Mass Spectrometry of Cyclonucleosides

SEI TSUBOYAMA AND JAMES A. MCCLOSKEY*

Institute for Lipid Research and Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77025

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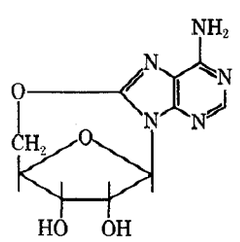
Mass spectra of pyrimidine cyclonucleosides containing 2,2′, 2,3′, 2′,6, and 5′,6 linkages were studied in order to determine the effects of differing positions of sugar and base linkage, and of anomeric configuration of the base, upon fragmentation reactions. In analogy to previously reported data for purine cyclonucleosides, the 2′- and 3′-linked compounds could be readily distinguished from the 5′ isomer but not from each other. The spectra of free cyclonucleosides were found to show numerous complex fragmentation paths and rearrangements, some of which are related to thermal changes during sample vaporization. Base + H and + 2H ions common to conventional nucleosides were observed, but the intact sugar fragment was not. Alternatively, trimethylsilylation provided derivatives which were sufficiently volatile for sample introduction by gas chromatograph, thereby avoiding thermal problems, and which exhibited fragmentation more clearly representative of structural details. Several major ions from trimethylsilyl derivatives showed evidence of an unusual exchange in which a single trimethylsilyl hydrogen had been replaced by hydrogen from the remainder of the molecule during the fragmentation sequence.

In recent years, a variety of cyclonucleosides have been synthesized[1] and used as models for studies of nucleoside conformation[2] and as key intermediates in the synthesis of nucleoside analogs.[1,3] Mass spectrometry would be expected to be a highly useful means of characterizing these compounds in view of its considerable utility in dealing with structural problems of conventional nucleosides.[4] A previous report on cyclonucleoside mass spectra was made by Ikehara and coworkers, who studied the mass spectra of a number of adenosine 8-cyclonucleosides.[5,5a] Their data indicated that the 8,5′ compound **1** could be differentiated from its 8,2′ or 8,3′ isomers (**2**, **3**), but the latter two could not be distinguished from each other. In addition, the mechanistic origins and structures of several prominent ions were not determined, although much information was obtained by high-resolution techniques and by examination of the analogous 8-*S*-cyclonucleosides. Based on the known fragmentation behavior of adenosine analogs,[6] we found the general similarity of spectra of **1**–**3**, as well as their apparent complexity, to be somewhat surprising. We have therefore undertaken a detailed study of the mass spectra of a number of pyrimidine cyclonucleosides in order to determine what structural information can be deduced from their spectra, and whether the same difficulties exist as for the purine cyclonucleosides. In addition, the mass spectra and gas chromatographic properties of the analogous trimethylsilylated compounds were examined as alternatives to the less volatile free cyclonucleosides.



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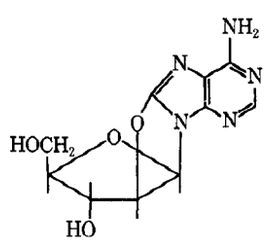

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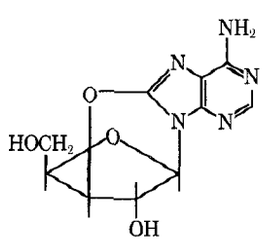


3

Mass Spectra of Free Cyclonucleosides.—Model compounds were chosen which would represent the effects of α,β anomerism [2,2′-anhydro-1-(β-D-arabinofuranosyl)uracil, **4**; its α anomer, **5**]; and differing points of attachment to the sugar [2,3′-anhydro-1-(β-D-xylofuranosyl)uracil, **6**; 5′,6-anhydro-1-(β-D-ribofuranosyl)-6-hydroxyuracil, **7**], and to the base [2′,6-anhydro-1-(β-D-arabinofuranosyl)-6-hydroxyuracil, **8**]. Mass spectra were acquired at the minimum possible vaporization temperatures which would produce an ion beam of moderate intensity (*ca.* 200–240°), since changes in ion abundance were observed to occur with either increased temperature, or over a period of time at lower temperatures. The spectrum of **4** shown in Figure 1 exhibits most of the basic ion types which were common to the series. In contrast to the mass spectrum of uridine,[7] all molecular ions show substantial abundance due to the increased cyclic nature of the molecules. The principal fragmentation pathway in the upper mass range proceeds by loss of a hydroxyl radical (*m/e* 209) followed by expulsion of CH_2O from the 5′ moiety to produce *m/e* 179. Plausible mechanisms can be written for both **4** (or **5**) and **6** which do not require opening of the ribose ring. Space-filling CPK nucleic acid models[8] indicate that O-4′ is sterically a suitable acceptor site for the hydrogen which is retained. This process is blocked in the 5′-linked compound **7**, which instead expels the elements of CHO

(1) See, for example, (a) M. Ikehara, *Accounts Chem. Res.*, **2**, 47 (1969); (b) J. J. Fox, *Pure Appl. Chem.*, **18**, 223 (1969).

(2) See, for instance, (a) T. R. Emerson, R. J. Swan, and T. L. V. Ulbricht, *Biochemistry*, **6**, 843 (1967); (b) M. Ikehara, M. Kaneko, K. Muneyama, and H. Tanaka, *Tetrahedron Lett.*, 3977 (1967); W. Voelter, G. Barth, R. Necords, E. Bunnenberg, and C. Djerassi, *J. Amer. Chem. Soc.*, **91**, 6165 (1969).

(3) For example, (a) R. E. Holmes and R. K. Robins, *J. Org. Chem.*, **28**, 3483 (1963); (b) E. A. Falco, B. A. Otter, and J. J. Fox, *ibid.*, **35**, 2326 (1970).

(4) J. A. McCloskey in "Basic Principles in Nucleic Acid Chemistry," P. O. P. Ts'o, Ed., Academic Press, New York, N. Y., in press.

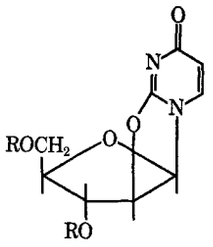
(5) M. Ikeda, Y. Tamura, and M. Ikehara, *J. Heterocycl. Chem.*, **7**, 1377 (1970).

(5a) NOTE ADDED IN PROOF.—D. Lipkin and J. A. Rabi [*J. Amer. Chem. Soc.*, **93**, 3309 (1971)] have recently commented on the principal features of 5′-link pyrimidine cyclonucleosides. Their conclusions agree with ours with exception of the even-electron b + 2H peak from **7** (*m/e*) which they report as being small or absent.

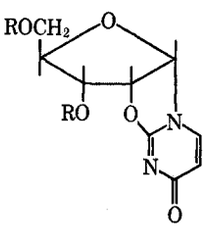
(6) S. J. Shaw, D. M. Desiderio, K. Tsuboyama, and J. A. McCloskey, *J. Amer. Chem. Soc.*, **92**, 2510 (1970).

(7) K. Biemann and J. A. McCloskey, *ibid.*, **84**, 2005 (1962).

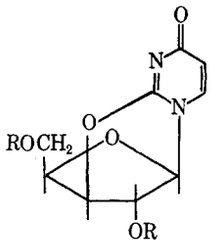
(8) W. L. Koltun, *Biopolymers*, **3**, 665 (1965).



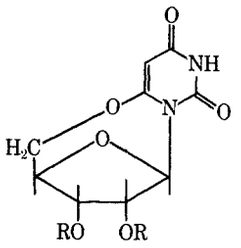
4, R = H
4a, R = $SiMe_3$



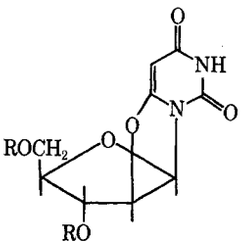
5, R = H
5a, R = $SiMe_3$



6, R = H
6a, R = $SiMe_3$

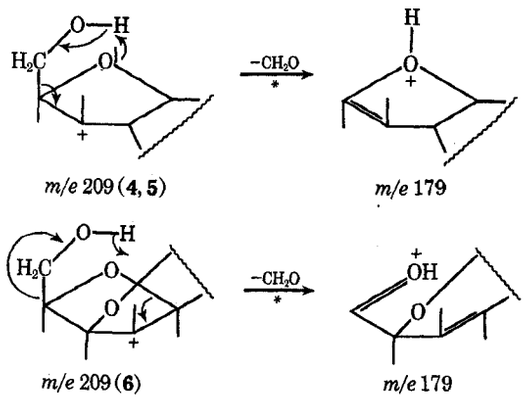


7, R = H
7a, R = $SiMe_3$



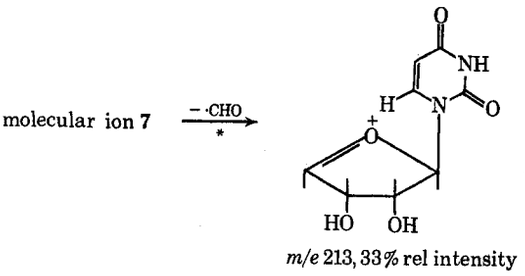
8, R = H
8a, R = $SiMe_3$

from 5′ (confirmed by measurement of exact mass), presumably with retention of hydrogen at the unsaturated C-6 in the base. Although the absence of a peak at M − 47 (OH + CH_2O) would appear to be diagnostic of a 5′-linked molecule, it is also absent in the spectrum of **8**. More useful is *m/e* 195 (Figure 1) which arises by simple loss of 5′-CH_2OH,[5] and is suitably absent in the spectrum of **7**. Further elimination of H_2O to form *m/e* 177 is marked by a metastable peak.



m/e 209 (**4**, **5**) *m/e* 179

m/e 209 (**6**) *m/e* 179



molecular ion **7** *m/e* 213, 33% rel intensity

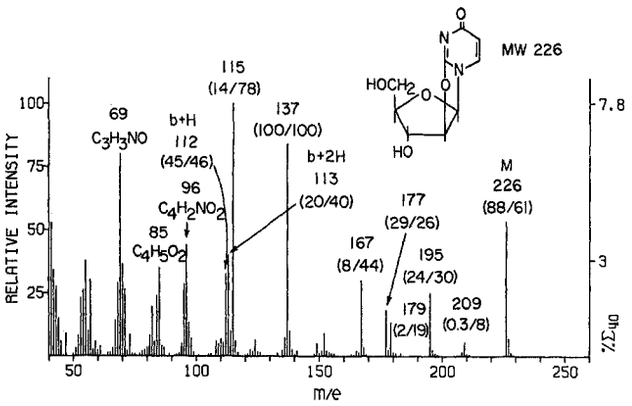
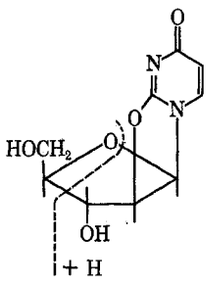


Figure 1.—Mass spectrum of 2,2′-anhydro-1-(β-D-arabinofuranosyl)uracil (**4**). Values in parenthesis refer to relative intensity values for the isomers **5** and **6**, respectively.

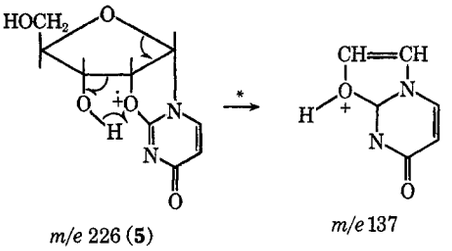
A more complex process is represented by the loss of 59 mass units (*m/e* 167, Figure 1), earlier determined by Ikehara[5] to involve elimination of C-4′,5′, the ribose ether oxygen, and one rearranged hydrogen. This assignment was confirmed by measurement of exact mass in the spectrum of **5**. The complex origin of this peak



m/e 167 (**4**)

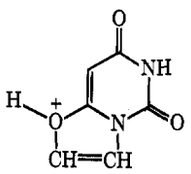
is indicated by its presence in the spectrum of the 5′-linked model **7** (*m/e* 183, rel intensity 17%), which requires the unfavorable rupture of the C-6,O-5′ bond. This ion is reportedly absent in the spectrum of **1**,[5] but our results cannot completely exclude the possibility that the skeletal atoms of C-3′ and -4′ are being lost in the case of compound **7**.

One of the most abundant ions in the spectra of **4**–**6** is the even-electron ion *m/e* 137, $C_6H_5N_2O_2$. The analogous ion was reported by Ikehara and coworkers,[5] who concluded only that it must contain the base and its heteroatom link to the sugar. Since the composition of *m/e* 137 requires inclusion of the base, the most reasonable structure consists of the base plus C-1′ and -2′. The spectrum of O-3′,O-5′-**4**-d_2 shows that *m/e* 137 contains one labile hydrogen rearranged from the sugar fragment which is lost. Unlike **4** and **6**, the α anomer **5** is



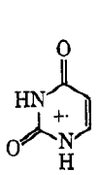
m/e 226 (**5**) *m/e* 137

conformationally capable of providing *m/e* 137 without ring opening, by transfer of hydrogen from O-3′. The analogous ion is also formed from **7**, indicating the occurrence of extensive bond breaking and making in its formation. Since the production of *m/e* 137 from the 3′-linked isomer **6** seemed particularly unlikely, metastable focussing was employed in order to determine the identities of its precursors. The results showed that the molecular ion (*m/e* 226), M − 31, and M − 59 all produced *m/e* 137, further testament to its relatively indiscriminate and multiple modes of formation.

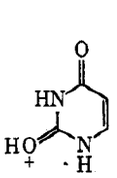


m/e 153 (**7**), 8.0% rel intensity
(**8**), 58% rel intensity

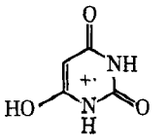
The principal fragmentation reaction in common with conventional nucleosides was found to be the ubiquitous[4] formation of the free base and its protonated forms, *m/e* 112 (b + H), 113 (b + 2H). As in the case of the cycloadenosines, their formation requires double and triple hydrogen rearrangements, respectively, in contrast to single and double transfers for nucleosides. In the formation of b + H and b + 2H from the 6-linked isomers **7** and **8**, the 5′- or 2′-O bond is broken in preference to the energetically less favorable 6-O bond, after which the oxygen at C-6 is free to abstract hydrogen from the ribose moiety. The b + H and b + 2H ions from 6-linked cyclonucleosides therefore retain the bridge oxygen and characteristically occur at *m/e* 128 and 129, 16 mass units higher than in the 2-linked isomers.[5a]



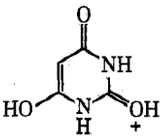
m/e 112



m/e 113



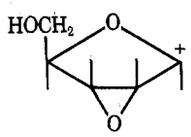
m/e 128
7, 42% rel intensity
8, 7.2% rel intensity



m/e 129
86% rel intensity
4.4% rel intensity

Other peaks in the spectrum of **8** cannot be represented as arising by any obvious mechanism, and may in part have thermal origins. Principal among these are *m/e* 168 ($C_7H_6NO_4$, 97% rel intensity) and *m/e* 150 ($C_7H_4NO_3$, 68% rel intensity), which differ by the elements of H_2O, and contain a portion of the base.

In spectra of conventional pyrimidine nucleosides, rupture of the glycosidic bond leads to a usually abundant ion (*m/e* 133 from ribonucleosides) consisting of the intact sugar fragment.[7] This ion is predictably absent from cyclonucleoside spectra, since its formation would require not only the breakage of a bond α to an unsaturated carbon (C-2 or -6), but also, in the case of **4**–**6**, transfer of hydrogen to the sugar from unsaturated carbons in the base (C-5 or -6). However, an important sugar-containing ion which is prominent in the spectra of the β-2,2′ and β-2,3′ isomers (**4**,**6**) is *m/e* 115, shown by measurement of exact mass to have the composition $C_5H_7O_3$. Examination of the spectrum of O-3′,O-5′-**4**-d_2 indicates a maximum of one labile hydrogen to be present, although the exact distribution could not be determined, due to the shift of *m/e* 113 and partial reexchange of the label during sample vaporization. However, these data indicate a structure isomeric with that shown below, although structural details as to the identity of oxygens or hydrogens are not available with the present evidence. Other numerous ions in the



m/e 115

low mass region of the spectrum in Figure 1 were shown at high resolving power to be multiplets, which mostly involve fragments of the base moiety. Compositions shown in Figure 1 for *m/e* 69, 85, and 96 represent the most abundant species in each case, as determined from the high-resolution spectrum of **5**.

The foregoing data reveal that mass spectra of isomeric free cyclonucleosides represent a number of complex processes which give rise to spectra which exhibit fewer differences than would be expected *a priori*. In particular, the presence of *m/e* 137 from other than 2′-linked models, and of the M − 59 ion from the 5′-linked model **7**, limits the usefulness of the spectra in a predictive sense, although other useful characteristic features are present. Two factors which are believed to play a role in this anomalous behavior are the high temperatures necessary for vaporization and the considerable ring strain inherent in the rigid tricyclic system. For example, on our LKB instrument, the vaporization of **4** commences at 150° (sample holder temperature), some 40° higher than uridine. It seems likely that many fragmentation processes are initiated by ring opening before fragmentation, thus reducing structural differences and increasing the opportunity for skeletal rearrangements.

Mass Spectra of Trimethylsilyl Derivatives.—Trimethylsilylation has been previously demonstrated to be an effective means of reducing the polarity of nucleosides[9] and nucleotides,[10] thereby enhancing their volatility.[11] The derivatization reaction is rapid, and easily applied on a microgram scale. The derivatives formed (*e.g.*, **4a**–**8a**) are sufficiently volatile for gas chromatography (see Experimental Section), permitting introduction of the sample into the mass spectrometer directly by gas chromatograph. The method is therefore potentially useful for the direct analysis of reaction mixtures, and provides an independent means of characterization of cyclonucleosides by their relative retention times. Of primary importance in the present study, the mass spectra of trimethylsilyl derivatives were found

(9) J. A. McCloskey, A. M. Lawson, K. Tsuboyama, P. M. Krueger, and R. N. Stillwell, *J. Amer. Chem. Soc.*, **90**, 4182 (1968).

(10) A. M. Lawson, R. N. Stillwell, M. M. Tacker, K. Tsuboyama, and J. A. McCloskey, *ibid.*, **93**, 1014 (1971).

(11) For leading references to the trimethylsilylation of nucleosides and nucleotides for gas chromatography, see ref 10 and C. W. Gehrke and C. D. Ruyle, *J. Chromatogr.*, **38**, 473 (1968).

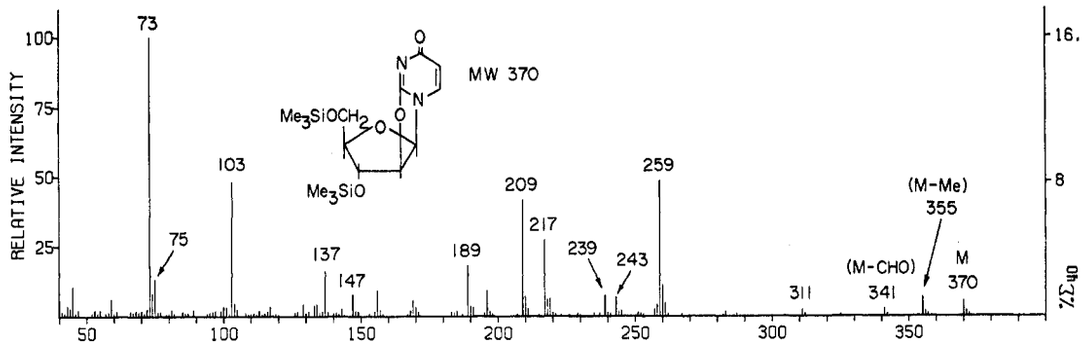


Figure 2.—Mass spectrum of the trimethylsilyl ether of 2,2′-anhydro-1-(β-D-arabinofuranosyl)uracil (**4a**).

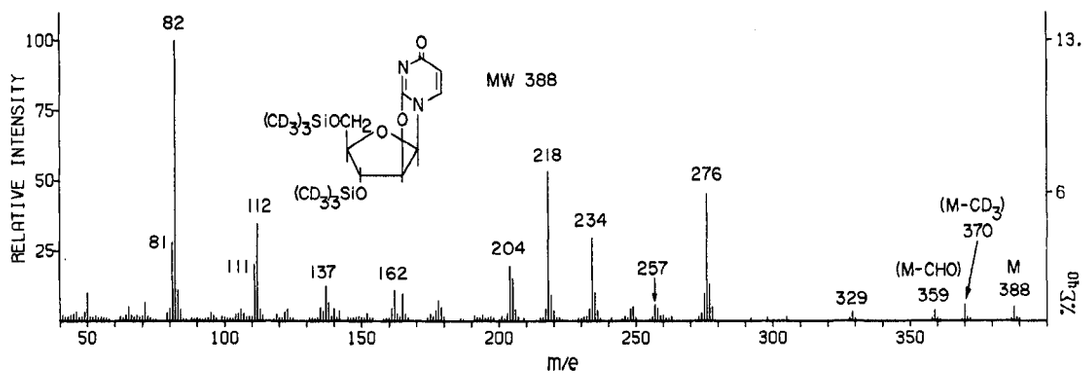
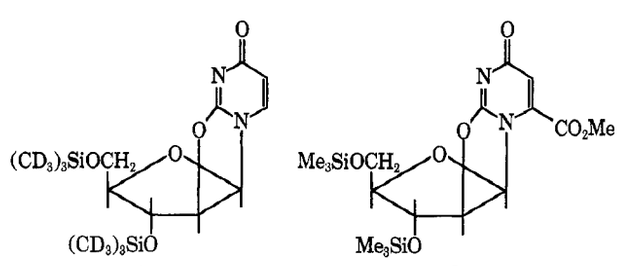
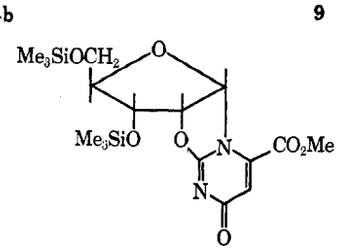


Figure 3.—Mass spectrum of the trimethylsilyl-d_9 ether of 2,2′-anhydro-1-(β-D-arabinofuranosyl)uracil (**4b**).

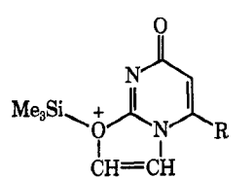
to be more truly representative of the parent cyclonucleoside structure than in the case of the free compounds.

Mass spectra of the uridine derivatives **4a–8a** are shown in Figures 2 and 4–6. Further correlations were made through the spectra of the trimethylsilyl derivatives of the anomeric cyloorotidine derivatives **9** and **10**, which were not sufficiently volatile for vaporization as free compounds. The corresponding trimethylsilyl-d_9 derivatives of each compound were also prepared and their mass spectra examined (*e.g.*, **4b**, Figure 3) as a highly useful means[12] of corroborating structural assignments and computer-derived elemental compositions obtained from exact mass data.

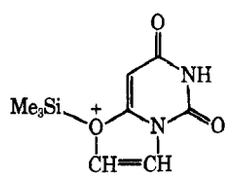




Many of the major ions represented in Figures 2–5 were found to be structurally, but not mechanistically, analogous to ions produced by the free compounds. Of principal importance is *m/e* 209 (Figures 2, 4), shown by deuterium labeling (*m/e* 218, Figure 3) and measurement of exact mass to consist of the base + C_2H_2 + $SiMe_3$. The ion is therefore analogous to *m/e* 137 in Figure 1, but bears a trimethylsilyl group, rather than hydrogen, transferred from O-3′ or O-5′.[13] The spectra of **9** and **10** show the same ion species, shifted 58 units higher to *m/e* 267. The corresponding ion from **8a** (Figure 6) is shifted 16 mass units (*m/e* 225), a characteristic of base-containing ions derived from the



m/e 209 R = H (**4a**, **5a**)
m/e 267 R = CO_2Me (**9**, **10**)



m/e 225 (**8a**)

6-linked cyclouridine derivatives which contain one additional oxygen atom. In the spectrum of the 3′-linked isomer (Figure 5), the mass 209 species is present (confirmed by measurement of exact mass) at greatly reduced intensity, and as *m/e* 225 from the 5′ isomer **7a** (intensity data, Figure 6). These intensity differences reflect the greater ease of formation of this ion from 2′-linked cyclonucleosides, and are therefore much more structurally diagnostic than in the case of the free compounds. Interestingly, the corresponding ion contain-

(12) J. A. McCloskey, R. N. Stillwell, and A. M. Lawson, *Anal. Chem.*, **40**, 233 (1968).

(13) The parallel tendency of H and $SiMe_3$ to rearrange in forming structurally similar ions is also found in the mass spectra of conventional trimethylsilyl nucleoside derivatives.[8]

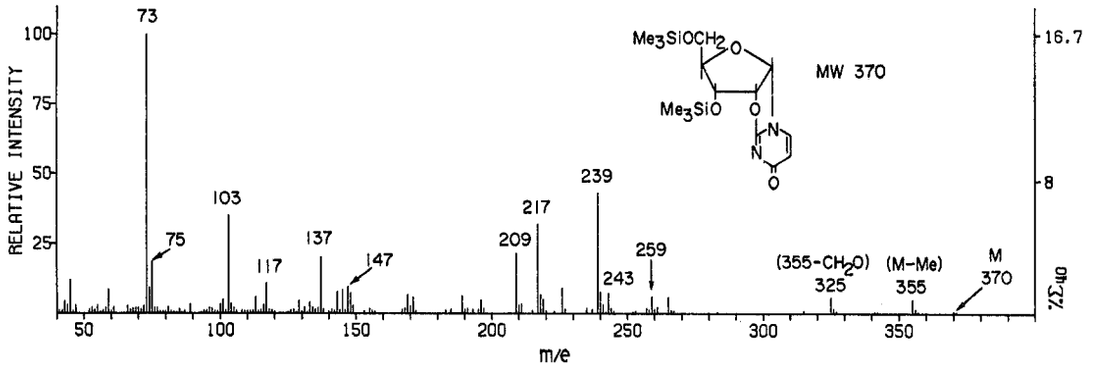


Figure 4.—Mass spectrum of the trimethylsilyl ether of 2,2′-anhydro-1-(α-D-ribofuranosyl)uracil (**5a**).

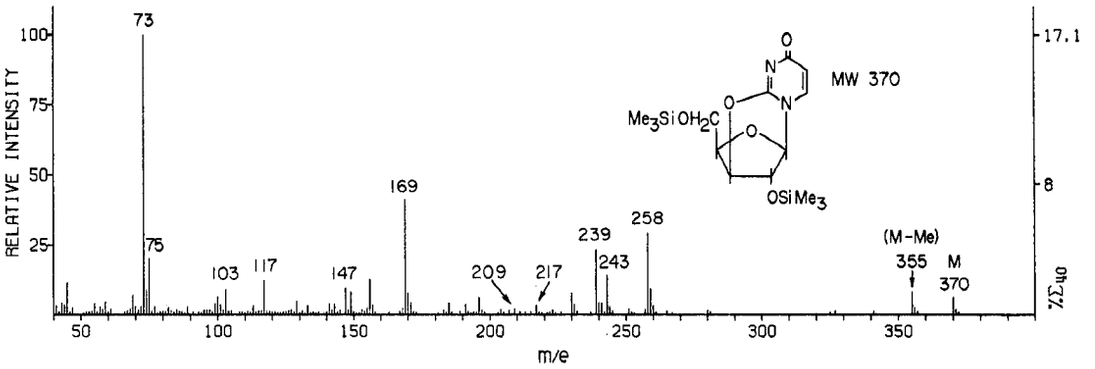
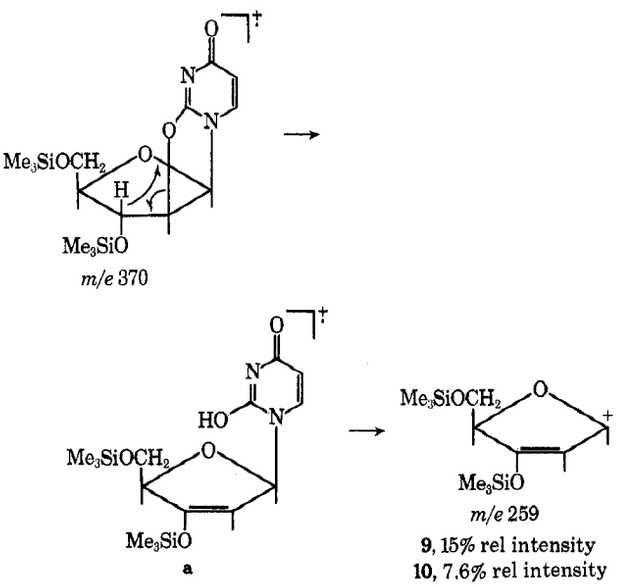


Figure 5.—Mass spectrum of the trimethylsilyl ether of 2,3′-anhydro-1-(β-D-xylofuranosyl)uracil (**6a**).

ing rearranged hydrogen rather than trimethylsilyl is also present, shifting 72 mass units lower (*m/e* 137, Figures 2–4), but is absent in the case of **6a** and **7a**. Deuterium labeling in the trimethylsilyl moiety (*i.e.*, **4b**) shows the ribose skeleton to be the source of rearranged hydrogen in *m/e* 137.

Fragmentation of the ribose ring with loss of C-4′, C-5′ and O-4′ is responsible for the ion of mass 239, which predominates in the α anomers **5a** and **10** (*m/e* 297, 44% rel intensity), and is structurally analogous to the M − 59 peak from free cyclonucleosides[5] (*m/e* 167, Figure 1). The composition $C_{10}H_{15}N_2O_3Si$, derived from exact mass data (**6a**) and deuterium labeling, requires that one hydrogen from C-4′ or -5′ be retained in *m/e* 239. Migration of the silyl function from O-5′ prior to rupture of the C-1′,O-4′ and C-3′,C-4′ bonds is also feasible, as evidenced by the occurrence of a peak at M − 59 in spectra of the 2′-linked models **4a** (*m/e* 311) and **8a** (*m/e* 327, Figure 6). Deuterium labeling in both instances reveals retention of two intact silyl groups. The absence of both ions in the spectrum of the 5′-linked model **7a** provides a further means of characterizing the 5′ linkage.

Further similarity to ions occurring in spectra of free cyclonucleosides is represented by *m/e* 259 (Figures 2, 4) shown by measurement of mass to be $C_{11}H_{23}O_3Si_2$. This ion, which is most abundant in spectra of 2,2′-linked cyclonucleosides, contains the entire ribose carbon skeleton, in analogy to *m/e* 115 in Figure 1. Unlike *m/e* 115, which bears only one labile hydrogen, *m/e* 259 retains both silyl ether moieties. As a plausible process we envision ring opening with abstraction of hydrogen from C-3′ to form the intermediate unsaturated species a, which further decomposes by cleavage of the glycosidic bond. The lower abundance of *m/e* 259 in the α anomers **5a** (Figure 4) and **10** may reflect the decreased availability of skeletal hydrogen after ring opening compared with **4a** or **9**. This well-stabilized ion is also prominent in the mass spectra of conventional nucleoside trimethylsilyl derivatives, where it is formed by elimination of Me_3SiOH and a methyl radical from the sugar moiety.[9] When the cyclic linkage is made at other positions, a related ion species (*m/e* 258) containing one less hydrogen is formed in preference to *m/e* 259 [Figures 5, 6 (**7a**)]. Further loss of CH_3 from *m/e* 258 to produce *m/e* 243 is a common feature, and is marked by metastable peaks in the spectra of **6a** and **7a**.



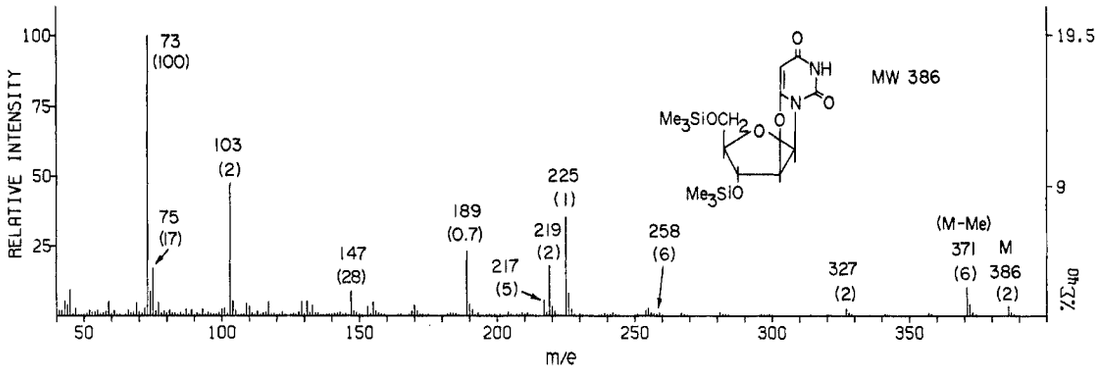


Figure 6.—Mass spectrum of the trimethylsilyl ether of 2′,6-anhydro-1-(β-D-arabinofuranosyl)-6-hydroxyuracil (**8a**). Numbers in parentheses refer to relative intensity values from the spectrum of **7a**.

When the mass spectra of trimethylsilyl-d_9 derivatives were examined to confirm the number of silicon atoms in *m/e* 258 or 259, mass shifts of primarily (>90%) 17 units rather than the expected 18 were found, as shown in Figure 3 (*m/e* 276), in those cases for which the shifts could be measured without interference from adjacent ions. The sole exception was compound **7a**, which showed more than 50% of the ion as the fully labeled d_{18} species. Although these unexpected results could be explained simply by loss of a trimethylsilyl hydrogen during formation of *m/e* 258 or 259, evidence from other ions (discussed below) indicates that *exchange* of one trimethylsilyl hydrogen has occurred at some point previous to formation of *m/e* 259 or 258. The daughter ion *m/e* 243 also shows replacement of one deuterium by hydrogen (again with the exception of **7a**) although to slightly less extent in each case than the corresponding *m/e* 258 ion.

As previously discussed, expulsion of CHO from the molecular ion was significant only in the case of the 5′-linked model **7**. This process still operates after ionization of trimethylsilyl derivatives, but, as is evident in Figures 2 and 5, occurs in other isomers as well, probably by migration of trimethylsilyl and hydrogen from the 5′ position, prior to loss of CHO. Elimination of the entire 5′ group as the elements of formaldehyde also occurs, primarily in the α anomers **5a** and **10**, following migration of trimethylsilyl and ubiquitous loss of a trimethylsilyl methyl radical (*m/e* 355 → 325, Figure 4).

The clearest indicator of the 5′ group is *m/e* 103, shown by previous studies of trimethylsilylated nucleosides[9] and mononucleotides[10] to be the intact 5′ moiety. This ion was found to be abundant in every case except **6a** (Figure 5), and was predictably absent in the case of the 5′ model **7a**. Deuterium labeling in most instances showed that substantial amounts of hydrogen from the trimethylsilyl moiety were exchanged prior to cleavage of the 4′–5′ bond, as shown by *m/e* 111 and 112 in Figure 3. The ratio *m/e* 111:*m/e* 112 from **4b** was examined as a function of ionizing electron en-

$CH_2{=}\overset{+}{O}Si(CR_3)_3$
m/e 103, R = H
m/e 112, R = D

$CH_2{=}\overset{+}{O}Si(CD_3)_2$
CD_2H
m/e 111

ergy, and was found to smoothly increase from 14 (ratio 0.75) to 70 eV (1.7). Although the exchange of a single hydrogen from C-5′ does not indicate that randomization in the usual sense has occurred, the tendency toward increased exchange at lower energies is characteristic of hydrogen randomization reactions, and has been attributed to increased ion lifetimes in the low-energy region.[14]

Exchange of a single trimethylsilyl hydrogen was also noted in *m/e* 217, an ion which occurs widely in the mass spectra of trimethylsilylated polyols such as carbohydrates and related compounds.[9,10,15] In the spectra

$\overset{+}{C}H{-}CH{=}CH$
$(CR_3)_3SiO$ $OSi(CR_3)_3$
R = H, *m/e* 217
R = D, *m/e* 235

$\overset{+}{C}H{-}CH{=}CH$
$(CD_3)_3SiO$ $OSi(CD_3)_2$
CD_2H
m/e 234

of deuterium-labeled models (*m/e* 234, Figure 3), the extent of hydrogen exchange could be measured without interference in every case but **6a** and **8a**. Shifts of 17 mass units accounted for over 80% of the ion species in each of the remaining cases except **7a**, which showed approximately 40% d_{17} and 60% d_{18} upon labeling. The substantially reduced exchange observed in **7a** for both *m/e* 217 and *m/e* 258, discussed previously, seems to implicate the 5′ position in the exchange mechanism. It is noteworthy that the exchange is apparently not extensive at the molecular ion stage, since none was indicated in any of the M − CH_3 ions from the seven labeled trimethylsilyl derivatives which were examined. The spectrum of **4a** shows a metastable peak in support of the transition *m/e* 259 → 217, which may in part account for the generally similar labeling pattern in the two ions.

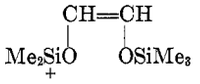
In the absence of skeletal rearrangements, *m/e* 217 should be a useful indicator of the proximity of hydroxyl groups in the parent cyclonucleoside. The very low abundance of *m/e* 217 in the spectrum of **6a** (Figure 5), which does not contain silyl ether functions within the requisite three skeletal carbons, seems to validate this hypothesis. However, the well-known tendency for trimethylsilyl group migration[16] imposes a note of caution in this interpretation. For example, the structurally similar two-carbon fragment *m/e* 189 (Figures 2, 6), whose structure as shown was supported by measurement of exact mass and deuterium labeling

(14) A. N. H. Yeo, R. G. Cooks, and D. H. Williams, *Chem. Commun.*, 1269 (1968).

(15) For example (a) O. S. Chizhov, N. V. Molodtsov, and N. K. Kochetkov, *Carbohyd. Res.*, **4**, 273 (1967); (b) G. Petersson and O. Samuelson, *Acta Chem. Scand.*, **21**, 1251 (1967); (c) W. R. Sherman, N. Ç. Eilers, and S. L. Goodwin, *Org. Mass Spectrom.*, **3**, 829 (1970).

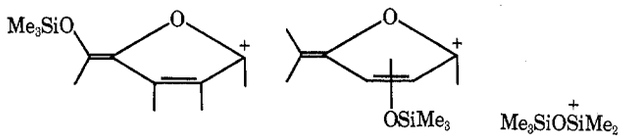
(16) See E. White, V. and J. A. McCloskey, *J. Org. Chem.*, **35**, 4241 (1970), and references cited therein.

obviously arises by trimethylsilyl migration (O-3′ to O-4′ or O-5′ to O-2′).



m/e 189

Other degradation processes in the sugar moiety include *m/e* 169,[10] which is abundant only in the spectrum of **6a**, and can be represented by either of the stable isomeric structures shown. Other ions characteristic of trimethylsilyl ethers[17] include the abundant trimethylsilyl ion *m/e* 73 ($SiMe_3^+$), *m/e* 75 (Me_2SiOH^+), *m/e* 117 ($C_2H_3O_2SiMe_2^+$),[18] and the rearranged species



m/e 169 *m/e* 147

m/e 147. All are certain to have multiple paths of formation, and were found to generally show only small amounts of hydrogen exchange in silyl methyl groups.

Experimental Section

Melting points (uncorrected) were measured on a Kofler hot-stage melting point apparatus. Uv spectra were determined using a Cary Model 15 instrument.

Low-resolution mass spectra were recorded on an LKB 9000 instrument, with sample introduction by direct probe (**4–8**) or through the gas chromatographic inlet (**4a–8a, 9, 10**); 6 ft, 1 ft or 6 in. × 0.25 in. (glass) 1% OV-17, temperature programmed at 5–10°/min from 150–200°; carrier gas separator temperature 250°, ion source 270°, probe temperatures 150–250°; accelerating voltage 3.5 kV, ionizing energy 70 eV. High resolution spectra of **5, 7, 8, 4a, 6a,** and **8a** were photographically recorded on a CEC 21-110B instrument, with sample introduction by direct probe after removal of solvents and reagents (for trimethylsilyl derivatives) in the direct inlet vacuum lock.

All trimethylsilyl derivatives showed sharp peaks with slight tailing on gas chromatography, and **7a** showed markedly decreased peak height at long retention times. Elution temperatures after programming at 10°/min from 200° (3 ft, 1% OV-17, 50 cc/min of N_2, Barber–Colman 5000 instrument): **7a** and **8a**, 235°; **4a**, 251°; **6a**, 256°; **5a** and **9**, 259°; **10**, 265°.

(17) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day, San Francisco, Calif., 1967, p 471.

(18) D. C. DeJongh, T. Radford, J. D. Hribar, S. Hanessian, M. Bieber, G. Dawson, and C. C. Sweeley, *J. Amer. Chem. Soc.*, **91**, 1728 (1969).

2,2′-Anhydro-1-(β-D-arabinofuranosyl)uracil (**4**), 2,2′-anhydro-1-(α-D-ribofuranosyl)uracil (**5**), 2,2′-anhydro-1-(β-D-arabinofuranosyl)-6-carbomethoxyuracil, and 2,2′-anhydro-1-(α-D-ribofuranosyl)-6-carbomethoxyuracil were purchased from Terra-Marine Bioresearch, La Jolla, Calif.

2,3′-Anhydro-1-(β-D-xylofuranosyl)uracil (**6**) and 2′,6-anhydro-1-(β-D-arabinofuranosyl)-6-hydroxyuracil (**8**) were supplied by Dr. J. J. Fox, Sloan-Kettering Institute for Cancer Research, Rye, N. Y.

O-3′,O-5′-**4**-d_2 was prepared by solution of **4** (5–10 μg) in D_2O in the direct probe glass sample holder. The sample was dried overnight, and introduced by direct probe simultaneously with CH_3OD from a reservoir inlet. The labeling pattern measured from the molecular ion was 11% d_0, 37% d_1, 39% d_2, 13% d_3, sufficient to determine the shifts of major fragment ions.

5′,6-Anhydro-1-(β-D-ribofuranosyl)-6-hydroxyuracil (**7**) was prepared following the outline of Lipkin, *et al.*[19] A solution of 5-iodouridine (68 mg) in 10 ml of dry DMSO was added rapidly to a solution of potassium *tert*-butoxide (20 mg) in 10 ml of dry *tert*-butyl alcohol under dry nitrogen. The solution was maintained at 60° with stirring for 24 hr. Excess potassium *tert*-butoxide was destroyed by water, the solution was applied to a water-washed Dowex 50 (H^+) (3 ml), and the eluate was concentrated to a syrup *in vacuo*. Recrystallization from aqueous ethanol afforded 21 mg (48%) of **7** in two crops: mp 283–285° dec (darkens above 275°) (lit.[19] mp 283–285° dec); $\lambda_{max}^{pH\ 7}$ 262 mμ (ε 12,200) (lit.[19] $\lambda_{max}^{pH\ 7}$ 262 mμ (ε 12,080)).

Compounds from all sources were checked for purity by gas chromatography–mass spectrometry of their trimethylsilyl derivatives, and by tlc (Eastman chromagram) using either 2-propanol–water (3:2) or water–saturated 1-butanol solvent systems.

Preparation of Trimethylsilyl Derivatives.—To a solution of cyclonucleoside (10–30 μg) in 30 μl of pyridine was added 30 μl of bis(trimethylsilyl)acetamide and 1 μl of trimethylchlorosilane (Pierce Chemical Co., Rockford, Ill.). The reaction mixture was allowed to stand for a short period (10–30 min) and then heated at 100° for 5–10 min. These conditions proved satisfactory and no further study of optimal conditions was made. Deuterium-labeled trimethylsilyl derivatives were prepared in a similar manner using bis(trimethylsilyl)acetamide-d_{18} and trimethylchlorosilane-d_9 (Merck Sharp and Dohme of Canada, Ltd., Montreal).

Registry No.—**4**, 3249-95-4; **4a**, 32414-34-9; **4b**, 32318-93-7; **5a**, 32318-94-8; **6a**, 32380-92-0; **8a**, 32380-93-1.

Acknowledgments.—This work was supported by grants from the National Institutes of Health (GM 13901) and the Robert A. Welch Foundation (Q-125), and computer facilities through NIH Grant RR 259. We are indebted to Mr. K. J. Lyman and Miss P. F. Crain for technical assistance, and to Dr. J. J. Fox of the Sloan–Kettering Institute for Cancer Research for generous gifts of samples.

(19) D. Lipkin, C. Cori, and M. Sano, *Tetrahedron Lett.*, 5993 (1968).