangeable hydrogen by deuterium did not take place easily. While cytosine, which also contains three replaceable protons, gave a mixture under our deuteration conditions in which the ratio of dideuterated to trideuterated product was less than 2:5, this ratio for hydroxymethyluracil was 5:4. The presence of the oxygen atom in the hydroxymethyl group and the hydrogen-bonded structure of this compound result in a fragmentation pattern significantly different from that of the simpler 2,4-dioxypyrimidines (Figure 14). The molecular ion, *m/e* 142, can eject a hydrogen radical, followed by successive expulsion of HNCO and CO; it can lose formyl radical, HCO, followed by loss of HNCO and possibly CO; or it can lose H_2O, with or without one atom of deuterium, as shown by metastable peaks in the spectrum of the deuterated compound at *m/e* 111.2 (145 → 127) and 109.4 (145 → 126). Loss of H_2O in this way may or may not result in ring expansion (Figure 14); it is followed by loss of CO or HNCO. The spectrum also displays a peak at *m/e* 82, which corresponds to loss of NCO radical after the initial dehydration reaction. The peaks below *m/e* 70 nearly disappear at 20 e.v., and interpretation of this region of the spectrum is hampered by the lack of metastable peaks.

The major mass spectrometric difference between this compound and other 2,4-dioxypyrimidines is that the 5-hydroxymethyluracil molecular ion never undergoes retro Diels–Alder fragmentation; loss of a water molecule, formyl radical, or hydrogen atom always occurs

Table I. Electron-Impact Fragmentation of the Thymine Moiety of Thymidine at 70 E.v. Ion Compositions Were Ascertained by High-Resolution Mass Measurement[a]

Parent ion			Daughter ion			Neutral fragment lost	Observed metastable peak (*m/e*)
Composition	*m/e*	Intensity	Composition	*m/e*	Intensity		
$C_5H_6N_2O_2$	126	32	C_4H_5NO	83	8	HNCO	54.7
C_4H_5NO	83	8	C_4H_4NO	82	5	H	81.0
C_4H_5NO	83	8	C_3H_5N	55	31	CO	36.5
C_3H_5N	55	31	C_3H_4N	54	15	H	53.1

[a] Compare Figures 5 and 9. Intensity is the height of a given peak relative to that of the most intense peak in the spectrum (100).

first. This indicates that the primary ionization process is different. Ionization of organic molecules by removal of a loosely bound, lone-pair electron from a nitrogen or oxygen atom is a highly probable result of electron bombardment,[18] and in amino- and dioxypyrimidines the primary ionization process is best interpreted as removal of a nonbonding electron from the extranuclear heteroatom, rather than the nitrogen atoms or the conjugated π-electron system of the ring. In this case the oxygen atom of the hydroxymethyl group, which is not conjugated with the ring, seems to ionize preferentially.

A similar situation occurs in the electron impact ionization of nucleosides. Biemann and McCloskey[3] showed that in these compounds the positive charge resides on the ribose moiety; transfer of the charge to the pyrimidine or purine moiety is accompanied by transfer of one or two hydrogen atoms as well, and by fission of the bond linking the ribose C-1′ to the pyrimidine N-1 or purine N-9. This explains the occurrence in the spectrum of peaks at *m/e* values corresponding to the mass of the pyrimidine or purine molecular ion, M, and at (M + 1). We have confirmed this result, and have found by high resolution mass measurement that the peaks at *m/e* 126 and 127 in the spectrum of thymidine consists of $C_6H_5N_2O_2^+$ (the composition of the thymine molecular ion) and $C_5H_7N_2O_2^+$, respectively. Primary ionization of the pyrimidine moiety apparently does not occur; the molecular ion (*m/e* 242) does not undergo the fragmentation processes characteristic of thymine. The thymine fragment ion at *m/e* 126, however, decomposes by the same paths as a 2,4-dioxypyrimidine molecular ion (Figure 5); the presence of more than 50 metastable peaks in the thymidine spectrum permits the identification of decomposition processes originating with the pyrimidine fragment. Many of these processes generate daughter ions containing a nitrogen atom; these can be positively identified as pyrimidine fragments by high-resolution mass measurement even when they are not abundant (Table I). This is a result of biochemical importance, for nucleosides can readily be obtained by enzymatic degradation of nucleic acids, while the pyrimidine–ribose bond is very resistant to hydrolysis.

We wish to point out in conclusion that this paper is not meant to be a comprehensive catalog of all the electron-impact fragmentation patterns possible for pyrimidines of biological interest. A substantial number of mass peaks and metastable ions have not been interpreted here, and alternant mechanisms may exist for some of the processes we have discussed. We have restricted our study to major fragmentation paths of potential interest to structure determination on derivatives of these compounds, especially those of biological origin. We are presently extending our studies to the purines and nucleosides.

Acknowledgment. M. B. wishes to thank the directors of Associated Electrical Industries Ltd. for providing him with the opportunity to work at Harvard. The authors are happy to acknowledge Professor Paul Doty's encouragement and support of this work.

(18) J. H. Beynon, ref. 6, p. 267.

The Synthesis and Spectra of α,β-Unsaturated Aliphatic Azo Compounds[1,2]

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Received June 8, 1965

The reaction of α-chloroaldehydes and α-chloro ketones with 2 equiv. of methylhydrazine has resulted in the formation of the previously unknown α,β-unsaturated aliphatic azo compounds. The ultraviolet absorption of this new chromophore has been established. The infrared and n.m.r. spectra of these new compounds were obtained. A mechanism for the formation of the unsaturated azo compounds is proposed and the synthetic limitations of the reaction are discussed briefly.

(1) This investigation was supported by Public Health Service Research Grant AI-02923 from the National Institute of Allergy and Infectious Diseases.

(2) Presented in part at the 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 1964, Paper No. 155, Abstract of Papers, p. 87S.