closely related to cob(II)alamin and give rise to a spectrum indistinguishable from that of the latter. The negative results in all experiments designed to trap cob(I)alamin or its equivalent with alkylating agents is consistent with the latter explanation although it might simply be due to the low steady-state concentration of the complex, and the stereochemical consequences of tight binding of the cobamide to the enzyme.

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References

Barker, H. A., Smyth, R. D., Weissbach, H., Toohey, J. I., Ladd, J. N., and Volcani, B. E. (1960), *J. Biol. Chem. 235*, 480.

Barker, S. B., and Summerson, W. H. (1941), *J. Biol. Chem. 138*, 535.

Beaven, G. H., Holiday, E. P., and Johnson, E. A. (1955), *in* The Nucleic Acids, Vol. 1, Chargaff, E., and Davidson, J. N., Ed., New York, N. Y., Academic Press, p 493.

Bernhauer, K., Renz, P., and Wagner, F. (1962), *Biochem. Z. 335*, 443.

Blakley, R. L. (1966), *J. Biol. Chem. 241*, 176.

Bray, G. A. (1960), *Anal. Biochem. 1*, 279.

Cleland, W. W. (1963), *Nature* (*London*) *198*, 463.

Clutterbuck, P. W., and Reuter, F. (1935), *J. Chem. Soc.*, 1467.

Firth, R. A., Hill, H. A. O., Pratt, J. M., Williams, R. J. P., and Jackson, W. R. (1967), *Biochemistry 7*, 2178.

Hamilton, J. A., Yamada, R., Blakley, R. L., Hogenkamp, H. P. C., Looney, F. D., and Winfield, M. E. (1971), *Biochemistry 10*, 347.

Hogenkamp, H. P. C., Ghambeer, R. K., Brownson, C., Blakley, R. L., and Vitolis, E. (1968), *J. Biol. Chem. 243*, 799.

Hogenkamp, H. P. C., and Rush, J. E. (1968), *Biochemical Prepn. 12*, 124.

Johnson, A. W., Mervyn, L., Shaw, N., and Smith, E. L. (1963), *J. Chem. Soc.*, 4146.

Klotz, I. (1953), *Proteins 1*, 727.

Ladd, J. N., Hogenkamp, H. P. C., and Barker, H. A. (1961), *J. Biol. Chem. 236*, 2114.

Müller, O., and Müller, G. (1962), *Biochem. Z. 336*, 299.

Nicolet, B. H., and Shinn, L. A. (1941), *J. Amer. Chem. Soc. 63*, 1456.

Orr, M. D., Panagou, D., and Blakley, R. L. (1971), *Anal. Biochem.* (in press).

Paulus, H. (1969), *Anal. Biochem. 32*, 91.

Rochat, C., Rochat, H., and Edman, P. (1970), *Anal. Biochem. 37*, 259.

Schrauzer, G. N., and Lee, L. P. (1970), *Arch. Biochem. Biophys. 138*, 16.

Schrauzer, G. N., and Sibert, J. W. (1970), *J. Amer. Chem. Soc. 92*, 1002.

Shelton, K. R., and Clark, J. M., Jr. (1967), *Biochemistry 6*, 2735.

Yamada, R., Shimizu, S., and Fukui, S. (1968), *Biochemistry 7*, 1713.

Mass Spectrometry of Trifluoroacetyl Derivatives of Nucleosides and Hydrolysates of Deoxyribonucleic Acid[*]

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ABSTRACT: Mass spectra of the trifluoroacetyl derivatives of ribonucleosides and deoxyribonucleosides have been examined in detail and shown to be useful for the determination of a number of structural features. Reaction conditions were employed in which sugar hydroxyls and amino or alkylated amino groups in the base were trifluoroacetylated. The most useful ions for determination of molecular mass and composition were a series of elimination reactions from the molecular ion involving trifluoroacetyl moieties. Breakage of the glycosidic bond with retention of charge in the base produced major ions representing the base and its substituents. Specific fragmentation reactions were found which permit recognition of N,N-dimethylation, and permit differentiation between purines methylated at N-1 and N^6. Trifluoroacetylated, unfractionated hydrolysates of DNA can be examined for the presence of minor deoxyribonucleosides. Exact mass values derived from photographically recorded high resolution mass spectra provide an independent and objective means of identifying components of a hydrolysate, which is not dependent upon ultraviolet absorbance or chromatographic mobility.

In recent years a number of studies have demonstrated the potential role of mass spectrometry for dealing with structural problems of nucleic acids and their analogs (McCloskey, 1971). The majority of these have involved the structure determination of modified nucleosides isolated from transfer RNA (*e.g.*, Baczynskyj *et al.*, 1968; Carbon *et al.*, 1968; Armstrong *et. al.*, 1970) which were examined as free (chemically underivatized) compounds. However, since constituents of nucleic

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acids are often of marginally sufficient volatility for mass spectrometry, various procedures for conversion to volatile derivatives (McCloskey, 1971) have provided a means of extending the technique to include the more polar nucleosides, such as guanosine (McCloskey *et al.*, 1968), and small nucleotides (Hunt *et al.*, 1968; Dolhun and Wiebers, 1969; Lawson *et al.*, 1971a), without thermal degradation. Although mass spectra of the four major 2′-deoxyribonucleosides have been reported (Biemann and McCloskey, 1962; Brown *et al.*, 1969; Tsunakawa, 1969; McCloskey, 1971), no systematic effort has been directed toward modified deoxyribonucleosides, or for procedures for the examination of hydrolysates of DNA.

As part of recent work in this laboratory to explore the mass spectrometry of nucleic acids (Lawson *et al.*, 1971a, and references therein), we have investigated the use of trifluoroacetyl derivatives of nucleosides and deoxynucleosides, with a particular view toward the examination of hydrolysates for biologically modified components. Several experimental approaches have been found suitable, depending upon the goals of the investigation. For the determination of nucleoside structure, the isolated compound can be trifluoroacetylated and its mass spectrum examined in detail. For the identification of known or suspected components of hydrolysates the mixture can be derivatized and subjected directly to low- or high-resolution mass spectrometry without fractionation. Individual components are then identified by characteristic fragment ions, or when using high resolution methods, by recognition of predetermined sets of structurally diagnostic exact mass values (Lawson *et al.*, 1971b). Since no reliance is placed upon chromatographic mobility or ultraviolet absorbance, this latter approach has the inherent advantage of being insensitive to interference from many types of impurities.

Materials and Methods

Materials. Nucleosides were obtained from the following sources: Calbiochem (Los Angeles, Calif.)—2′-deoxycytidine, 5-methyl-2′-deoxycytidine, 2′-deoxyadenosine, 2′-deoxyguanosine, 2′-deoxyuridine, 5-hydroxymethyl-2′-deoxyuridine, 5-methylamino-2′-deoxyuridine, thymidine, 3-methylthymidine, cytidine, guanosine, uridine, 5,6-dihydrouridine, ribosylthymine, inosine, 1-methylinosine; Sigma Chemical Co. (St. Louis, Mo.)—3′-deoxyadenosine, N^6,N^6-dimethyl-3′-amino-3′-deoxyadenosine, adenosine, N^6-methyladenosine, N^6,N^6-dimethyladenosine, 1-methyladenosine, 7-methylguanosine, N^2-methylguanosine, N^2,N^2-dimethylguanosine, pseudouridine, 7-methylinosine, 7-methylxanthosine; Pierce Chemical Co. (Rockford, Ill.)—5-bromo-2′-deoxycytidine, 2′-deoxyinosine; Cyclo Chemical (Los Angeles, Calif.)—N^6-methyl-2′-deoxyadenosine, 7-methyl-2′-deoxyguanosine, 1-methylguanosine. 4′-Thioadenosine, N^6-(3-methyl-2-butenyl)-adenosine, 9-β-psicofuranosyladenine, and 6-methyluridine were supplied by the Cancer Chemotherapy National Service Center of the National Institutes of Health. 2′-*C*-Methyladenosine, 3′-*C*-methyladenosine and 5′,5′-di-*C*-methyladenosine were gifts from Dr. F. W. Holly (Merck, Sharp and Dohme, Rahway, N. J.); 5-hydroxymethyluridine and uridin-5-oxyacetic acid from Dr. S. Nishimura (National Cancer Center Research Institute, Tokyo, Japan). Nucleosides were checked for purity by gas chromatography or mass spectrometry of their trimethylsilyl derivatives (Hancock and Coleman, 1965; Sasaki and Hashizume, 1966; McCloskey *et al.*, 1968).

DNA from calf thymus (type V) and from *Micrococcus lysodeikticus* (type X) was purchased from Sigma Chemical Co. Alkaline phosphatase (BAPC) and venom phosphodiesterase (VPH) were obtained from Worthington Biochemical Corp. (Freehold, N. J.), and deoxyribonuclease from Pierce Chemical Co. DEAE-polyacrylamide (Bio-Gel DM-2) was supplied by Calbiochem.

Hydrolysis of DNA. The digestion mixture consisted of 0.5-ml total volume, which contained 2 mg of DNA, 200 μg of deoxyribonuclease, 50 μg of phosphodiesterase, 40 μg of alkaline phosphatase, 1.2 μmoles of magnesium acetate, and 200 μmoles of ammonium acetate (pH 8.8). Incubations were conducted at 37° for 18 hr. The incubation mixture was passed through a microcolumn (0.5 × 2 cm) of DEAE-polyacrylamide in a syringe fitted with a Swinny adapter and a 1.2 μ Millipore filter to exclude mechanical contamination of the sample by resin, followed by sufficient H_2O (4–5 ml) to effect complete recovery of deoxyribonucleosides. The extent of hydrolysis was determined by recovery of A_{260} units. The sample was then freeze dried, then dried over P_2O_5 at 80° *in vacuo* for 2–3 hr prior to conversion into trifluoroacetates. On occasion, low molecular weight contaminants, in particular traces of glucose, were found to interfere with derivatization and produced abnormally high pressures during vaporization of the sample in the mass spectrometer. These effects were prevented by dialysis of the DNA and enzymes before hydrolysis. DNA and venom phosphodiesterase were dialyzed against 0.1 M NaCl; deoxyribonuclease and alkaline phosphatase were dialyzed against 10 mM ammonium acetate (pH 8.8).

Preparation of Trifluoroacetyl Derivatives. The most satisfactory procedure for both individual nucleosides and hydrolysates consisted of heating the free nucleosides, usually totals of 50–100 μg or proportionally less, with 100 μl of CH_2Cl_2 and 7 μl of trifluoroacetic anhydride in a sealed ampoule or very tightly closed screw-capped vial with a Teflon liner in the cap, for 30 min at 100°. Nucleosides of guanine showed generally poor conversion under these conditions but were improved when the reaction mixture was instead heated at 150° for 5 min. However, the latter conditions were not used as a general procedure because at that temperature (or with longer heating periods) some cleavage of the gylcosidic bond resulted. Under those conditions, particularly in the case of 7-methylpurine nucleosides and many 2′-deoxynucleosides, the mass spectrum of the corresponding acylated base was obtained in addition to that of the nucleoside.

Mass spectra of the model compounds listed previously show that under the above conditions (100°), acylation occurs at all hydroxyl groups of the ribose or deoxyribose moieties and at amino functions which bear active hydrogens, while enolizable carbonyl groups in general do not react. For example, 2′-deoxycytidine, 2′-deoxyguanosine, and uridine each add three trifluoroacetyl groups, while N^6-(3-methyl-2-butenyl)adenosine, N^2-methylguanosine, and cytidine incorporate four groups. Exceptions to this behavior are dihydrouridine and 7-methylinosine, which form mixtures of tri- and tetraacyl derivatives. 2′-Deoxyadenosine also forms small amounts of the lower diacyl derivative, while N^2,N^2-dimethylguanosine produces a trace of the higher derivative containing four groups. In addition to the expected tetrakis(trifluoroacetate), 1-methyladenosine also forms a substantial amount (∼25%) of a pentaacyl derivative which contains two trifluoroacetyl groups in the base. The two derivatives are easily separated on a packed gas chromatographic column such as 3 ft, 1% SE-30, the higher derivative eluting first (188°), followed by the tetraacetate (195°), but both later than the isomeric derivative of N^6-methyladenosine (121°). Molecules which bear free (nonenolic) hydroxyl groups in the base will undergo reaction at these sites, as, for example, 5-hydroxymethyluridine; however the carboxyl group of uridin-5-oxyacetic acid did not react under the standard conditions employed.

Determination of Mass Spectra. Low-resolution mass spectra were acquired of the trifluoroacetyl derivatives of all compounds listed under Materials, using an LKB 9000 instrument. Ionizing electron energy was 70 eV, and ion source and carrier gas separators 250° unless specified otherwise. The direct inlet probe was used for hydrolysates of DNA, and for all model compounds except derivatives of 1-methyladenosine, N^6-methyladenosine, N^6,N^6-dimethyladenosine, N^6-(3-methyl-2-butenyl)adenosine, thymidine, 3-methylthymidine, ribosylthymine, 5-methyl-2′-deoxycytidine, 1-methylinosine, 5-bromo-2′-deoxyuridine, and 5,6-dihyrouridine. Derivatives of the foregoing compounds were introduced by gas chromatograph, using 1% SE-30 stationary phase (0.25 in. × 3 ft). Probe samples were prepared by placing several microliters of the reaction mixture in a glass sample tube; reagents were removed under slight vacuum in a desiccator with care taken to avoid splattering of the sample during evaporation.

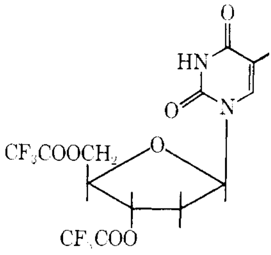
High resolution mass spectra were photographically recorded using a CEC 21-110B instrument, with ion source temperatures 220–260°, 70 eV ionizing energy, and sample introduction by direct probe. Resolving power was set at 17,000–20,000; Ilford Q2 photoplates were developed 15 min (20°) in Kodak Microdol-X for model compounds, and for 10 min (20°) in Kodak D-19 for sensitivity studies and hydrolysates. For hydrolysates, exposure periods of 2–3 min were employed, during which time vaporization of individual components of the mixture occurs. Ion beam levels of 10^{-9}–10^{-10} A usually resulted, with integrated exposure levels of $\sim 10^{-9}$ C. Exact mass measurements were made for all ions of derivatives of guanosine, 7-methylguanosine, adenosine, 4′-thioadenosine, 5′,5′-di-*C*-methyladenosine, 5,6-dihydrouridine, and of selected ions from derivatives of 2′-deoxyadenosine, thymidine, and uridine.

The basic computer system and procedure used for identification of individual nucleosides in hydrolysates by recognition of exact mass values have been previously described (Lawson *et al.*, 1971b). More recently, the incremental magnetic tape recorder was replaced by a PDP 8/I computer which is on line to a central facility (IBM 360-50). Under the present system the data acquisition time for a full high-resolution mass spectrum is approximately 5 min, which typically involves 150 lines for single components, or about 800 lines for a hydrolysate of DNA.

Results and Discussion

The most severe limitation in the applications of mass spectrometry to constituents of nucleic acids is their high polarity and therefore low volatility. Trifluoroacetylation offers a useful means of conversion of nucleosides into relatively volatile derivatives on a micro scale, and may be considered as a technique complementary to trimethylsilylation (McCloskey *et al.*, 1968), phenylboronation, permethylation, or 2′,3′-*O*-isopropylidene formation (Dolhun and Wiebers, 1969). The primary utility of these derivatives in mass spectrometry lies in the ability to make unambiguous structure–spectra correlations. We have therefore examined a relatively large number of model compounds to determine which fragmentation processes may be useful for the determination of structural modification.

Interpretation of Mass Spectra. Mass spectra of the major deoxyribonucleoside derivatives from DNA (I–IV) are shown in Figures 1–4. The principal peaks from spectra of the major ribonucleosides and selected modified derivatives are given in Table I. The conclusions and discussions in this section are based in general on mass spectra of model compounds listed in the materials section. Spectra of those compounds not presented in figures or Table I are available upon request from the authors.



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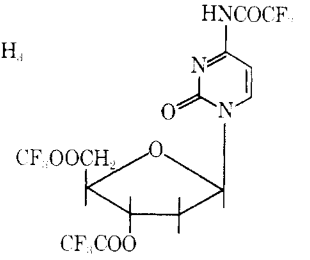

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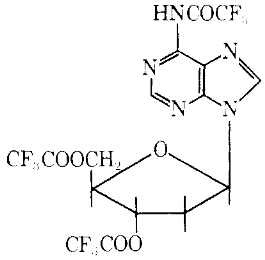

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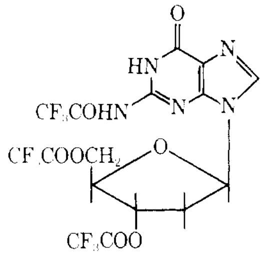


IV

Molecular ion (M) stabilities of both ribonucleoside and deoxyribonucleoside derivatives vary widely, as shown in Table I. In some instances, most notably the trifluoroacetates of dihydrouridine and cytidine, a tendency to form M + H ions through pressure-induced intermolecular hydrogen-transfer reactions was observed. This behavior, as well as the low abundances of molecular ions of some compounds, does not interfere with the recognition of M and the corresponding establishment of molecular weight because of the presence of numerous fragment ions which are numerically related to M. The most useful of these are due to losses of F and CF_3 radicals (19 and 69 mass units) from the molecular ion, forming a characteristic pattern in the upper mass range. The abundance data in Table I consistently show that M − CF_3 is greatly suppressed in those molecules which do not contain an *N*-trifluoroacetyl function in the base. From this we conclude that loss of CF_3 from the molecular ion occurs predominantly from the base rather than sugar, and is due to charge localization and subsequent stabilization from nitrogen, as, for example, in Figure 2. Other prominent ions are associated with losses of intact or partial trifluoroacetyl groups from the molecular ion. As shown in Figures 1–4, these reactions include losses of CF_3CO (97 mass units), CF_3CO_2 (113), CF_3CO_2H (114), $CF_3CO_2H + CF_3$ (183), $CF_3CO_2H + CF_3CO_2$ (227), and $2CF_3CO_2H$ (228), as well as other combinations. Although all possible members of these series are not observed in the spectrum of any given compound, a sufficient number are found to provide an extensive cross-check of the value of M. As discussed in a later section, these ions are also important for the identification of individual components of unresolved mixtures.

In parallel with the behavior of free nucleosides and other

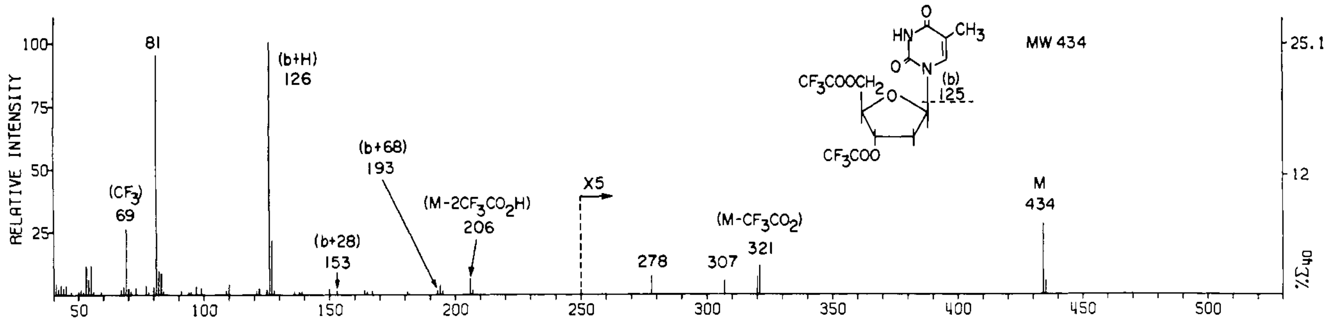


FIGURE 1: Mass spectrum of O-3′,5′-bis(trifluoroacetyl)thymidine.

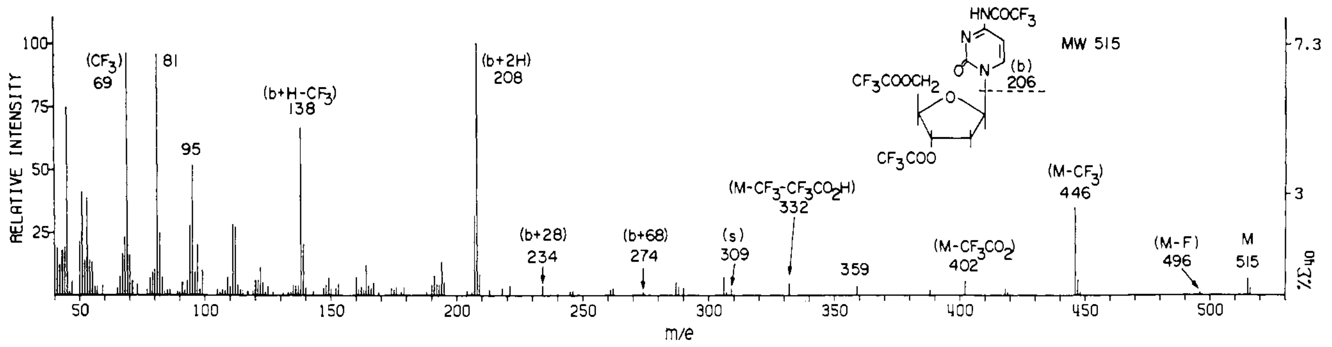


FIGURE 2: Mass spectrum of N^4,O-3′,5′-tris(trifluoroacetyl)-2′-deoxycytidine.

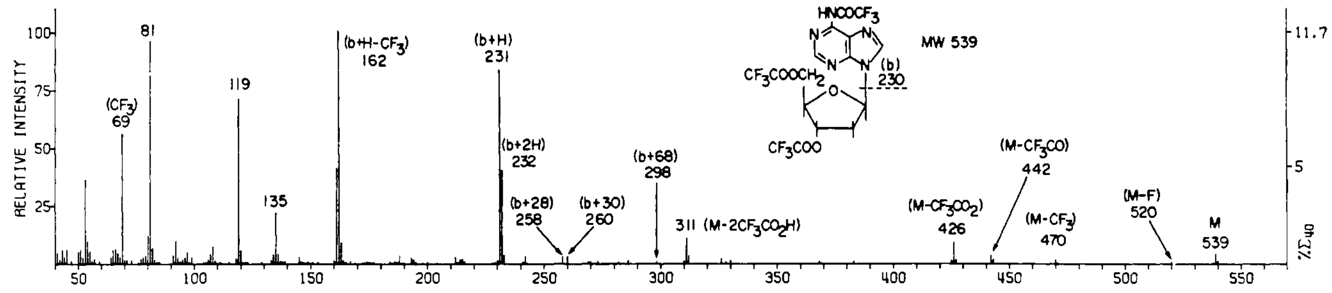


FIGURE 3: Mass spectrum of N^6,O-3′,5′-tris(trifluoroacetyl)-2′-deoxyadenosine.

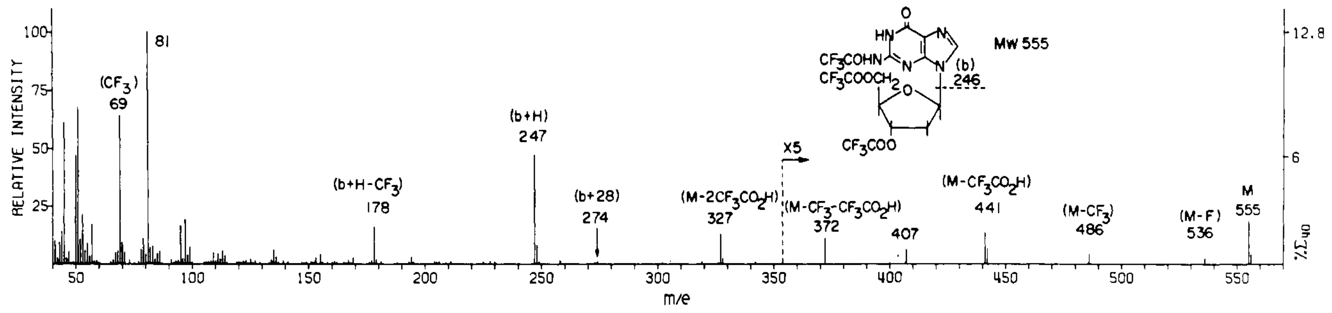


FIGURE 4: Mass spectrum of N^2,O-3′,5′-tris(trifluoroacetyl)-2′-deoxyguanosine.

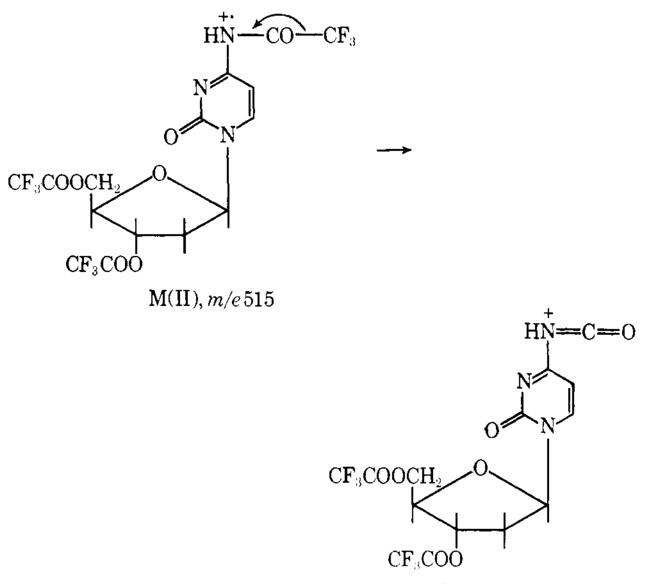
derivatives (McCloskey, 1971), cleavage of the glycosidic bond is a major and diagnostically important process. The base fragment itself (ion b) is of generally low abundance or is absent, but the rearranged species b + H or b + 2H serve as primary indicators of the mass of the base. In the mass spectra of free nucleosides the relative abundances of b + H *vs.* b + 2H were found to have some correlation with the nature of the base (purine or pyrimidine) and sugar (ribose or deoxyribose), and the sources of hydrogen were shown to be primarily the hydroxyl hydrogens (Biemann and McCloskey, 1962). In the case of trifluoroacetyl derivatives the source of rearranged hydrogen in these ions is the ribose carbon skeleton, with the result that no predictable values of the ratio (b + H):(b + 2H) were obtained.

As a general process, b + H further loses a CF_3 radical if the base is acylated. For example, in the trifluoroacetate of 2′-deoxycytidine (Figure 2), charge localization at N^4 in the b + H ion leads to *m/e* 138 by a mechanism similar to that proposed above for the formation of M − CF_3. In many instances, ions corresponding to the mass of b + H − CF_3

TABLE I: Selected Ions from the Mass Spectra of Nucleoside Trifluoroacetyl Derivatives.

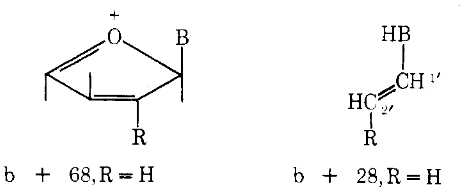
Parent Nucleoside	No. of Trifluoroacetyl Groups	Mass (Relative Abundance)								
		M	M − F	M − CF_3	M − 227	b + H	b + H − CF_3	s	s − $2CF_3CO_2H$	Other Diagnostic Ions
2′-Deoxyuridine	2	420 (2.0)	401 (0.0)	351 (0.0)	193 (1.2)	112 (12)	*a*	309 (1.7)	81 ((100)	113 (23),[c] 264 (1.2), 307 (1.4)[b]
2′-Deoxyinosine	2	444 (4.4)	425 (0.4)	375 (0.3)	217 (48)	136 (69)	*a*	309 (0.0)	81 (100)	69 (43), 216 (18),[d] 331 (1.3)[b]
3-Methylthymidine	2	448 (2.9)	429 (0.2)	379 (0.0)	221 (0.6)	140 (100)	*a*	309 (0.0)	81 (24)	83 (13), 335 (1.3)[b]
Uridine	3	532 (2.0)	513 (5.5)	463 (0.0)	305 (24)	112 (12)	*a*	421 (29)	193 (100)	165 (14), 418 (8.2),[f] 419 (6.9)[b]
Pseudouridine	3	532 (0.2)	513 (5.3)	463 (0.0)	305 (33)	112 (16)	*a*	421 (0.0)	193 (2.4)	69 (100), 191 (51), 418 (9.0)[f], 419 (2.5)[b]
5,6-Dihydrouridine	3	534 (0.0)[g]	515 (5.0)	465 (0.0)	307 (28)	114 (5.7)	*a*	421 (6.0)	193 (18)	69 (100), 392 (14), 420 (11),[f] 461 (0.5)
5-Hydroxymethyl-2′-deoxyuridine	3	546 (5.0)	527 (0.5)	477 (0.0)	319 (2.4)	238 (12)	169 (0.7)	309 (11)	81 (100)	125 (48), 194 (24), 433 (9.0)[b]
Ribosylthymine	3	546 (12)	527 (5.9)	477 (0.0)	319 (6.1)	126 (90)	*a*	421 (15)	193 (100)	82 (33), 413 (1.5), 432 (36)[f]
Inosine	3	556 (52)	537 (7.1)	487 (0.0)	329 (8.6)	136 (100)	*a*	421 (4.4)	193 (65)	69 (82), 137 (27),[c] 215 (8.5),[h] 443 (9.5)[b]
7-Methyl-2′-deoxyguanosine	3	569 (0.2)	550 (0.6)	500 (3.1)	342 (1.1)	261 (100)	192 (99)	309 (0.5)	81 (58)	149 (40), 341 (2.8),[d] 359 (9.5)[e]
*N*⁶,*N*⁶-Dimethyladenosine	3	583 (3.8)	564 (4.7)	514 (0.5)	356 (1.8)	163 (21)	*a*	421 (0.0)	193 (12)	134 (100), 148 (31), 162 (58), 554 (15), 568 (1.0)
*N*²,*N*²-Dimethylguanosine	3	599 (100)	580 (7.7)	530 (0.0)	372 (2.0)	179 (77)	*a*	421 (0.0)	193 (12)	150 (37), 164 (20), 261 (10), 570 (3.1)
Uridin-5-oxyacetic acid	3	606 (6.7)	587 (3.8)	537 (0.0)	379 (1.4)	186 (41)	*a*	421 (6.1)	193 (56)	69 (100), 141 (32), 447 (25), 492 (1.3),[f] 561 (1.8)
Cytidine	4	627 (0.1)[g]	608 (5.6)	558 (31)	400 (18)	207 (1.8)	138 (100)	421 (2.6)	193 (46)	69 (80), 247 (15), 444 (28)
Adenosine	4	651 (5.1)	632 (4.2)	582 (2.1)	424 (6.4)	231 (24)	162 (38)	421 (1.1)	193 (88)	69 (100), 232 (58),[c] 260 (38), 310 (9.4),[h] 538 (21)[b]
1-Methyladenosine	4	665 (0.2)	646 (1.6)	596 (44)	438 (00)	245 (1.0)	176 (100)	421 (0.0)	193 (6.2)	69 (22), 246 (1.6),[c] 55 (1.6)[b]
*N*⁶-Methyladenosine	4	665 (8.2)	646 (2.5)	596 (5.1)	438 (1.4)	245 (3.5)	176 (19)	421 (0.3)	193 (30)	148 (100), 246 (13),[c] 274 (11), 568 (19)
Guanosine	4	667 (34)	648 (3.4)	598 (0.0)	440 (6.1)	247 (89)	178 (20)	421 (2.4)	193 (92)	69 (100), 326 (8.0),[h] 484 (42), 553 (20)[f]
1-Methylguanosine	4	681 (48)	662 (6.0)	612 (6.0)	454 (2.8)	261 (68)	192 (39)	421 (2.7)	193 (96)	69 (100), 164 (3.6), 340 (6.6),[h] 498 (41)
*N*²-Methylguanosine	4	681 (95)	662 (9)	612 (1.8)	454 (5.2)	261 (69)	192 (18)	421 (2.5)	193 (87)	69 (100), 164 (55), 205 (30), 350 (15), 584 (10)
7-Methylguanosine	4	681 (1.0)	662 (0.4)	612 (1.7)	454 (1.6)	261 (100)	192 (91)	421 (12)	193 (58)	69 (100), 149 (74), 472 (18), 567 (4.9)
*N*⁶-(3-Methyl-2-butenyl)adenosine	4	719 (23)	700 (0.8)	650 (2.3)	492 (0.0)	299 (2.8)	230 (5.0)	421 (0.4)	193 (26)	162 (34), 202 (100), 582 (16), 622 (76), 704 (14)

[a] Occurrence not possible since the base contains no trifluoroacetyl group. [b] M − CF_3CO_2. [c] b + 2H. [d] M − $2CF_3CO_2H$. [e] M − $C_4F_6O_3$. [f] M − CF_3CO_2H. [g] M + H from ion-molecule reactions usually observed. [h] M − $2CF_3CO_2H$ − CF_3CO_2.



are present in the mass spectra of derivatives which contain no trifluoroacetyl group in the base. These ions differ in elemental composition from b + H − CF_3 and are generally less prominent than those cases in which the *N*-trifluoroacetyl group is present in the base. In summary, both b + H − CF_3 and M − CF_3 provide a strong indication of the presence or absence of an *N*-trifluoroacetyl function, and therefore of an NH group in the parent nucleoside.

A number of other fragment ions occur as a general series of ions in which the base is attached to fragments of the sugar moiety. Although these ions are of low abundance, their identities can be confirmed in questionable cases by measurement of exact mass. In addition to establishing the identity of the base they are useful indicators for cases of sugar modification, such as thiation or alkylation. The more important of these minor ions occur at base (ion b) + 68, b + 28, and b + 30 mass units in deoxyribonucleoside derivatives, and at b + 180, b + 140, and b + 30 in ribonucleoside spectra. The b + 68 ion (Figures 2 and 3) formally corresponds to loss of the elements of $CF_3CO_2CH_2$ plus CF_3CO_2H from the molecular ion. The implied absence of C-5′ in this ion is confirmed by its shift 14 mass units higher in the spectra of 2′- and 3′-*C*-methyladenosine derivatives than in adenosine (*m/e* 410, 1.3% relative intensity), as well as 126 mass units in 9-β-psicofuranosyladenine, and its failure to shift in the spectrum of 5′,5′-di-*C*-methyladenosine. This ion is therefore isomeric with the structure shown in Chart I.

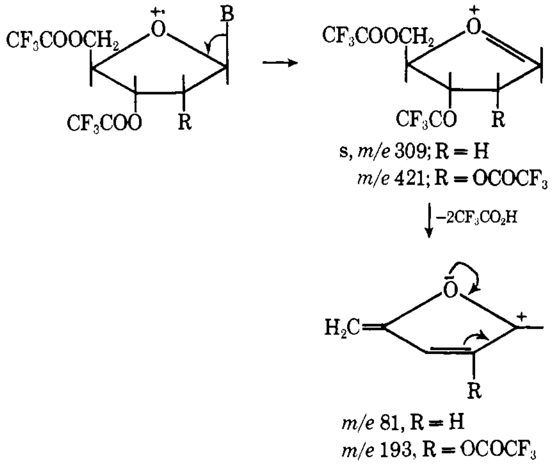


b + 68, R = H b + 180, R = OCOCF3

b + 28, R = H b + 140, R = OCOCF3

Retention of carbons-1′ and -2′ and hydrogen transferred from the fragment which is lost lead to ion b + 28 (2′-deoxy derivatives) or b + 140 (ribonucleosides), which has analogy in the spectra of nucleoside trimethylsilyl derivatives (McCloskey *et al.*, 1968). This ion (Figures 1–4) is sensitive to substitution at C-1′ or -2′, as demonstrated by its shift to appropriately higher values on substitution by methyl at C-2′ in adenosine (*m/e* 384, 1.5% relative intensity) or hydroxymethyl at C-1′ (*m/e* 496, 0.2% relative intensity). In the case of *O*-3′,5′-bis(trifluoroacetyl)thymidine (I), b + 28 (*m/e* 153.0664) forms the minor member (1:2) of a doublet with *m/e* 153.0159, a minor fragment from the sugar moiety, as determined from the high-resolution mass spectrum. There is presently no evidence for the location of the rearranged hydrogen. The representation shown above in which hydrogen has migrated to the base is in analogy to rearrangement processes in free nucleosides (Shaw *et al.*, 1970). Another highly useful ion consists of the base plus CH_2O. This ion is prominent in the mass spectra of most nucleosides and their derivatives (McCloskey, 1971), but in the case of trifluoroacetyl derivatives appears most consistently in nucleosides of adenine. The presence of O-4′ in the b + 30 ion is demonstrated by the shift of b + 30 from the adenosine derivative (*m/e* 260, Table I) to b + 46 in the spectrum of the trifluoroacetate of 4′-thioadenosine (*m/e* 276, 5% relative intensity). Further decomposition of b + 30 in the spectrum of the adenosine derivative proceeds by loss of CO to form b + 2H (*m/e* 232) and is marked by a metastable peak at *m/e* 207.6. Deoxynucleoside derivatives frequently show a peak at b + 153 which has no counterpart in the mass spectra of the ribose derivatives. Measurement of its exact mass in the spectrum of I (*m/e* 278.0508) supports the composition $C_{10}H_9N_2O_4F_3$, or the general composition M − (CF_3CO_2H + CH_2CO). Other minor fragment ions derived from fragmentation in the sugar moiety include b + 182 (294 for ribonucleoside derivatives) resulting from loss of CH_2OCOCF_3 by simple cleavage of the C-4′,5′ bond. Another fragment often observed in these spectra is b + 40 (C_3H_4) or b + 152 (C_3H_3 + CF_3CO_2) for deoxyribose or ribose derivatives, respectively. These ions formally represent the combined loss of CF_3CO_2H, CH_2CO, and CF_3CO_2 from the molecular ion, and have retained C-2′ and -3′, based on mass shifts in the spectra of 2′- and 3′-*C*-methyladenosine derivatives.

SCHEME I



Cleavage of the glycosidic bond with retention of the charge in the sugar moiety leads to ion s, at *m/e* 309 or 421 (see Scheme I). This ion tends to be low in abundance in spectra of deoxyribonucleoside derivatives, but varies widely in the case of ribonucleosides, as shown in Table I. For most nucleosides derived from nucleic acids these mass values will not vary, but will change in special cases involving heteroatom substitution, such as the puromycin nucleoside, N^6,N^6-dimethyl-3′-amino-3′-deoxyadenosine (s = *m/e* 420, 1.8% relative intensity). Expulsion of two neutral molecules of trifluoroacetic acid from ion s yields a highly stabilized ion of mass 81 or 193, whose generalized structure is represented

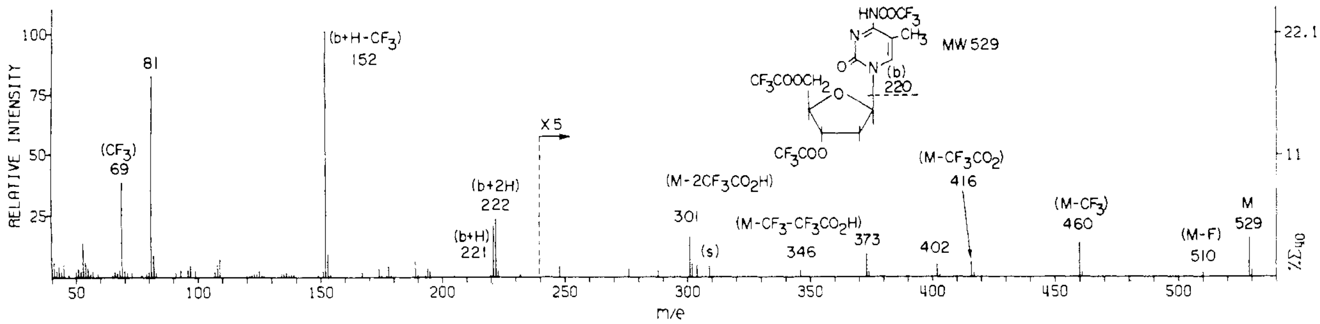


FIGURE 5: Mass spectrum of N^4,O-3′,5′-tris(trifluoroacetyl)-5-methyl-2′-deoxycytidine.

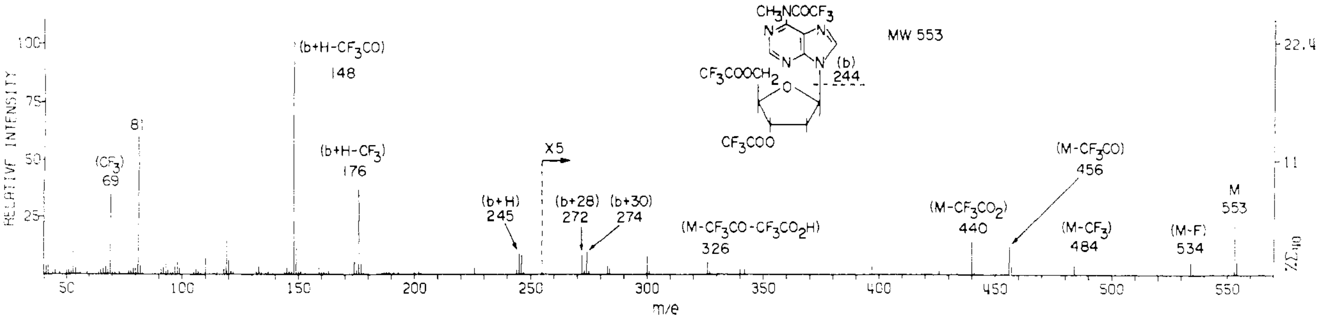
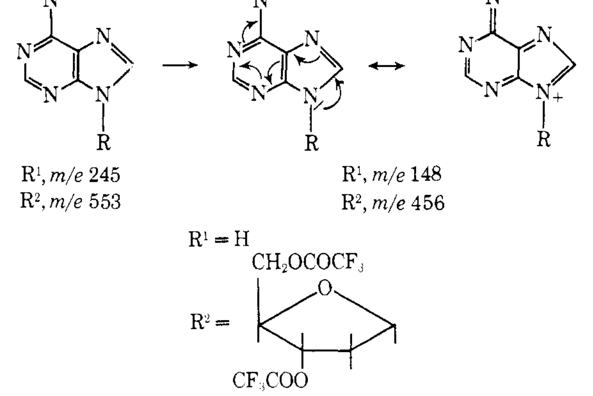


FIGURE 6: Mass spectrum of N^6,O-3′,5′-tris(trifluoroacetyl)-N^6-methyl-2′-deoxyadenosine.

above. This ion is the base peak in many spectra (Table I), and serves as the primary feature to distinguish nucleosides of DNA from those of RNA. Many ribonucleoside derivatives in addition show a characteristic peak at *m/e* 165. A metastable transition in the spectra of the trifluoroacetates of guanosine and uridine shows that *m/e* 165 is generated by expulsion of CO from *m/e* 193. 4′-Thioadenosine further reveals the 4′-heteroatom to be lost in that transition.

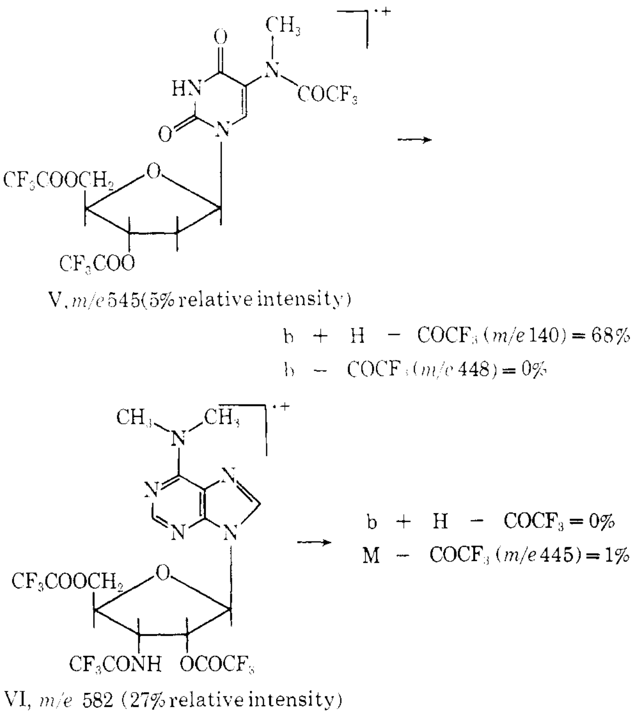
Mass Spectra of Modified Nucleosides. Methylation, the most common form of biological modification in nucleic acids, leads to changes in mass spectra which were found to be highly informative in terms of the position of alkylation. Of the model nucleosides examined in the present study, C-methylation in the base produced the most subtle effects, while aminomethylation resulted in highly characteristic and diagnostic fragment ions. An example of the former is given by the trifluoroacetyl derivative of 5-methyl-2′-deoxycytidine (Figure 5). In comparison with Figure 2, the addition of a methyl group is clearly shown by the upward shift of M and its related ions (M − F, M − CF_3, etc.), as well as b + 2H, and b + H − CF_3, by 14 mass units. Similar conclusions hold for comparisons of the derivatives of thymidine and 2′-deoxyuridine, and ribosylthymine and 6-methyluridine, which indicate that although such compounds are easily distinguished by mass spectrometry, location of the site of methylation is difficult in the absence of reference mass spectra.

SCHEME II



Methylation of amino groups in the base was found to lead to prominent ions resulting from loss of a trifluoro-

SCHEME III



V, *m/e* 545 (5% relative intensity)

VI, *m/e* 582 (27% relative intensity)

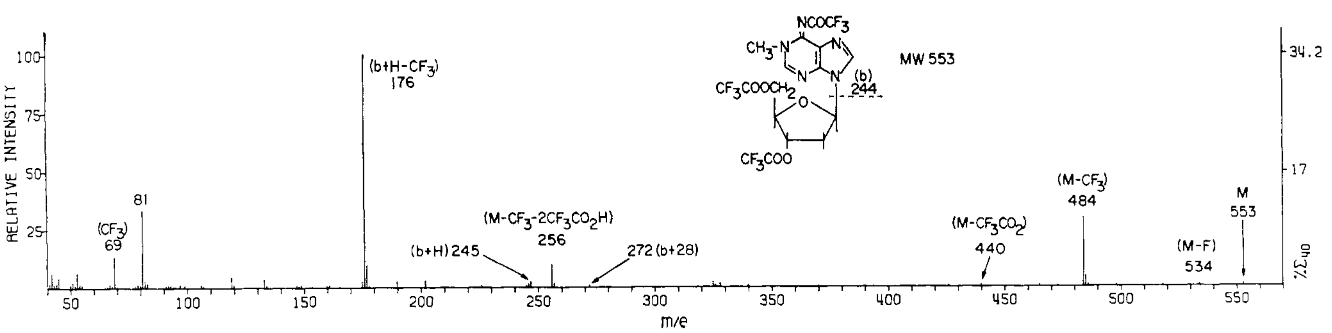
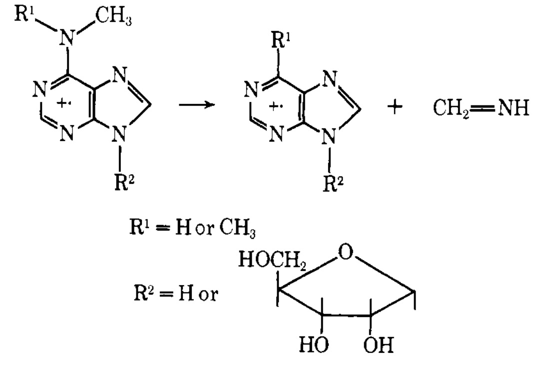


FIGURE 7: Mass spectrum of N^6,O-3′,5′-tris(trifluoroacetyl)-1-methyl-2′-deoxyadenosine.

acetyl radical (97 mass units) both from the molecular ion and from b + H. These characteristic ions are found at *m/e* 456 and 148 in the spectrum of the trifluoroacetyl derivative of N^6-methyl-2′-deoxyadenosine, shown in Figure 6. The corresponding ions appear in the spectra of trifluoroacetates of N^6-methyladenosine (*m/e* 568, 148), N^6-(3-methyl-2-butenyl)-adenosine (*m/e* 622, 202), and N^2-methylguanosine (*m/e* 584, 164), as indicated in Table I. We attribute the stabilities of these ions primarily to extensive resonance stabilization by the electron-rich aromatic base shown in Scheme II. The stability of the trifluoroacetyl radical which is lost is also an important factor, as shown by the substantial decrease in abundance of *m/e* 148 and 568 for N^6,N^6-dimethyladenosine (loss of CH_3) compared with the N^6-methyladenosine derivative (loss of $COCF_3$), as indicated in Table I. In the spectrum of the trifluoroacetate of 5-methylamino-2′-deoxyuridine (V), b + H − $COCF_3$ (Scheme III) is observed but M − $COCF_3$ is not, evidently reflecting the lower stabilizing ability of the pyrimidine nucleus compared with purines. This latter effect may also play a role in the low intensity of M − $COCF_3$ in Figure 2, along with the absence of inductive effects provided by N-methylation. In the case of the nucleoside from puromycin (VI) which contains an amino group at C-3′, the poorly stabilized M − $COCF_3$ ion is of low abundance, while b + H (*m/e* 163) cannot produce a b + H − $COCF_3$ peak.

Earlier work has shown that methylation at N^6 in the adenine moiety leads to a characteristic elimination of methylenimine (Eggers *et al.*, 1966; Shannon and Letham, 1966; Rahamim *et al.*, 1967; Shaw *et al.*, 1970) (Scheme IV). In the present study it was found that these highly diagnostic reactions occur as major processes both from M and b + H ions of N,N-dimethyl derivatives as in the trifluoroacetates of N^6,N^6-dimethyladenosine (*m/e* 570 and 150). The elimination reaction in monomethyl derivatives (Figure 6) is blocked by the presence of the trifluoroacetyl group on nitrogen. The occurrence of peaks at M − 29 and b + H − 29 therefore serves as a highly specific indication of N,N-dimethylation.

SCHEME IV



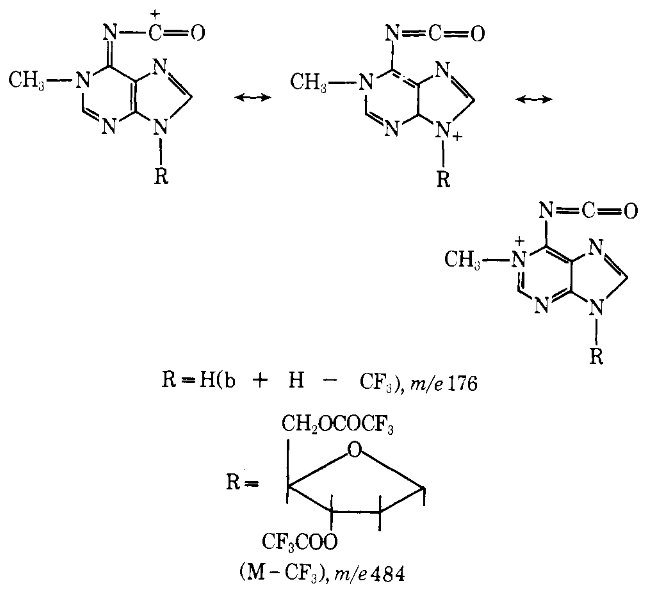
R^1 = H or CH_3

R^2 = H or

For comparison with N^6-methylation it is of interest to examine the spectrum of the trifluoroacetate of 1-methyl-2′-deoxyadenosine, shown in Figure 7. The substantial differences between Figures 6 and 7 are also found in the corresponding ribose derivatives (Table I), providing a clear means of distinguishing the two isomers. In Figure 7 the spectrum is dominated by *m/e* 484 and 176, representing loss of CF_3 from the molecular ion and b + H ion, respectively. The high stability of these ions is attributed to the imino structure which allows charge delocalization throughout the purine nucleus, *e.g.*, Scheme V. The pentaacyl derivative of 1-methyladenosine which is formed in addition to the tetrakis(trifluoroacetate) (see Materials and Methods) also produces very intense b + H − CF_3 and M − CF_3 peaks (100 and 9%). Therefore, although the position of the second trifluoroacetyl group in the base is not known, the imino structure is evidently retained. Since as indicated previously the derivative of N^6-methyladenosine and the two derivatives of 1-methyladenosine can be separated gas chromatographically, the use of this latter technique for sample introduction to the mass spectrometer provides an additional means of distinguishing between 1- and N^6-methyladenosine.

The mass spectra of trifluoroacetyl derivatives of 7-methyl-2′-deoxyguanosine and 7-methylguanosine (Table I) require special comment, because of their unique betaine structure (VII) (Jones and Robins, 1963; Ts'o *et al.*, 1969). Assuming

SCHEME V

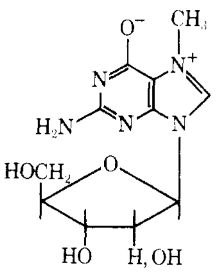


R = H(b + H − CF_3), *m/e* 176

R =

($M - CF_3$), *m/e* 484

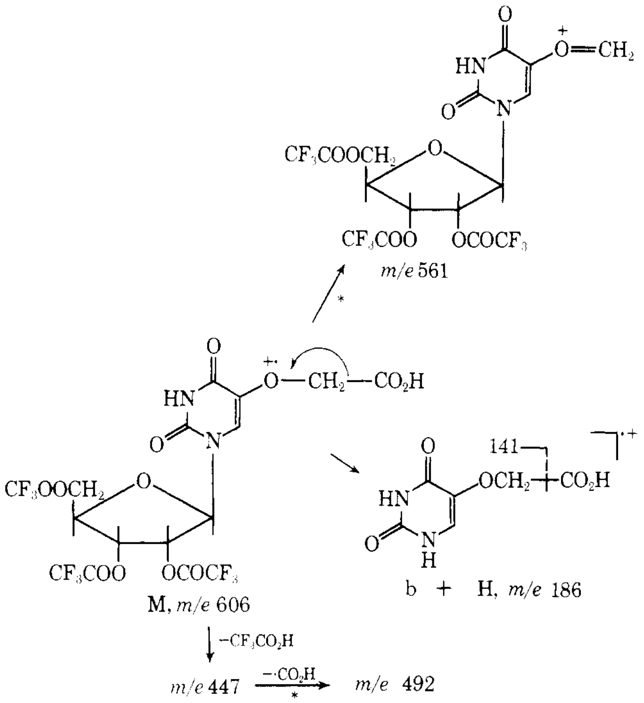
that the single trifluoroacetyl group which is added to the base during derivatization is at N^2, it seems questionable whether such a highly polar molecule is sufficiently volatile to permit vaporization without first rearranging, although mass spectra of other less polar (mp <190°) betaines have been successfully acquired (*e.g.*, Goudie *et al.*, 1969; Dougherty *et al.*, 1970; Ikeda *et al.*, 1971). Previous attempts to obtain mass spectra of free 7-methylguanosine (Hecht *et al.*, 1969) have evidently resulted in pyrolysis. The intense b + H − CF$_3$ peaks observed for these derivatives and indicated in Table I suggest that N^2 may be in the imine form, in analogy to the behavior of 1-methyladenosine. Although we presently have no data to support a detailed structure for these derivatives, their mass spectra permit unambiguous characterization, and differentiation from other isomers.



VII

The presence of unusual functional groups in the base can often be ascertained by the presence of ions in the mass spectrum which do not correspond to the usual patterns of losses from the molecular ion. An interesting example is uridin-5-oxyacetic acid, a constituent of *Escherichia coli* tRNA$_1^{\mathrm{Val}}$ (Murao *et al.*, 1970). Consistent with the usual behavior of nucleosides, the molecular ion and b + H ions decompose in the same manner: both lose the carboxyl group (45 mass units) to yield *m/e* 561 (Scheme VI) and 141 as a result of the favorable location of oxygen at C-5. The same process also occurs in combination with elimination of trifluoroacetic acid from the sugar as a major fragmentation pathway to produce an intense peak at *m/e* 447 (Table I).

SCHEME VI



The similarity in behavior of side-chain fragmentation in the b + H and molecular ions is also seen in the spectrum of the trifluoroacetate of *N*^{6}-(3-methyl-2-butenyl)adenosine, in which the loss of CF$_3$ is followed in each case by expulsion of C$_5$H$_8$ to yield *m/e* 162 and 582 (Table I). The hydrocarbon moiety which is lost in this process can be formally represented as 1,1-dimethylallene, but formulation of an exact mechanism would require deuterium labeling in the 3-methyl-2-butenyl side chain.

The triacyl of dihydrouridine also produces peaks in its mass spectrum not found in those of the common nucleosides. The major ion of mass 420 (see Table I) due to expulsion of CF$_3$CO$_2$H from the molecular ion further eliminates CO to form *m/e* 392. This reaction is marked by a metastable peak and supported by the high-resolution mass spectrum, but with the presently available evidence the exact source of CO is not known. A small peak at M − 73 (*m/e* 461) which is also found in the spectrum of the tetraacyl derivative (*m/e* 557, 1.2% relative intensity) was shown by a weak metastable peak and measurement of exact mass to have originated from M − F (*m/e* 515) by elimination of C$_3$H$_2$O, presumably from the C-4,5,6 portion of the base.

The unambiguous differentiation of isomeric nucleosides by mass spectrometry is preferably achieved by distinct differences in mass of major fragment ions, while differences in ion abundance alone are somewhat less reliable. An example of this situation is found in the mass spectra of *O*-2′,3′,5′-tris-(trifluoroacetyl)pseudouridine and its uridine isomer. Since cleavage of the gylcosidic bond on electron impact in pseudouridine is energetically unfavorable (Rice and Dudek, 1969), ion s (*m/e* 421) and its product *m/e* 193 are greatly suppressed in the mass spectrum of trifluoroacetylated pseudouridine, as shown in Table I. This difference is clearly sufficient to distinguish the two isomers when dealing with isolated compounds, but not in a hydrolysate, in which case *m/e* 421 and 193 would be produced from other components. Although not entirely satisfactory, this problem can in part be circumvented by the use of more extreme derivatization conditions (150° for 5 min) which results in formation of about 50% of a tetraacyl derivative of pseudouridine, but not uridine. The tetraacylated pseudouridine exhibits numerous unique peaks in its spectrum not found in that of the uridine derivative: *m/e* 244, 37%; *m/e* 287, 61%; *m/e* 445, 9%; *m/e* 609, 14%.

Effects of Ion Source Temperature. The inherently great reproducibility of mass spectra is generally useful for characterization of known compounds by comparison with reference spectra obtained under similar conditions. The effects of decreased ion source temperature, namely, decreased fragmentation and increased molecular ion abundance, upon mass spectra have previously been documented (Spiteller-Friedmann *et al.*, 1964), and attributed to changes in energy content of the molecular ion. These effects are usually regarded as minor under normal operating conditions, although little is known about the behavior of relatively large molecules (mol wt >400) in this respect. In the present study we have often observed changes in relative abundances of several per cent or more, which we attribute at least in part to minor differences in ion source and sample temperature. The variations in abundance of selected ions from the mass spectrum of guanosine trifluoroacetate as a function of temperature are shown in Table II. These rather large changes indicate that sample temperature is an important factor although

TABLE II: Variations in Abundance of Selected Ions from the Mass Spectra of the Trifluoroacetyl Derivative of Guanosine as a Function of Ion Source Temperature.

Ion (m/e)	Abundance (% Σ)		
	205°	250°	290°
M (667)	8.3	3.9	2.1
M − F (648)	0.79	0.39	0.21
M − CF_3CO_2H (553)	1.1	2.3	2.8
M − CF_3CO_2H − CF_3CO_2 (440)	0.58	0.71	0.96
M − CF_3CO_2H − CF_3 (484)	2.2	4.9	8.0
b + H (247)	14.0	12.0	8.5

thermally induced structural changes cannot be completely excluded. The intensities of peaks associated with loss of CF_3CO_2H rise with temperature, although under the same conditions the M − CF_3CO_2H − CF_3CO_2 ion from *O*-2′,3′,5′-tris(trifluoroacetyl)uridine shows a drop with temperature. Under normal operating conditions these effects impose some minor limitations on comparisons with reference spectra, but do not influence the interpretation of spectra for the determination of certain structural features as outlined in the preceding section.

Examination of Hydrolysates of DNA. Although individual nucleoside components can be derivatized and subjected to mass spectrometry after fractionation, a DNA or oligonucleotide hydrolysate can be qualitatively examined for composition without the necessity of prior separation. Since trifluoroacetyl derivatives of some nucleosides such as cytidine and guanosine or their 2′-deoxy analogs were found to be too polar for gas chromatography, gas chromatography–mass spectrometry cannot be used as a general technique for work involving hydrolysates. If gas chromatographic separation of nucleosides is preferred, trimethylsilyl derivatives are suitable (*e.g.*, Sasaki and Hashizume, 1966) and the technique can be used in direct combination with mass spectrometry. However, trimethylsilyl derivatives of deoxyribonucleosides, in particular those containing pyrimidine bases, produce molecular ions and related fragments of very low abundance (McCloskey *et al.*, 1968). Unless the mass spectrum is substantially free of minor extraneous peaks, which is often not possible, the important process of molecular ion identification is difficult. By contrast, the trifluoroacetyl derivatives exhibit numerous ions in the middle and upper mass region which facilitate the determination of molecular weight even in the presence of other components.

Using the direct probe as a means of sample introduction for trifluoroacetyl derivatives, the resulting mass spectrum obtained by conventional magnetic scanning reflects the composition of the vapor at the time the spectrum is recorded. Figure 8 shows the vaporization profile of a standard mixture of deoxyribonucleoside derivatives recorded over a period of about 4 min after initial sample introduction. The higher response for the molecular ion of thymidine trifluoroacetate reflects the relatively greater percentage of total ion current carried by its molecular ion. Mass spectra must be recorded several times during the period of vaporization in order to include nucleosides of different volatility. Model experiments conducted on mixtures of various modified ribonucleosides have shown that on our instrument (LKB) when set for high sensitivity, about 25 ng of each component can be observed with a signal:noise ratio ≥20. This value is in terms of the underivatized nucleoside, and assumes complete derivatization.

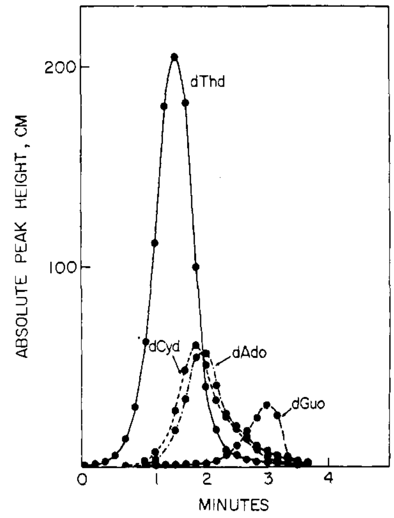


FIGURE 8: Vaporization profile of a standard mixture of 2′-deoxyribonucleoside trifluoroacetyl derivatives, introduced by direct probe. Values plotted are absolute molecular ion intensities, presented in terms of the most sensitive galvanometer trace. Samples are equivalent to 1.3 μg (∼60 mμmoles) of (free) nucleoside, corresponding to the following percentages of total ion current: dThd, 1.4%; dCyd, 0.46%; dAdo, 0.76%; dGuo, 0.47%.

The standard reaction conditions for trifluoroacetylation developed for model ribonucleosides and deoxyribonucleosides were applied to hydrolysates of DNA, and were found to be generally satisfactory. Figure 9 shows the upper mass region of a mass spectrum from a hydrolysate of calf thymus DNA, equivalent to 25 μg of free nucleosides. The four major deoxynucleosides are readily recognized by their molecular ions and characteristic fragments. The minor component 5-methyl-2′-deoxycytidine is shown by m/e 529 and 460. Based on a level of 0.5% of the total base composition (Chargaff *et al.*, 1953), the quantity of 5-methyl-2′-deoxycytidine represented in Figure 9 is approximately 0.13 μg, or 0.5 nmole. The lower mass region which is not shown is dominated by the usual ions associated with the four major deoxynucleosides (Figures 1–4). The characteristic m/e 152 ion from the 5-methyl-2′-deoxycytidine component (see Figure 5) was also observed in the correct abundance, in support of the m/e 529 and 460 shown in the upper mass range of Figure 9. The diagnostic upper mass region was found to be generally free from interference by artifacts, permitting assignment of essentially all peaks over mass 400. However, in some instances, peaks from traces of 1,2,3,4,6-penta-*O*-(trifluoro-

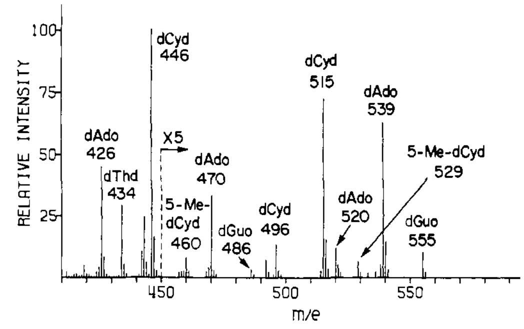


FIGURE 9: Partial mass spectrum of a trifluoroacetylated hydrolysate of DNA from calf thymus. See Figures 1–5 for structural identities of individual peaks.

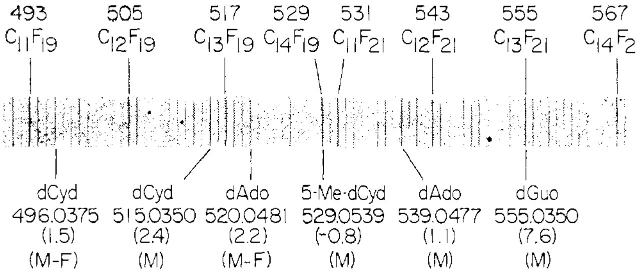


FIGURE 10: Upper mass section of a photographically recorded high-resolution mass spectrum of a trifluoroacetylated hydrolysate of calf thymus DNA, equivalent to 20 μg of free nucleosides. Numbers in parentheses refer to the difference (in millimass units) between theoretical exact mass values and the found values which are shown.

acetyl)glucose (*e.g.*, *m/e* 641 (M − F), 547, 433, and 319) were observed during the initial period of vaporization. This contaminant was found in spectra derived from a number of commercial sources of DNA, and although it did not materially interfere with the analysis of the spectra, unsuitably high ion source pressures occasionally resulted from sudden initial vaporization of the relatively volatile glucose component.

The certainty of identification of individual components in a hydrolysate can be increased through the use of high-resolution techniques, based on recognition of predetermined sets of exact mass values which are unique to each component. If photographic recording methods are employed, the entire high-resolution spectrum (∼*m/e* 28–706 at 8 kV) can be integrated over the period of sample evaporation. The spectrum is then converted into exact mass values using a standard computer programs for general high-resolution work. Sets of identifying exact masses for deoxyribonucleoside derivatives are listed in Table III. These values were chosen on the basis of their uniqueness and abundance, with increased emphasis on ions of higher mass in order to minimize interference from unresolved multiplets. Based on the spectra of numerous model compounds as discussed in the previous section, characteristic mass values can be predicted for the addition of new compounds for which reference spectra are not available, for the expansion of Table III. The exact mass list generated from the hydrolysate can either be searched manually, using the list from Table III arranged in numerical order, or by computer. In our laboratory the hydrolysate spectrum is searched by means of a time-shared computer system using remote terminals located in the mass spectrometry laboratory. The procedural operation for execution of the program (named MIXTURE) and description of the computer system have been previously published (Lawson *et al.*, 1971b).

TABLE III: Characteristic Exact Mass Values for Identification of Deoxyribonucleosides as Their Trifluoroacetyl Derivatives by High-Resolution Mass Spectrometry.

Parent Nucleoside	Exact Mass[a]
2′-Deoxyuridine	420.0392, 307.0541, 264.0358, 113.0351
Thymidine	434.0548, 321.0698, 320.0620, 307.0541 278.0514, 206.0691, 126.0429
2′-Deoxyinosine	444.0504, 425.0520, 331.0654, 216.0647 136.0385
2′-Deoxycytidine	515.0374, 496.0390, 446.0422, 402.0524 359.0340, 208.0334, 207.0255 138.0303
5-Methyl-2′-deoxycytidine	529.0531, 460.0579, 416.0681, 346.0650 301.0674, 222.0490, 221.0412, 152.0460
2′-Deoxyadenosine	539.0487, 520.0503, 470.0535, 442.0585 426.0636, 311.0630, 231.0368, 162.0416
1-Methyl-2′-deoxyadenosine	553.0643, 534.0659, 484.0691, 256.0834 176.0572
N^6-Methyl-2′-deoxyadenosine	553.0643, 534.0659, 484.0691, 456.0742 440.0792, 245.0524, 148.0623
2′-Deoxyguanosine	555.0436, 536.0452, 486.0484, 441.0507 327.0579, 247.0317, 178.0365
7-Methyl-2′-deoxyguanosine	569.0592, 500.0640, 359.0840, 341.0735 261.0473, 192.0521

[a] Exact molecular mass listed first for each compound; see Figures 1–7 and Table I for abundances and structural identities of the remaining ions.

An example of the original data produced by this procedure is reproduced in Figure 10, which shows a short (3 cm) section of a photographic plate which contains the high resolution mass spectrum of a calf thymus DNA hydrolysate. The amount of material introduced was equivalent to 35 μg of free nucleosides. With the exception of *O*-3′,5′-bis-(trifluoroacetyl)thymidine, whose molecular ion appears in the lower mass section of the plate (*m/e* 434.0552, found), the presence of other major deoxynucleosides and 5-methyl-2′-deoxycytidine is indicated by exact masses of their molecular ions. The minor M − F ions for 2′-deoxyguanosine and 5-methyl-2′-deoxycytidine derivatives were below the preset intensity threshold level of the recording microdensitometer, but their presence was supported by numerous other ions in the lower mass region. For reasons of low intensity or incomplete resolution from other ions of the same nominal mass, all of the values listed for any given compound in Table III may not be found, but particularly at high mass, positive identification can generally be based on two or three found values. Normally, experimentally found mass values deviate from theoretical values by less than 4 mmass units. Deoxyguanosine, as shown in Figure 10, was often found to exhibit higher errors. Although this may be in part due to its vaporization rather late in the exposure, close examination of the photoplate shown in Figure 10 showed that the position of the $C_{13}F_{21}$ marker line was shifted due to the presence of another unresolved line. If a correction factor is applied, which is not normally part of the routine calculation procedure, the error shown in Figure 10 is reduced from 7.6 to 3.7 mmass units.

The grainy appearance of the plate results from intentional overdevelopment, used to enhance sensitivity. Under these conditions the minimum detectable limit for major fragment ions is estimated to be in the range 20–50 ng of each nucleoside (Lawson *et al.*, 1971b). Since the maximum sample that can be introduced in our instrument without defocusing of the ion beam is 30–40 μg, the lower limits of identification of a minor nucleoside component are about 0.1–0.2% of the total nucleoside content. While these limits are sufficient for the identification of modified components of tRNA (Lawson *et al.*, 1971b), they may be insufficient for detection of modified bases in many types of DNA because of their generally lower level of occurrence. In those cases, prefractionation of the hydrolysate prior to mass spectrometry would be necessary.

At any stage of purification, the use of high resolution mass spectrometry for the examination of nucleic acid hydrolysates may be advantageous since it provides an independent and objective means of identification which does not rely upon ultraviolet absorbance or chromatographic mobility, and is therefore complementary to conventional procedures.

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References

Armstrong, D. J., Evans, P. K., Burrows, W. J., Skoog, F., Petit, J.-F., Dahl, J. L., Steward, T., Strominger, J. L., Leonard, N. J., Hecht, S. M., and Occolowitz, J. (1970), *J. Biol. Chem. 245*, 2922.

Baczynskyj, L., Biemann, K., and Hall, R. H. (1968), *Science 159*, 1481.

Biemann, K., and McCloskey, J. A. (1962), *J. Amer. Chem. Soc. 84*, 2005.

Brown, P., Pettit, G. R., and Robins, R. K. (1969), *Org. Mass Spectrom. 2*, 521.

Carbon, J., David, H., and Studier, M. H. (1968), *Science 161*, 1146.

Chargaff, E., Crampton, C. F., and Lipschitz, R. (1953), *Nature* (*London*) *172*, 289.

Dolhun, J. J., and Wiebers, J. L. (1969), *J. Amer. Chem. Soc. 91*, 7755.

Dougherty, R. C., Foltz, R. L., and Kier, L. B. (1970), *Tetrahedron 26*, 1989.

Eggers, S. H., Biedron, S. I., and Hawtrey, A. O. (1966), *Tetrahedron Lett.*, 3271.

Goudie, R. S., Preston, P. N., and Palmer, M. H. (1969), *Org. Mass Spectrom. 2*, 953.

Hancock, R. L., and Coleman, D. L. (1965), *Anal. Biochem. 10*, 365.

Hecht, S. M., Gupta, A. S., and Leonard, N. J. (1969), *Anal. Biochem. 30*, 249.

Hunt, D. F., Hignite, C. E., and Biemann, K. (1968), *Biochem. Biophys. Res. Commun. 33*, 378.

Ikeda, M., Tsujimoto, N., and Tamura, Y. (1971), *Org. Mass Spectrom. 5*, 61.

Jones, J. W., and Robins, R. K. (1963), *J. Amer. Chem. Soc. 85*, 193.

Lawson, A. M., Koenig, W. A., Smith, L. C., Earle, N. R., and McCloskey, J. A. (1971b), *Advan. Mass Spectrom. 5* (in press).

Lawson, A. M., Stillwell, R. N., Tacker, M. M., Tsuboyama, K., and McCloskey, J. A. (1971a), *J. Amer. Chem. Soc. 93*, 1014.

McCloskey, J. A. (1971), *in* Basic Principles in Nucleic Acid Chemistry, Ts'o, P. O. P., Ed., New York, N. Y., Academic Press, Chapter IX.

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

Murao, K., Saneyoshi, M., Harada, F., and Nishimura, S. (1970), *Biochem. Biophys. Res. Commun. 38*, 657.

Rahamin, Y., Sharvit, J., Mandelbaum, A., and Sprecher, M. (1967), *J. Org. Chem. 32*, 3856.

Rice, J. M., and Dudek, G. O. (1969), *Biochem. Biophys. Res. Commun. 35*, 383.

Sasaki, Y., and Hashizume, T. (1966), *Anal. Biochem. 16*, 1.

Shannon, J. S., and Letham, D. S. (1966), *N. Z. J. Sci. 9*, 833.

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

Spiteller-Friedmann, M., Eggers, S. H., and Spiteller, G. (1964), *Monatsh. Chem. 95*, 1740.

Ts'o, P. O. P., Kondo, N. S., Robins, R. K., and Broom, A. D. (1969), *J. Amer. Chem. Soc. 91*, 5625.

Tsunakawa, S. (1969), *Bull. Chem. Soc. Jap. 42*, 1.