THE STUDY AND CHARACTERIZATION OF 2'-ANHYDRONUCLEOSIDES AND THEIR DERIVATIVES BY MASS SPECTROMETRY

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by

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TO MY WIFE

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ABSTRACT

Cyclonucleosides are important analogues of natural nucleosides. The 2'-anhydronucleosides derived from the major bases, uracil, thymine, adenine, guanine, xanthine and hypoxanthine, and some of their derivatives were studied by mass spectrometry. Involatile or thermally unstable compounds of this class were derivatized, mainly by trimethylsilyation (TMS) and sometimes by trifluoroacetylation (TFAc) techniques. Deuterium and substituent labelling were employed to assist in clarification of fragmentation patterns. Metastable ions were studied whenever possible.

The mass spectra of the 2'-anhydronucleosides are quite distinct from those of natural nucleosides. Their mass spectra show characteristic ions which retain the structure of the base moiety plus the anhydro-ring (represented as A). These ions are $(A+H)^+$, $(A+31)^+$ and their analogues such as $(A+TMS)^+$and $(A+30+TMS)^+$. The formation of $(A+H)^+$ and $(A+TMS)^+$ require migration of hydrogen and the TMS group respectively. $(A+31)^+$ and $(A+30+TMS)^+$ require an anhydro-ring expansion which is more favorable in O-anhydro compounds. Another ion that retains the anhydro-linkage is C^+ (see text). Other diagnostically important ions are M^+, B^+, $(B+H)^+$, $(B+2H)^+$ and $(S'-H)^+$, where B represents the base of the corresponding natural nucleoside, and S' represents the sugar moiety of the anhydronucleoside. For the purine anhydronucleosides, two other distinct ions A^+ and $(A+13)^+$ were observed.

This mass spectrometric study indicates that 3' and/or 5' substituents on pyrimidine anhydronucleosides can be distinguished, as demonstrated by the mass spectra of various synthetically useful derivatives of 2,2'-anhydrouridine. CH_3CO_2 or CF_3CO_2 groups are eliminated exclusively from the 3' position. The 5'-pivaloyl group is responsible for the formation of an ion with m/e=193 in the mass spectra of 2,2'-anhydrouridine derivatives. Other characteristic ions were also observed. Derivatization is required for compounds that tend to rearrange thermally.

It was found that the purine 3'-anhydronucleosides studied gave spectra different from their 2'-isomers. The intensities of ions $(A+H)^+$, $(A+TMS)^+$, $(A+13)^+$, $(A+31)^+$, $(A+30+TMS)^+$, $(S'-2H)^+$, $(S'-H)^+$and $(S'-17)^+$ in both isomers were compared and each isomer can be identified.

TABLE OF CONTENTS

TABLE OF CONTENTS continued

LIST OF SCHEMES AND TABLES

LIST OF FIGURES

LIST OF FIGURES continued

LIST OF FIGURES continued

CHAPTER ONE: INTRODUCTION

Recently an immense effort has been made to understand how the living cell functions at the molecular level. From a chemical viewpoint it appears that sophisticated analytical techniques on a micro-scale will be a valuable addition to the techniques available for such studies. It is hoped that this study will play some part in the development of these analytical techniques.

It is generally accepted that DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) play an important role in the living cell. In 1953, Watson and Crick achieved a major breakthrough in establishing the right-handed double helical structure for DNA from X-ray data[1,2]. Since then, judging from the amount of material published[3-5], extensive studies on nucleic acid chemistry have been made. On examining the literature, one finds that the synthetic nucleosides, nucleotides, oligonucleotides and polynucleotides play an outstanding part in providing the starting materials for the investigations. For example, in deciphering the triplet genetic code for protein synthesis, the sixty four (4^3) possible triribonucleotides from the four major nucleosides guanosine (G), adenosine (A), cytidine (C) and uridine (U) were synthesized and tested for their specificities by using the ribosomal binding method[6].

Chemical methods of synthesis of oligoribonucleotides and oligodeoxyribonucleotides of predetermined sequences have been devised[6-11].

Improvements in synthetic methods are being sought with the ultimate aim that RNA and DNA of any desired sequence can be produced. Cyclonucleosides may prove to be useful intermediates in such syntheses. They are characterized by having in addition to the N- glycoside linkage a covalent linkage either directly or via bridging atoms between the 2', 3' or 5' carbons of the sugar and a carbon or nitrogen atom (other than the nitrogen of the glycoside bond) or the purine or pyrimidine ring. Their uses in nucleotide synthesis have been reported[12-14]. They have been detected as intermediates in proposed prebiotic syntheses[15,16]. Two important biologically active compounds, arabinocytidine and cordycepin (3'-deoxyadenosine), and an analogue to cordycepin, 3'-deoxyguanosine can be synthesized via cyclonucleosides[17-20]. Cyclonucleosides have been used as model compounds in conformational studies of naturally occurring nucleosides by optical rotatory dispersion (ORD) and circular dichroism (CD)[21-25]. A cyclonucleoside has been used in studies on enzyme specificity[26]. Chemically, controlled hydrolysis of cyclonucleosides by acid or base usually leads to ribo, arabino or xylo nucleosides[27]. Nucleophilic displacement of the cyclo-linkage leads to nucleosides modified on the sugar moiety[27-29] and sometimes on the base moiety[19,27,30,31].

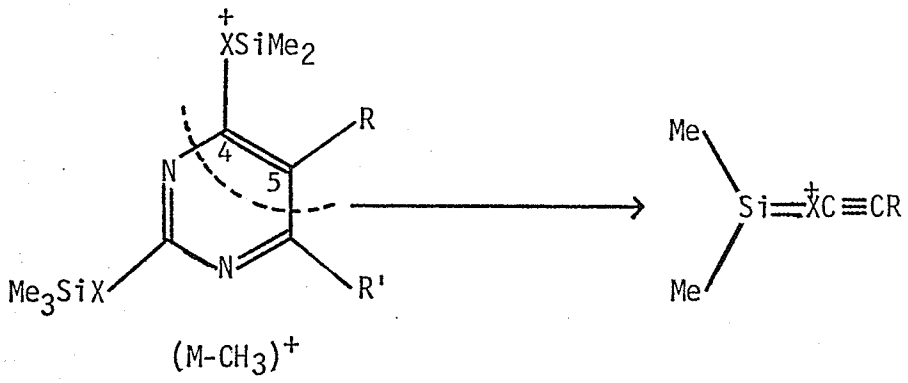
Because of the interest in cyclonucleosides, numerous syntheses have been reported[19,20,32-34]. Mass spectrometry has occasionally been used as an aid in compound identification in these reports but only one detailed systematic mass spectral study[35] of this class of compounds

has been reported to date. Such studies will help to identify and characterize these compounds and assist in elucidating their structures.

Mass spectrometry has been used frequently in various fields of research. Its basic principles are described in detail in a variety of books[36-41]. A brief description was given in this author's M.Sc. thesis[42]. Reviews and recent developments on different branches of mass spectrometry are regularly reported[43-46], but it is not until recently that mass spectrometry has been used in nucleic acid chemistry research. In 1962, Biemann and McCloskey published their now classical paper on nucleosides[47]. Since then the applications of mass spectrometry to nucleoside chemistry have been growing. The literature up to June 1970 has been reviewed[48] and a brief progress report [49] has been given. Thus far, this active field of research can be roughly divided into three areas.

1. The base. The most recent article[50] in this area deals with the mass spectra of trimethylsilyl (TMS) derivatives of pyrimidine and purine bases. Thirty three bases were studied. Both high resolution and deuterium-labelling techniques were used. These bases were characterized, and one of the most significant points of this study showed that the position of thiation (C-2 vs. C-4) or methylation (C-5 vs. C-6) in pyrimidine can be easily distinguished by observing a major characteristic ion containing C-4, C-5 and their attached groups.

For example:



X = O, S, NH

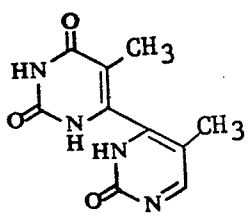
R = CH_3, R' = H

or R = H, R' = CH_3

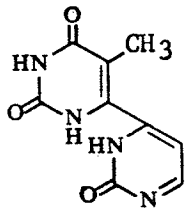
The major bases from RNA and DNA were characterized without derivatization by mass spectrometry in earlier studies[51,52]. The rare bases that are occasionally encountered in biological systems have often been identified with the aid of their mass spectra[48, 53,54].

Recent interest in the photochemistry of the bases has led to the isolation of different pyrimidine dimers and trimers. Their mass spectra have been reported[55-59]. The problem of low abundance or absence of molecular ion has been solved by the use of TMS derivatization techniques or a field ionization source[58]. It is possible to obtain structural information from their mass spectra, but due to their sometimes not so obvious fragmentation patterns, often only the information from the molecular ions is used. For example, both 6-[4'-(5'-methyl-

pyrimid-2'-one)] -thymine (a) and 6-[4'-(pyrimid-2'-one)]-thymine (b) lose ammonia to form $(M-17)^+$ ions but 6-[4'-(pyrimid-2'-one)]-uracil (c)



(a)



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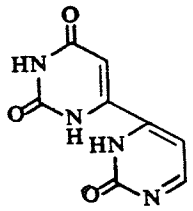


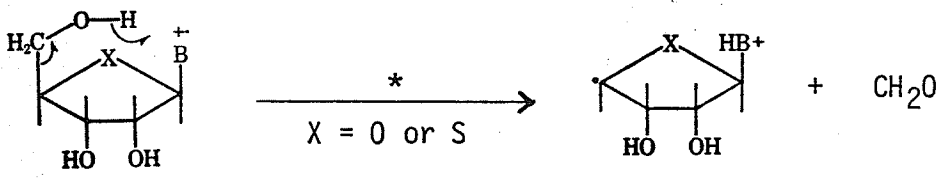
(c)

does not. This suggests that in such dimers, a saturated alkyl substituent is required at C-5 for the formation of the $(M-17)^+$ ion[59]. No doubt further studies will be carried out on similar compounds so that their structures can be elucidated.

2. Nucleosides and mononucleotides. This is probably the most active area. The main objective is to be able to identify components of nucleic acid material either as a pure isolated compound or as a mixture. The major difficulties encountered in this field of research are the involatility of many nucleosides and mononucleotides and sometimes the absence of the molecular ion from the mass spectrum. These problems are being overcome by different techniques such as trimethylsilylation[48,60], trifluoroacetylation[61,62], alkylation[62,63], field ionization[64], chemical ionization[65] and most recently field desorption[66,67].

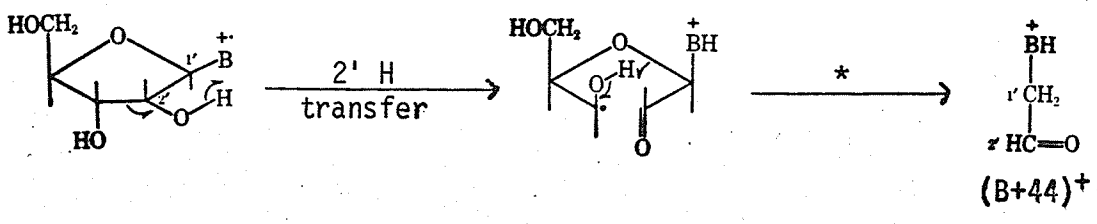
Mass spectra of underivatized nucleosides from tRNA have been recorded as reference spectra for structural confirmation of known

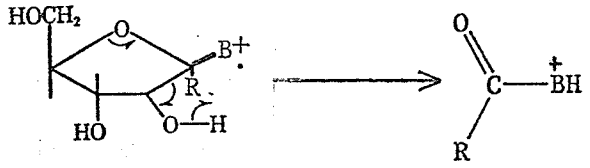
nucleosides and for structural elucidation of unknown, modified nucleosides by comparing their fragmentation patterns[68]. Detailed studies of adenosine, its analogues and some other major nucleosides have been carried out[47,69]. Fragmentation mechanisms were proposed after examining metastable transitions, spectra of deuterium- and substituent-labelled compounds and high resolution mass spectra. The most significant ions are $(M-30)^+$, $(B+44)^+$, $(B+30)^+$, $(B+60)^+$, $(B+2H)^+$, $(B+H)^+$, B^+ and S^+ where B represents the base moiety and S represents the sugar moiety. The formation of the $(M-30)^+$ ion requires the presence of the 5' hydroxyl group, and is envisaged as shown



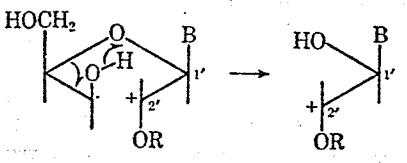
(* signifies the presence of metastable ion $M^* = \frac{M^2_{daughter}}{M_{mother}}$ [70])

Ions B^+, $(B+H)^+$, and $(B+2H)^+$ help to identify the nucleoside. The origin of the second hydrogen has to be explained with caution since these ions are most likely formed through multiple pathways. The ion S^+ signifies the sugar fragment and is usually more intense in pyrimidine than in purine nucleosides. The most likely routes for the formation of the ions $(B+44)^+$, $(B+30)^+$ and $(B+60)^+$ were explained as follows:

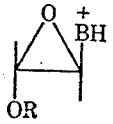




$(B+30)^+$, when R = H

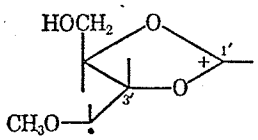


or



$(B+60)^+$, when R = H

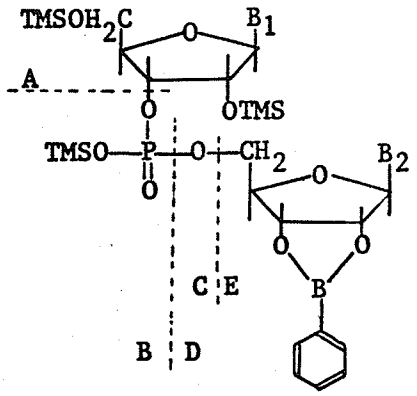
The abundance of the $(B+30)^+$ ion is substantially increased by the presence of a 2'-hydroxyl group, whereas $(B+60)^+$ requires the presence of a 3'-hydroxyl group. There were other alternate routes suggested for the formation of the $(B+44)^+$ ion that do not require the presence of a 2'-hydroxyl group. Also reported was a characteristic ion for nucleosides with a 2'-O-methyl group. One of its most likely structures was given as a secondary carbonium ion,



Thus O-methylation or lack of hydroxyl group in any of the C-1', C-2', C-3' or C-5' positions in a nucleoside can easily be recognized by examining these ions. Information gathered in these studies has been used successfully to analyse nucleosides isolated from various sources[48,49,71-73].

Detailed studies along similar lines as shown above , i.e. correlating the mass spectra with their structures, were carried out for trimethylsilyl derivatives of major nucleosides and mononucleotides [60,74] and for trifluoroacetyl derivatives of nucleosides[75] and analogues of adenosine differing in the sugar moiety[76]. The basic knowledge about the fragmentation patterns of these derivatized compounds has contributed to different areas of nucleic acid research[48,75,77] and will likely continue to do so.

3. Polynucleotide sequencing. This remains one of the least explored areas in nucleic acid research by mass spectrometry. Biemann et al first reported success in sequencing diribonucleotides after trimethylsilylation[78,79]; however their method relied on intensity differences. An improved method was later reported using double derivatization techniques[62,80]. The dinucleotide was first reacted with phenylboronic acid in pyridine forming a 2',3'-O-phenylboronic ester at the cis-glycol position of the 3' terminal and then trimethylsilylation was performed. Thus sequence isomers can easily be distinguished from the mass spectra by observing the ions A, B, C, D and E.



Sequence analysis of nucleotides of more than two units by mass spectrometry is not as successful as in polypeptides. For tri-, tetra or at the utmost decanucleotides, stepwise degradation pretreatment had to be employed[81,82]. Simpler and faster methods will definitely be welcomed by nucleic acid chemists.

Other methods employed in studying nucleosides include NMR, ORD, CD, X-ray diffraction, IR, Raman, UV, fluorescence and phosphorescence spectroscopies and they have been treated in an excellent review article by Paul O.P. Ts'o[83]. These methods do not undermine the usefulness of mass spectrometry which is a relatively new tool in nucleic acid chemistry research, whose impact has yet to be fully realized. The results from mass spectral studies on structure-correlation certainly seem fruitful. A similar study on cyclonucleosides, which will be presented in the remainder of this thesis, may provide useful information that will promote further interest in these compounds.

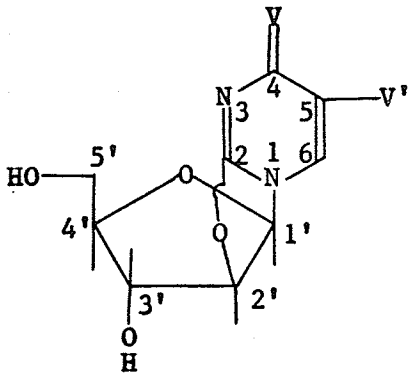
Before introducing the study of the cyclonucleosides by this author, it should be mentioned that a brief report of this work was presented at the Twentieth Annual Conference on Mass Spectrometry and Allied Topics[85]. The first part of this work has been accepted for publication[86]. While the accepted article was in press, an article on mass spectrometry of some cyclonucleosides was published by Tsuboyama and McCloskey[35] but only one of the cyclonucleosides studied here was reported namely, 2,2'-anhydrouridine. In their

work, model compounds of the pyrimidine cyclonucleosides and their trimethylsilyl derivatives were studied. The seven compounds studied include 2,2'- and 2,3'-anhydrouridine; 2',6- and 5',6-anhydro-6-hydroxyluridine; 2',6-anhydroorotidine and some of their α anomers. It was reported that the 2'- and 3'-linked compounds could be readily distinguished from the 5' isomers but not from each other. The characteristic ions reported for the 2'-cyclonucleosides are in good agreement with that obtained in the present investigation and therefore will not be presented separately. The differences and similarities in the observations and intepretations will be cited appropriately in the later chapters of this thesis. Some partial mass spectra of some 2',8-, 3',8 and 5',8 cycloadenosines were reported[87-89], but neither the structures of the prominent ions nor their origins were discussed.

Anhydronucleosides are cyclonucleosides which differ from a naturally occurring nucleoside by the loss of the elements of water. The main anhydronucleosides and their various derivatives studied in this work are derived from all the major bases except cytosine (whose derivatives are not presently readily available). The main compound types are listed in SCHEME 1. The complete list is presented in the next chapter. Some 3'-anhydronucleosides were also studied and will be discussed in conjunction with their 2'-isomers. The 2'-anhydronucleosides and some of their derivatives are of particular interest since the anhydro-ring

SCHEME I

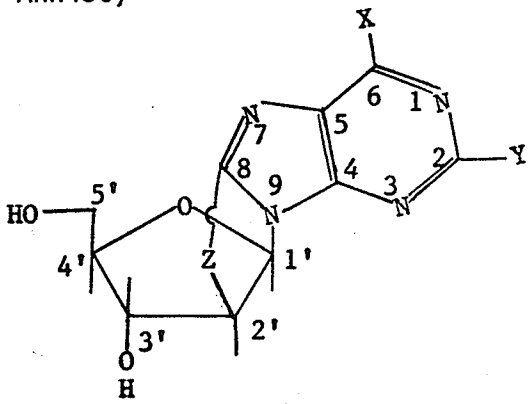
The major anhydronucleosides



I V=O; V'=H; 2,2'-anhydro-1-(β-D-arabinofuranosyl)-uracil; (2'AnhU)

II V=O; V'=CH_3; 2,2'-anhydro-1-(β-D-arabinofuranosyl)-thymine; (2'AnhT)

III V=S; V'=H; 2,2'-anhydro-1-(β-D-arabinofuranosyl)-4-thio-uracil; (2'Anh4SU)



IV Z=O; X=NH_2; Y=H; 8,2'-anhydro-8-oxy-9-(β-D-arabinofuranosyl)-adenine; (8,2'-O-AnhA)

V Z=S; X=NH_2; Y=H; 8,2'-anhydro-8-mercapto-9-(β-D-arabino-furanosyl)-adenine; (8,2'-S-AnhA)

VI Z=NH; X=NH_2; Y=H; 8,2'-anhydro-8-amino-9-(β-D-arabinofuranosyl)-adenine; (8,2'-N-AnhA)

VII Z=O; X=OH; Y=H; 8,2'-anhydro-8-oxy-9-(β-D-arabinofuranosyl)-hypoxanthine; (8,2'-O-AnhI)

VIII Z=S; X=OH; Y=H; 8,2'-anhydro-8-mercapto-9-(β-D-arabino-furanosyl)-hypoxanthine; (8,2'-S-AnhI)

SCHEME I (continued)

IX Z=NH; X=OH; Y=H; 8,2'-anhydro-8-amino-9-(β-D-arabinofuranosyl)-hypoxanthine; (8,2'-N-AnhI)

X Z=O; X=OH; Y=NH_2; 8,2'-anhydro-8-oxy-9-(β-D-arabinofuranosyl)-guanine; (8,2'-O-AnhG)

XI Z=S; X=OH; Y=NH_2; 8,2'-anhydro-8-mercapto-9-(β-D-arabino-furanosyl)-guanine; (8,2'-S-AnhG)

XII Z=NH; X=OH; Y=NH_2; 8,2'-anhydro-8-amino-9-(β-D-arabinofuranosyl)-guanine; (8,2'-N-AnhG)

XIII Z=S; X=OH; Y=OH; 8,2'-anhydro-8-mercapto-9-(β-D-arabino-furanosyl)-xanthine; (8,2'-S-AnhX)

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effectively acts as a blocking group for the 2' position of the sugar moiety leaving the 3' and/or 5' positions free for construction of oligonucleotide chain. Their mass spectra are interesting in that they are substantially different from those of naturally occurring nucleosides. This study characterizes these compounds so that their chemical, photochemical, enzymatic or other reaction products can be analysed and novel anhydronucleosides can be identified. It should also form a basis for any further work on combined gas chromatography mass spectrometry and oligocyclonucleotide sequencing.

Deuterium labelling, metastable peaks and closely related compounds were utilized as much as possible. The versatility of high resolution mass spectrometry is realized[84], but taking into account that not every laboratory possesses such an expensive instrument and its non-availability to this laboratory, this work was done on a medium resolution instrument.

A brief report of this work was presented at the Twentieth Annual Conference on Mass Spectrometry and Allied Topics[85]. The first part of this work has been accepted for publication[86]. While the accepted article was in press, an article on mass spectrometry of some cyclonucleosides was published but only one of the cyclonucleosides studied here was reported namely I, 2'AnhU and its mass spectrum was in general agreement with that obtained in the present investigation[35]. Some partial mass spectra of a few cycloadenosines, IV, V and VI were reported but no systematic interpretation of the spectra was given[87-89].

The experimental conditions and the physical properties of the compounds studied will be presented next, followed by the results and discussions. The latter will be divided roughly into two parts, the 2,2'-anhydropyrimidine nucleosides with their derivatives and the 8,2'-anhydropurine nucleosides, their derivatives and some of their 8,3'-isomers.

CHAPTER TWO EXPERIMENTAL PROCEDURES AND PHYSICAL PROPERTIES OF COMPOUNDS

The mass spectra were recorded on a Hitachi RMU-6D single-focussing mass spectrometer. Samples were inserted into the mass spectrometer on the direct insertion probe. The sample temperature was raised steadily until the rate of vaporization was sufficient to give good spectra. The sample temperature was recorded by a thermocouple mounted on the side of the sample heater block with a reproducibility of $\pm 3^{o}$ and a probable accuracy of $\pm 10^{o}$. The electron energy was 50eV and the ionization chamber temperature usually 250^{o}. The mass spectra thus obtained were measured manually from the chart record and then normalized and a line graph plotted utilizing a computer program developed for this purpose (see Appendix). Metastable peaks were matched with a program reported in this author's M.Sc. thesis [42].

The anhydronucleosides and all their derivatives are listed in TABLE I along with some physical properties. The compounds were prepared as reported in the literature and appropriate references are cited in TABLE I wherever necessary. Derivatizations which include deuteration, acetylation (Ac), trifluoroacetylation (TFAc), trimethysilylation (TMS), trimethyl-d_9-silylation (dTMS) and dimethylsilylation (DMS) were carried out in the following manner:

Deuteration: The 3' and 5' hydroxyl groups of I and II were deuterated by several refluxings with D_2O. The mass spectrometer was flushed with D_2O before, and during, recording their spectra. Although complete

TABLE I

Sample temperature, melting point and the UV absorption maxima of the anhydronucleosides and their derivatives. (The short notation in SCHEME I and the following are used: $Si(CH_3)_3$ = TMS, $Si(CD_3)_3$ =dTMS, CH_3CO = Ac, CD_3CO = dAc, CF_3CO = TFAc, $(CH_3)_3CCO$ = Pivaloyl = Piv, $(C_6H_5)_3C$ = Trityl = Tri and $(CH_3)_2HSi$ = DMS. All derivatives are O- and/or N- substituted. See Experimental section for details.)

	COMPOUND	SAMPLE TEMP. ^oC	M.P. ^oC	λmax., nm (solvent)
I	2'AnhU[95]	220	245-7	250, 223 (H_2O) 248, 223 (EtOH)
Ia	3',5'-d_2-2'AnhU	260	-	-
Ib	3,5'-di-Ac-2'AnhU	134	184-5	250, 223 (H_2O)
Ib'	3,5'-di-dAc-2'AnhU	143	-	-
Ib''	3'-Ac-5'-dAc-2'AnhU	140	-	-
Ib'''	3'-dAc-5'-Ac-2'AnhU	140	-	-
Ic	3',5'-di-TFAc-2'AnhU	120	-	-
Id	3',5'-di-Tri-2'AnhU[94]	262	141-3	249(sh), 223(sh) (EtOH)
Ie	3',5'-di-Piv-2'AnhU[91]	134	227-31	248, 226 (EtOH)
If	3',5'-di-TMS-2'AnhU	115	-	-
If'	3',5'-di-dTMS-2'AnhU	95	-	-
Ig	3',5'-di-DMS-2'AnhU	120	-	-
Ih	5'-Tri-2'AnhU[92]	221	218-220	249(sh) (EtOH)
Ii	3'-Ac-5'-Tri-2'AnhU[92]	91	98-104	249(sh) (EtOH)

TABLE I continued

Ij	3'-Ac-2'AnhU[92]	144	204-9	249 (H_2O, EtOH)
Ik	5'-Ac-2'AnhU[92]	135	164-7	249 (H_2O, EtOH)
Il	3'-Ac-5'-TMS-2'AnhU	91	-	-
Il'	3'-Ac-5'-dTMS-2'AnhU	91	-	-
Im	3'-TMS-5'-Ac-2'AnhU	113	-	-
Im'	3'-dTMS-5'-Ac-2'AnhU	113	-	-
Io	3'-Ac-5'-TFAc-2'AnhU	123	-	-
Ip	3'-TFAc-5'-Ac-2'AnhU	135	-	-
Iq	3'-Ac-5'-Piv-2'AnhU[91]	124	198-202	248 (EtOH)
Ir	3'-Piv-5'Ac-2'AnhU[91]	130	170-3	248 (EtOH)
Is	5'-Piv-2'AnhU[91]	120	210-5	249 (H_2O, EtOH)
It	3'-Piv-2'AnhU[91]	144	240-2	249 (H_2O, EtOH)
Iu	3'-TMS-5'-Piv-2'AnhU	144	-	-
Iu'	3'-dTMS-5'Piv-2'AnhU	144	-	-
Iv	3'-Piv-5'-TMS-2'AnhU	137	-	-
Iv'	3'-Piv-5'-dTMS-2'AnhU	137	-	-
Ix	3'-TFAc-5'-Piv-2'AnhU	111	-	-
Iy	3'-Piv-5'-TFAc-2'AnhU	166	-	-
II	2'AnhT[96]	220	227-8	254, 224 (H_2O)
IIa	3',5'-d_2-2'AnhT	248	-	-
IIb	3',5'-di-Ac-2'AnhT	120	-	-
IIb'	3',5'-di-dAc-2'AnhT	130	-	-
IIc	3',5'-di-TFAc-2'AnhT	95	-	-

TABLE I continued

IIf	3',5'-di-TMS-2'AnhT	91	-	-
III	2'Anh4SU[97]	251	150-170	332, 254, 217 (H_2O)
IIIb	3',5'-di-Ac-2'Anh4SU	241	160-3	332, 241 (EtOH)
	Uridine	150	166-7	261 (H_2O)
	2'-Deoxythymidine	127	183	267 (H_2O)
	Adenosine	117	237	259, 206 (H_2O)
IV	8,2'-O-AnhA[104]	170	d*170	261 (H_2O)
IVf	8,2'-O-AnhA-$(TMS)_3$	47	-	-
IVf'	8,2'-O-AnhA-$(dTMS)_3$	95	-	-
V	8,2'-S-AnhA[33]	190	159-62	276.5, 222 (H_2O)
Vf	8,2'-S-AnhA-$(TMS)_3$	52	-	-
Vf'	8,2'-S-AnhA-$(dTMS)_3$	68	-	-
VI	8,2'-N-AnhA[98]	215	d*260	273 (H_2O)
VIf	8,2'-N-AnhA-$(TMS)_4$	80	-	-
VIf'	8,2'-N-AnhA-$(dTMS)_4$	80	-	-
VII	8,2'-O-AnhI[106]	-	d* 232	253 (H_2O)
VIIf	8,2'-O-AnhI-$(TMS)_3$	134	-	-
VIII	8,2'-S-AnhI[33]	dec*	d* 217	265 (H_2O)
VIIIf	8,2'-S-AnhI-$(TMS)_3$	115	-	-
VIIIf'	8,2'-S-AnhI-$(dTMS)_3$	65	-	-
IX	8,2'-N-AnhI[98]	-	d* 210	263 (H_2O)
IXf	8,2'-N-AnhI-$(TMS)_4$	134	-	-
IXf'	8,2'-N-AnhI-$(dTMS)_4$	105	-	-

TABLE I continued

X	8,2'-O-AnhG[107]	-	d* 210	283, 247 (H_2O)
Xf	8,2'-O-AnhG-$(TMS)_4$	109	-	-
XI	8,2'-S-AnhG[33]	dec*	d* 256	268, 283(sh) (H_2O)
XIf	8,2'-S-AnhG-$(TMS)_4$	86	-	-
XIf'	8,2'-S-AnhG-$(dTMS)_4$	137	-	-
XII	8,2'-N-AnhG[98]	-	d* 203	258, 290(sh) (H_2O)
XIIf	8,2'-N-AnhG-$(TMS)_5$	109	-	-
XIII	8,2'-S-AnhX[106]	dec*	d* 260	273 (H_2O)
XIIIf	8,2'-S-AnhX-$(TMS)_4$	115	-	-
XIV	8,3'-O-AnhA[104]	-	266-7	262 (EtOH)
XIVf	8,3'-O-AnhA-$(TMS)_3$	40	-	-
XV	8,3'-S-AnhA[105]	-	166-8	287, 223 290(sh), 276(sh) (H_2O)
XVf	8,3'-S-AnhA-$(TMS)_3$	75	-	-
XVI	8,3'-O-AnhI[106]	-	d* 229	253 (H_2O)
XVIf	8,3'-O-AnhI-$(TMS)_3$	62	-	-
XVII	8,3'-S-AnhI[106]	-	d* 218	272, 218 278(sh) (H_2O)
XVIIf	8,3'-S-AnhI-$(TMS)_3$	35	-	-
XVIII	8,3'-S-AnhG[20]	-	d* 183	272, 283(sh) (H_2O)
XVIIIf	8,3'-S-AnhG-$(TMS)_4$	131	-	-

d* - decomposition occurs at temperatures greater than

dec* - no spectrum could be obtained, upper temperature reached = 300°C

deuteration was not achieved (88% for Ia, 66% for IIa) the spectra also showed partial incorporation of a third deuterium atom into the molecules (25% for Ia, 15% for IIa). This probably occurs at C-5 or C-6 of the base[90].

Acetylation: Two methods were used. (i) Samples of Ib were prepared on the 100-1000 mg. scale from I by reaction with acetic anhydride in dry pyridine overnight at room temperature. The products were purified by precipitation, recrystallization and thin layer chromatography[91]. (ii) Small scale preparation of samples for mass spectral studies was achieved as follows: 1 mg. of sample was placed in a 0.3 ml. "Reacti-vial" (Pierce Chemical Company) which was sealed with a butyl rubber septum. 10 µl. of dry pyridine, followed by 50 µl. of acetic anhydride or acetic anhydride-d_6 (99%D, Stohler Isotope Chemicals) were then injected with a syringe. The reaction vial and contents were then maintained at 50-60^0 in a water bath for 2 hours. 20 µl. of the reaction mixture was withdrawn, placed in specially prepared glass tubing which was to become the sample tube, and volatile materials removed under high vacuum overnight. The glass tubing was cut to size, and inserted into the mass spectrometer sample tube holder prior to mass spectral analysis of the sample. Separate paper chromatographic analyses of reaction mixtures confirmed that this procedure caused complete acetylation of starting materials. Samples of Ib'' and Ib''' were prepared by this procedure, but using 3'-O-acetyl- or 5'-O-acetyl-2'-anhydrouridine[92] as starting materials. IIIb was prepared by treating Ib with phosphorus pentasulfide[91].

Trifluoroacetylation: Procedure (ii) described above for acetylation was used with the following changes. The reaction was performed at room temperature for 1 hour and trifluoroacetic anhydride was substituted for acetic anhydride. (Extensive reaction appeared to occur immediately on adding the anhydride). The mass spectra indicated complete reaction though this could not be confirmed by paper chromatography due to the lability of the trifluoroacetyl group.

Trimethylsilylation: Approximately 0.5 mg. of a sample was placed in a 0.3 ml. "Reactivial" which was then sealed with a butyl rubber septum. 20 µl. of dry pyridine followed by 100 µl. of N,O-bis-trimethylsilyl-trifluoroacetamide (BSTFA) and 15 µl. of trimethylchlorosilane (TMCS) (Pierce Chemical Company) were then added with a syringe. After standing overnight, or after heating to 60^{o} for 30 minutes, 20 to 30 µl. of the reaction mixture were removed, and prepared for the mass spectrometer as described in procedure(ii) for the acetyl derivatives. Complete trimethylsilylation (i.e. one hydrogen of each OH, NH or NH_2 group of sugar and base moieties was replaced by a TMS group[93]) was achieved at room temperature in all cases except for IX, 8,2'-N-AnhI. Four trimethylsilyl groups were incorporated on warming to 60^{o} but only three trimethylsilyl groups were incorporated at room temperature.

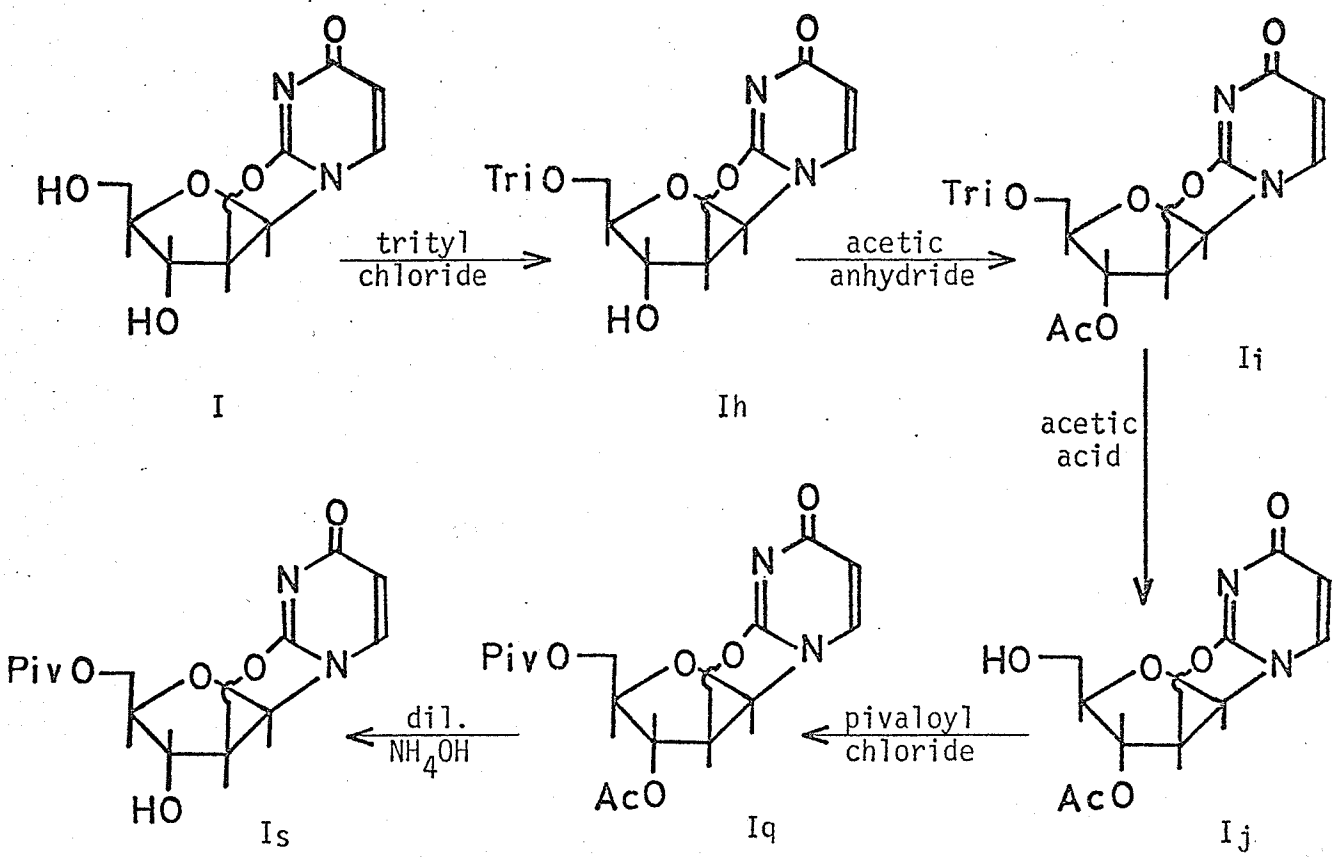
Trimethyl-d_9-silylation and Dimethylsilylation: Trimethyl-d_9-silyl (dTMS) derivatives were prepared in a similar way to trimethylsilylation. Half quantities were used and the reagents used were N,O-bis-trimethyl-

silylacetamide-d_{18} (BSA) and TMCS-d_9 (Merck, Sharpe and Dohme, Montreal, Canada) instead of BSTFA and TMCS. The reagents used in dimethylsilylation were N,O-bis-dimethylsilylacetamide and dimethylchlorosilane (Pierce Chemical Company).

CHAPTER THREE: RESULTS AND DISCUSSION (PART I)

The Pyrimidine 2,2'-Anhydronucleosides and Their Derivatives

Most of the derivatives studied were of value as, or closely related to, intermediates in a program of synthetic work. For example, in the case of 2,2'-anhydrouridine, I, by utilizing the greater reactivity of the 5'-OH group, than the 3'-OH group, to trityl chloride, base-labile groups can be placed on either the 3'- or 5'- oxygen atoms as illustrated by the scheme[91]:



I Ih Ii Ij Iq Is

Phosphorylation of Ih, Ij and Is can be readily achieved and the nucleotides may then be used in oligonucleotide synthesis. The protecting groups may then be removed under mild conditions. To allow for the maximum flexibility in the synthesis of oligonucleotides it is essential to have available acid- and base-labile protecting groups for the 3'- and 5'-positions. It is also essential to be able to readily distinguish between isomeric pairs. As the results will show, the mass spectra of the isomers are indeed different and each isomer can be identified. However, the di- and tri-methylsilyl ether and trifluoroacetyl derivatives were also studied because of their enhanced volatility with respect to their parent compounds rather than because of any anticipated synthetic value. Indeed, not all of the compounds studied, for example the 2'-anhydropurine nucleosides (see TABLE I), were sufficiently volatile to investigate without these derivatization techniques. TABLE I also shows that the anhydronucleosides are much less volatile than the corresponding normal nucleosides. (Compare sample temperatures for uridine, deoxythymidine, adenosine with those for I, II, IV, V, and VI.)

2,2'-anhydropyrimidine-nucleosides

The mass spectra of 2'-AnhU (I), 2'-AnhT (II), and 2'-Anh4SU (III) are shown in Figure 1. These spectra show a number of prominent peaks whose relative intensities differ considerably from corresponding peaks in the mass spectra of pyrimidine and purine nucleosides[47,64,68,69,99-101].

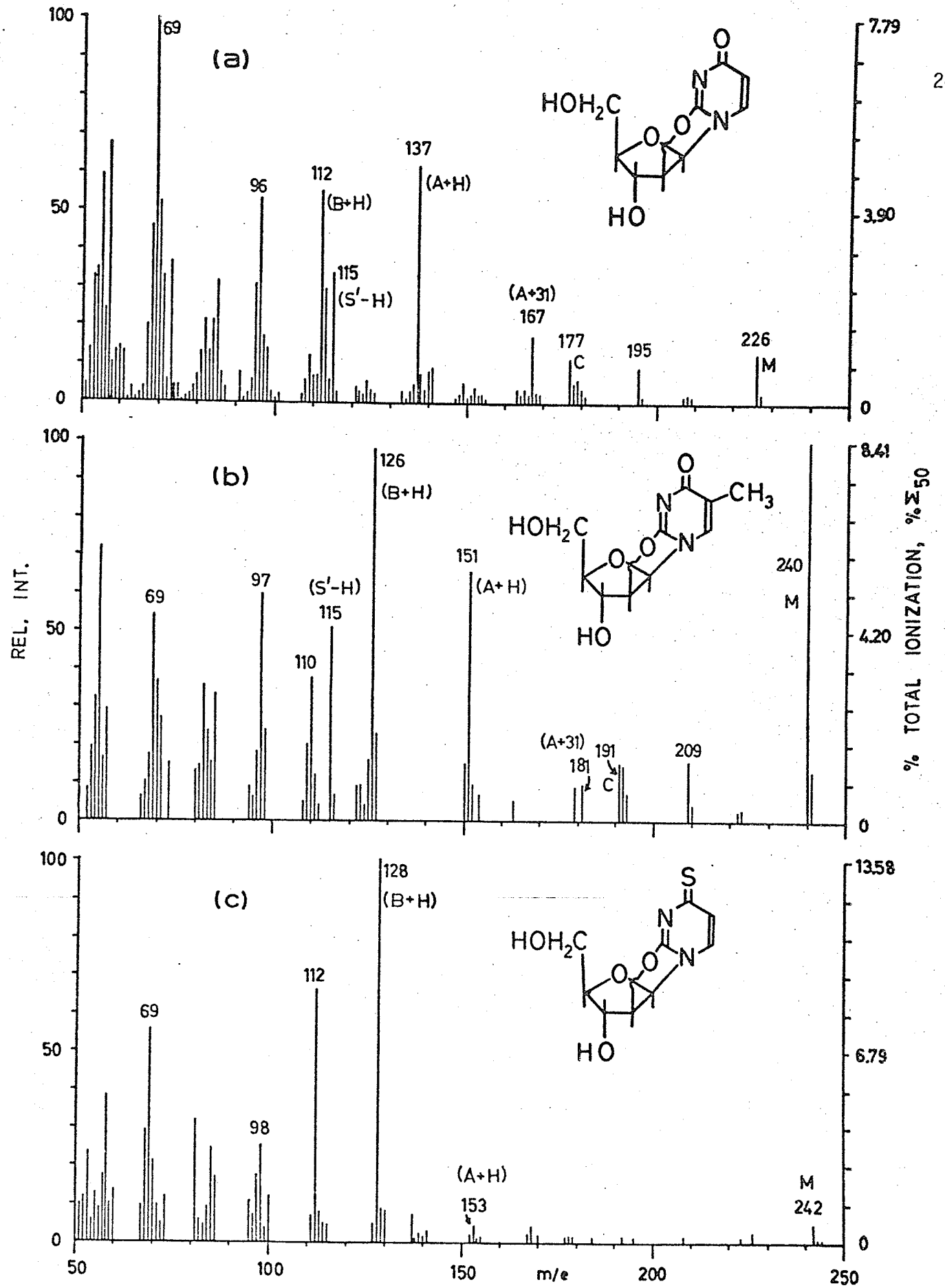


Figure 1. Mass Spectra of (a) 2'AnhU, (I); (b) 2'AnhT, (II); and (c) 2'Anh4SU, (III).

TABLE II

Relative intensities, and deuterium shifts, of some diagnostically important peaks in the mass spectra of 2,2'-anhydronucleosides, and comparison with spectra of other nucleosides.

Ion	mass-to-charge ratio/relative intensity						
	I	II	III	Uridine	Deoxy-thymidine	Adenosine	Deuterium shift for I and II
M^+	226/51.7	240/100	242/5.1	244/3.1	242/6.3	267/5.0	+2
$(M-30)^{+*}$	196/2.0	210/$\leq$1.8	212/ -	214/0.70	212/0.40	237/11.0	-
$(M-31)^+$	195/23.0	209/15.5	211/ -	213/1.3	211/1.1	236/1.6	+1
$(M-49)^+$ or C^+	177/21.7	191/15.0	193/ -	195/3.8	193/1.4	218/0.78	0
$(A+31)^+$	167/41.3	181/9.8	183/ -	-	-	-	+2
$(A+H)^+$	137/100	151/66.0	153/4.3	-	-	-	+1
$(M-89)^+$	-	-	-	155/6.9	153/5.3	178/62.0	-
S^{+*}	-	-	-	133/65.2	117/100	133/2.0	-
$(S-H)^+$	-	-	-	132/10.1	116/ -	132/ -	-

TABLE II continued

$(S'-H)^+$	115/55.3	115/51.1	115/4.5	-	-	-	+1
$(B+30)^+$	-	-	-	141/23.1	155/1.1	164/100	-
$(B+2H)^{+*}$	113/32.3	127/18.6	129/4.6	113/100	127/30.6	136/24.3	+2
$(B+H)^+$	112/65.0	126/97.7	128/100	112/41.2	126/66.4	135/100	+2
B^+	111/10.3	125/16.1	127/4.5	111/2.7	125/ -	134/2.4	+1
$(B+2H-O)^+$	97/16.0^t	111/12.1	113/8.2	97/12.4	111/2.8	120/1.1	0,+1?
$(B+H-O)^+$	96/44.3^t	110/37.9	112/66.3	96/7.0	110/14.0	119/8.1	0
$(B-O)^+$	95/34.7^t	109/20.1	111/7.2	95/1.3	109/3.7	118/ -	0
$(B-OH)^+$	94/15.7^t	108/5.2	110/12.3	94/ -	108/ -	117/ -	0
$\%\Sigma_{50}$/R.I.	0.081	0.084	0.136	0.114	0.162	0.185	-

NOTE: B has the same identity in nucleosides and anhydronucleosides, namely the base moiety of the nucleoside. S is the sugar moiety of a nucleoside. S' is the sugar moiety of an anhydronucleoside.

* Relative intensities of these peaks have been corrected for ^{13}C content of peak one mass unit lower.

t Includes contributions from fragments of ribose moiety.

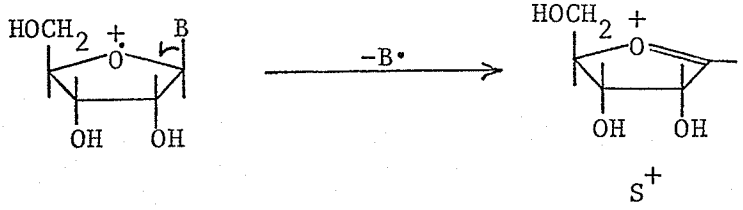
Comparative data, obtained in this study, for the principal, or diagnostically important, peaks in the mass spectra of compounds I, II and III, and also uridine, deoxythymidine (i.e. thymine-2-deoxyriboside) and adenosine, are shown in TABLE II. The spectra for the latter three compounds agree favorably with those in the literature[47,64,69,99,100].

$(M-30)^+$ and $(M-31)^+$

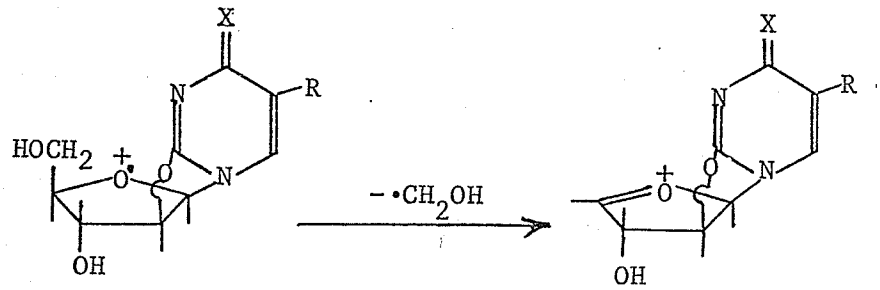
The relative intensities of these ions are of interest. For purine nucleosides the presence of the $(M-30)^+$ ions has been taken as a structural indicator for a 5' hydroxyl group since the H of the hydroxyl group can be transferred to the charge carrying base moiety with subsequent elimination of CH_2O[69] (also see Introduction). Two requirements for this reaction are (i) it must be energetically preferred, (ii) in the excited ion the 5' hydroxyl group and the base must be sterically accessible to each other. Initially, consideration was given to steric conditions for transfer of the active hydrogen of the 5' hydroxyl group to the base moiety. Proton magnetic resonance studies on 2,2'-anhydrouridine in aqueous solution at room temperature or above indicate that rotation about the 4', 5' carbon-carbon bond is rapid[102]. Models show that such an internal rotation permits the H of the 5' hydroxyl group to approach within van der Waals distances of the O atom of the anhydro-ring and the N-1 and C-2 atoms of the base respectively. If any of these sites are able to accept the hydrogen atom then transfer may occur. For purine nucleosides, where

the probability of localization of the positive charge on the purine base moiety is high, the transfer of the H of the 5' hydroxyl group readily occurs, though it does not occur to any significant extent in purine anhydronucleosides where the probability of localization of the positive charge on the base is also high (see Figures 20, 21, 22 and reference 87). Two possibilities are immediately apparent. The anhydro-ring locks the base into a given position. This position may be such that the site (not specified in the original proposal[69]) to which the H atom transfers in the purine nucleoside is unfavorably positioned in the anhydronucleoside. Alternatively, this site may be that involved in the formation of the anhydro-ring itself. For pyrimidine nucleosides and anhydronucleosides the probability of localization of the positive charge on the base moiety is much lower (compare the relative intensities of peaks characteristic of sugar fragments, e.g. S^+, $(S'-H)^+$ and those characteristic of base fragments, e.g. $(B+H)^+$, $(B+2H)^+$, in TABLE II, and also in published spectra[47,64,69,99,100]).

For the pyrimidine compounds, fragmentations initiated by localization of the charge either on the heteroatom of the sugar moiety, or the anhydro-ring, are relatively more important. For pyrimidine nucleosides, elimination of the base moiety is a favored process:



In 2,2'-anhydronucleosides this easy fragmentation is precluded by the presence of the fused ring between the base and sugar moieties. Here, transfer of the H of the 5' hydroxyl group to the base is less favorable than elimination of a hydroxymethyl radical[35,87-89]:



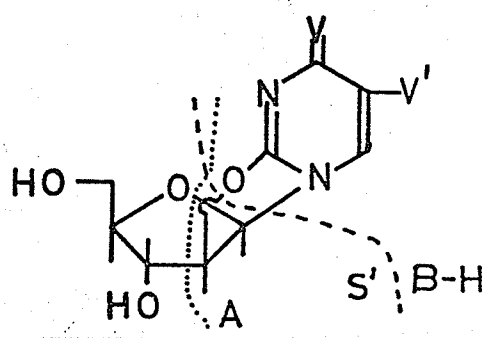
$(M-31)^+$

(Deuterium labelling, see TABLE II, shows the loss of one active hydrogen.) The foregoing observations have important consequences to the interpretation of some of the major peaks in the spectra.

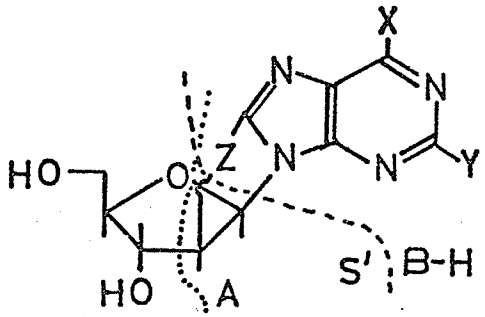
$\underline{(A+H)^+}$

This ion, in which A represents the anhydro-ring plus base as shown below, appears to be diagnostically characteristic of anhydronucleosides (both pyrimidine and purine types) and all of their derivatives that were studied. As is the case for natural nucleosides it corresponds to the loss of m/e=89 from the molecular ion of underivatized ribose nucleosides, but for anhydronucleosides its composition and structure must be different. Corresponding ions have also been observed from

volatile derivatives of the 2'-anhydropurine nucleosides listed in TABLE I.

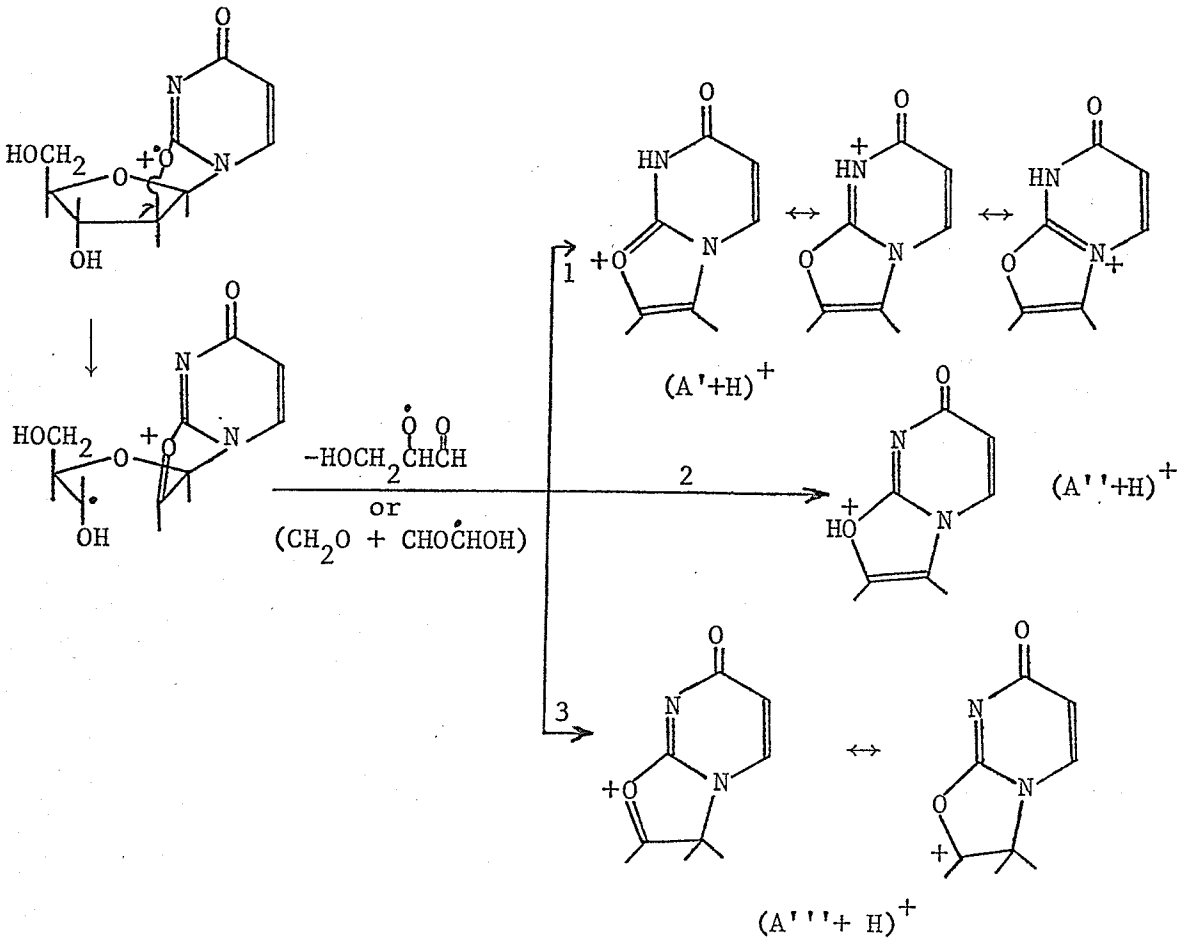


I, II, III



IV, V, VI

There is little doubt that the atomic composition of this ion involves the atoms of the base plus the C-1' and C-2' atoms of the sugar plus the heteroatom (i.e. O,S or N) of the anhydro-ring. For 8,2'-S-AnhA (V) the atomic composition determined by high resolution mass spectrometry supports this assignment[87]. Furthermore, deuterium labelling, see TABLE II, shows that migration of an active hydrogen atom is involved in the formation of these ions. The relative intensity of this peak for the 2'-anhydropyrimidine nucleosides parallels the intensity of the $(S'\text{-}H)^+$ peak characteristic of the sugar moiety and is believed to arise from fragmentations initiated by localization of the positive charge on the oxygen atom of the anhydro-ring. Possible reactions, illustrated for 2'-AnhU, I, consistent with these observations are:



Each of these reactions could involve H transfer from either 3' or 5' OH, or both. Reaction 3 would occur via a six-membered cyclic transition state. Reaction 1 must also be considered because molecular models indicate that once the C-2', C-3' bond is broken either labile hydrogen atom can approach to within van der Waals distances of N-3, in contrast to the situation discussed earlier with respect to the $(M-30)^+$ and $(M-31)^+$ ions. Reaction 2 is also a possibility[35]. Of the three possibilities, reaction 1 would appear to lead to the most stable ion product because of the more extensive delocalization

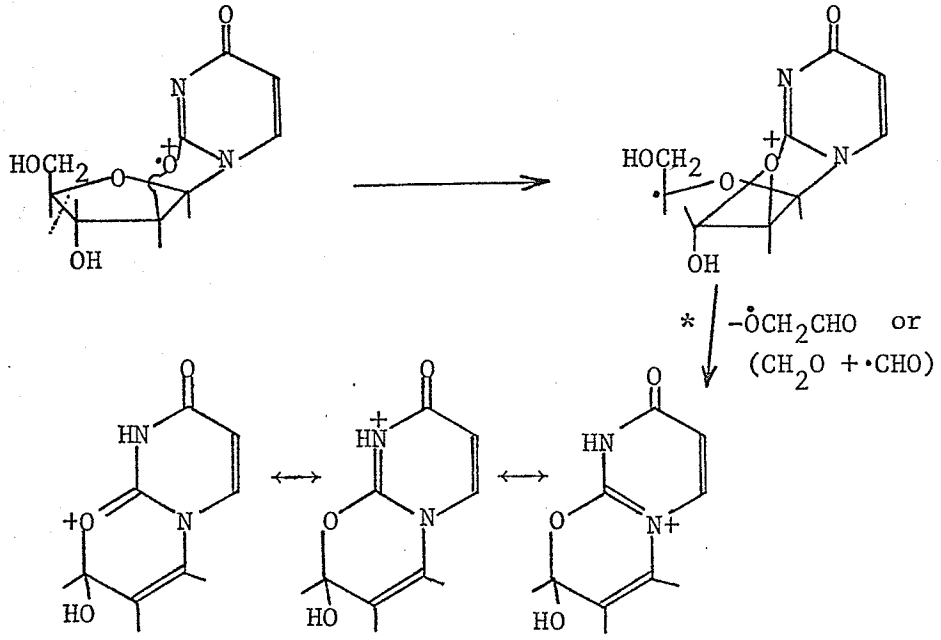
of positive charge. The molecular models support the proposed charge delocalization as suggested for $(A'+H)^+$. They show that one of the p orbitals of the oxygen atom of the anhydro-ring is nearly perpendicular to the plane of the pyrimidine ring and is thus at a favorable angle to overlap with the π system of the base. Furthermore, in the neutral molecule, such interaction of the oxygen p orbital with the π orbitals of the base would lower the ionization energy of an electron from this orbital and increase the probability of fragmentations initiated by initial localization of positive charge at the oxygen atom.

A bicyclic structure is considered likely for the $(A+H)^+$ ion. It is evidently of high stability because of its regular occurrence and high abundance in the spectra of the 2'-anhydronucleosides that were studied. To propose ions of reasonable non-bicyclic structure in which charge delocalization would be extensive, or which do not have a diradical character is difficult.

$\underline{(A+31)^+}$

This ion can also be represented as $(M-59)^+$ [35]. Deuterium labelling, see TABLE II, indicates that both the labile hydrogens of the 3' and 5' hydroxyl groups are incorporated into its structure. For I, a metastable peak confirms that the molecular ion is a precursor (for 226→167, m* (calc) = 123.4, m* (obs) = 123.5). The ion could arise from fragmentations initiated by localization of the positive charge

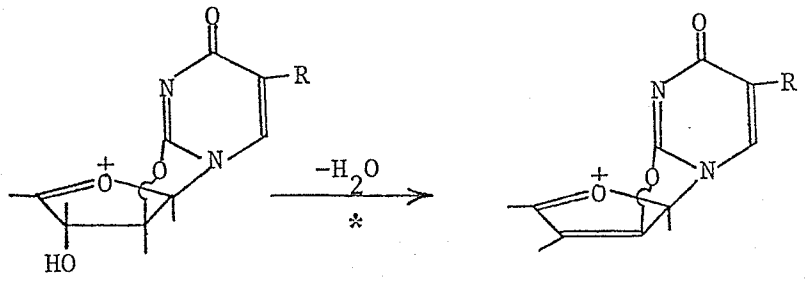
on the oxygen atom of the anhydro-ring, or possibly the O atom of the 3' hydroxyl group. Molecular models indicate the feasibility of the H transfer from the 5' OH group. Of the possibilities considered, the reaction below would seem to lead to the most stable ion product, and precedents for this type of mechanism have been proposed[103].



$(A + 31)^+$

C^+ or $(M-49)^+$

Since this ion does not contain either labile H atom (see TABLE II) the following route, supported in I by a metastable peak at 160.5 (195→177, m* (calc) = 160.7), to its formation is proposed[35]:



$(M-31)^+$ C^+

$(B+2H)^+$, $(B+H)^+$, B^+

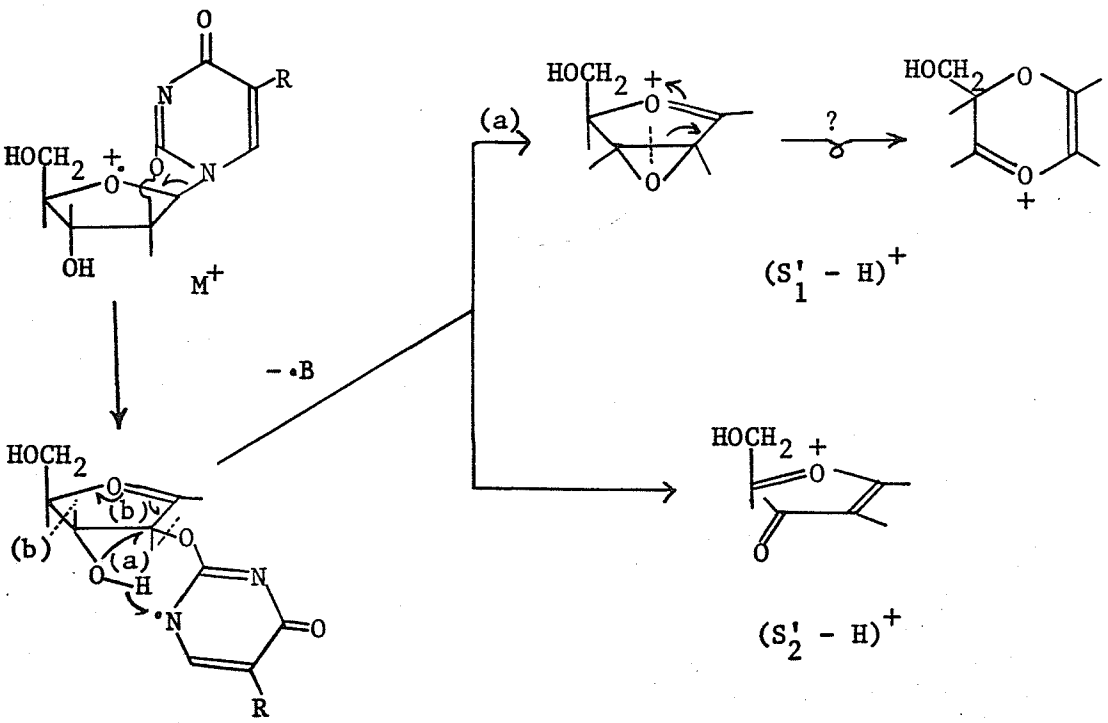
In these ions B represents the base moiety of the corresponding natural nucleoside. If the heteroatom of the anhydro-ring is regarded as part of the base then the base of an anhydronucleoside is represented by B-H, as shown in I-VI. Thus, ions of the same atomic composition $(B+2H)^+$, $(B+H)^+$ and B^+ are found in the mass spectra of both anhydronucleosides and natural nucleosides (compare Figure 1, TABLE II and references[47,64,68,69,73,99-101]). In the present case however, 3, 2, and 1 hydrogen transfers to the base are required respectively. Deuterium labelling (see TABLE II) indicates that both the labile hydrogens of the 3' and 5' OH groups are transferred in the formation of $(B+2H)^+$ and $(B+H)^+$, and in the former case at least one non-labile hydrogen from the ribose skeleton is involved. Although these ions must be formed by complex multiple hydrogen transfer reactions they are usually of high abundance in the mass spectra of 2'-anhydronucleosides[35,87-89].

$(B+2H-O)^+$, $(B+H-O)^+$, $(B-O)^+$, $(B-H-O)^+$

These ions, differing in the number of hydrogens transferred in their formation are more abundant in the spectra of anhydronucleosides than of natural nucleosides. Deuterium labelling indicates that while there is some transfer of active hydrogen into the ions $(B+2H-O)^+$ and $(B+H-O)^+$ it is not extensive and most of the hydrogen transferred into the ion products originates from the ribose skeleton. For I, interpretation is complicated because fragment ions from the ribose moiety occur at the same m/e values.

$(S'-H)^+$

The ion occurring at m/e = 115 is characteristic of unmodified anhydro-(arabinofuranosyl)-nucleosides since no mass shift occurs as the base moiety changes. (S' is used instead of S to avoid confusion with the fragment ion characteristic of the sugar moiety of a natural nucleoside). Deuterium labelling (see TABLE II) shows that one of the labile hydrogens of the 3' or 5' OH groups is lost in the formation of this ion. Of the reactions considered the following are thought to be the most plausible (other reactions involved multiradical transition states or migrations of groups other than hydrogen). These reactions lead to ion and neutral products expected to be stable and molecular models show that the hydrogen transfer processes are feasible [35]



Other ions

The ions occurring below m/e = 100 are considered of little diagnostic value. Some of the prominent ions can be produced by fragmentations involving the base or sugar moieties and deuterium labelling indicated that no single reaction accounted for their formation.

3',5'-di-O-acetyl-2,2'-anhydropyrimidine nucleosides

The mass spectra of the 3',5'-di-Ac-2'AnhU (Ib), 3',5'-di-dAc-2'AnhU (Ib'), 3'-Ac-5'-dAc-2'AnhU (Ib''), 3'-dAc-5'-Ac-2'AnhU (Ib'''), 3',5'-di-Ac-2'AnhT (IIb), 3',5'-di-dAc-2'AnhT (IIb') and 3',5'-di-Ac-2'Anh4SU (IIIb) are shown in Figure 2. The identity and structure of a number of ions are solved by the appropriate mass shifts as the base moiety changes and by considering the spectra of the deuterium labelled compounds Ib, Ib', Ib'', Ib''', IIb, IIb'. Significant points which arise are considered in the discussion of each ion.

M^+

Loss of H from the molecular ion is minimal for Ib and IIb. The enhanced relative intensities of ions of 1, 2 and 3 m/e units below M^+ for Ib', Ib'', Ib''' and IIb' indicates some conversion of D to H either during the acetylation reactions, or in the ion source. (The $(CD_3CO)_2O$ used in the acetylation reactions was supplied as 99% D (Stohler Isotope Chemicals). Its deuterium content was checked by

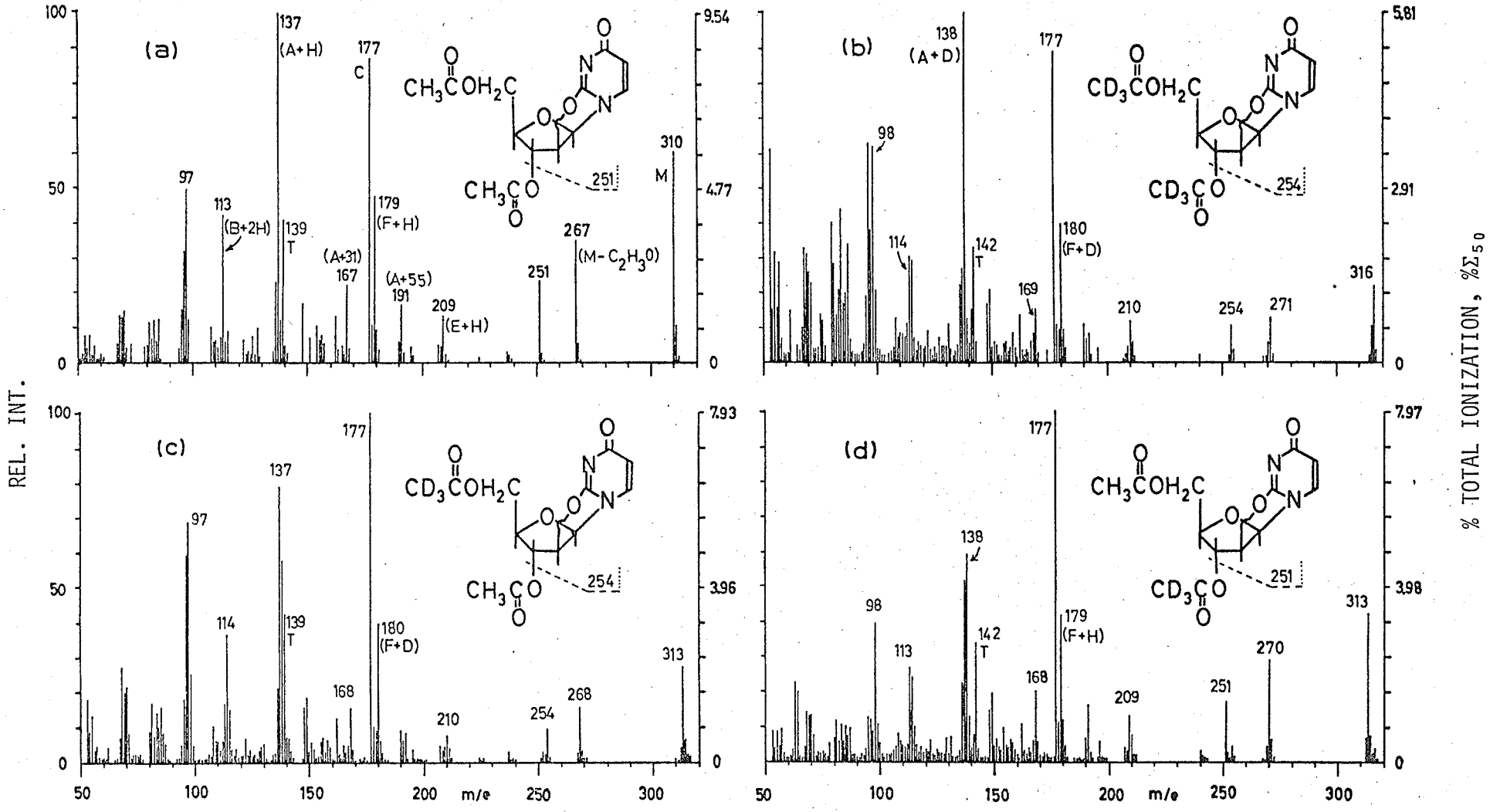


Figure 2. Mass Spectra of (a) 3',5'-di-Ac-2'AnhU, (Ib); (b) 3',5'-di-dAc-2'AnhU, (Ib'); (c) 3'-Ac-5'-dAc-2'AnhU, (Ib'') and (d) 3'-dAc-5'-Ac-2'AnhU, (Ib''').

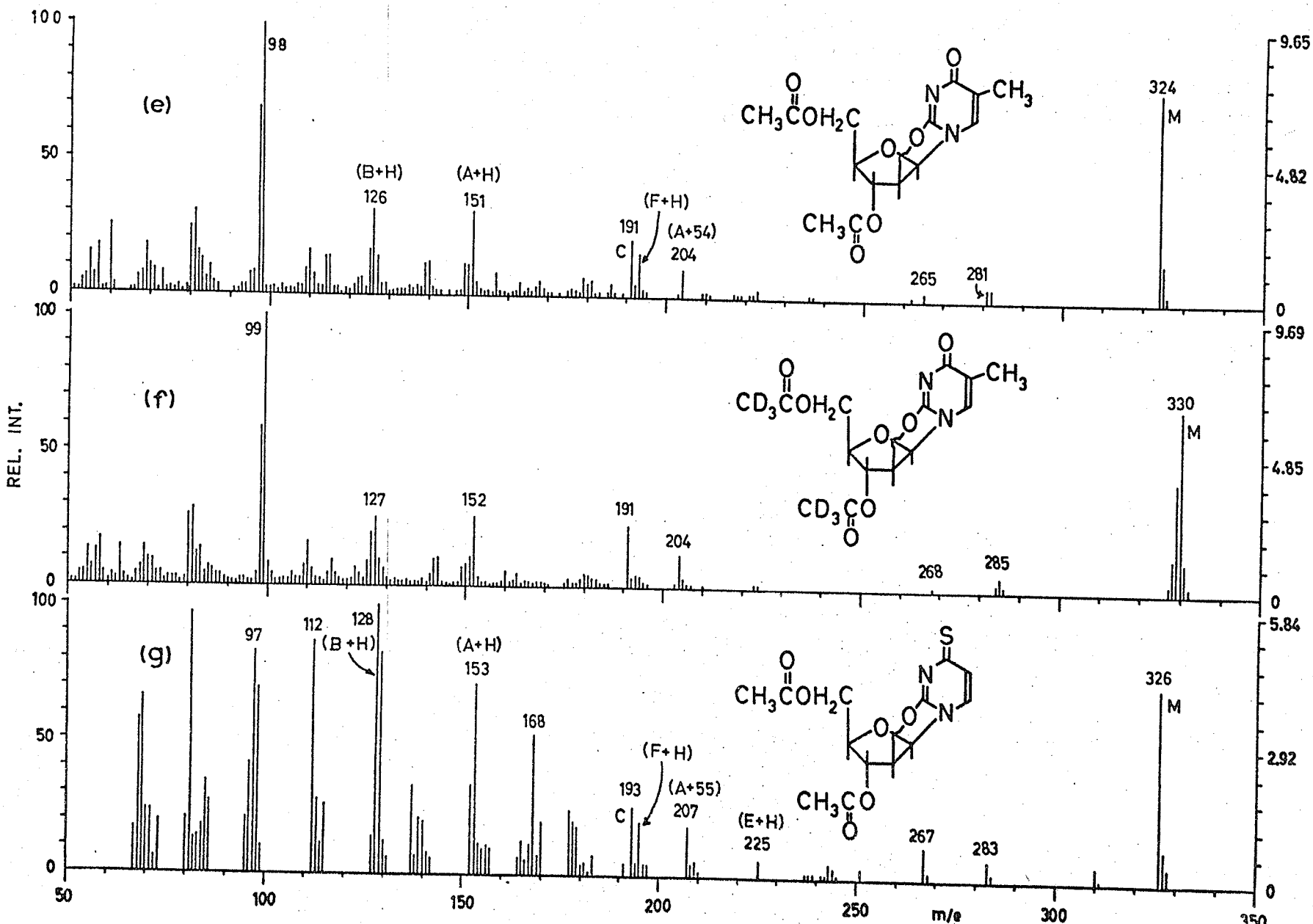


Figure 2, continued. Mass Spectra of (e) 3',5'-di-Ac-2'AnhT, (IIb); (f) 3',5'-di-dAc-2'AnhT, (IIb') and (g) 3',5'-di-Ac-2'Anh4SU, (IIIb).

converting it to methyl acetate. Mass spectral analysis indicated the D content to be a minimum of 97%.)

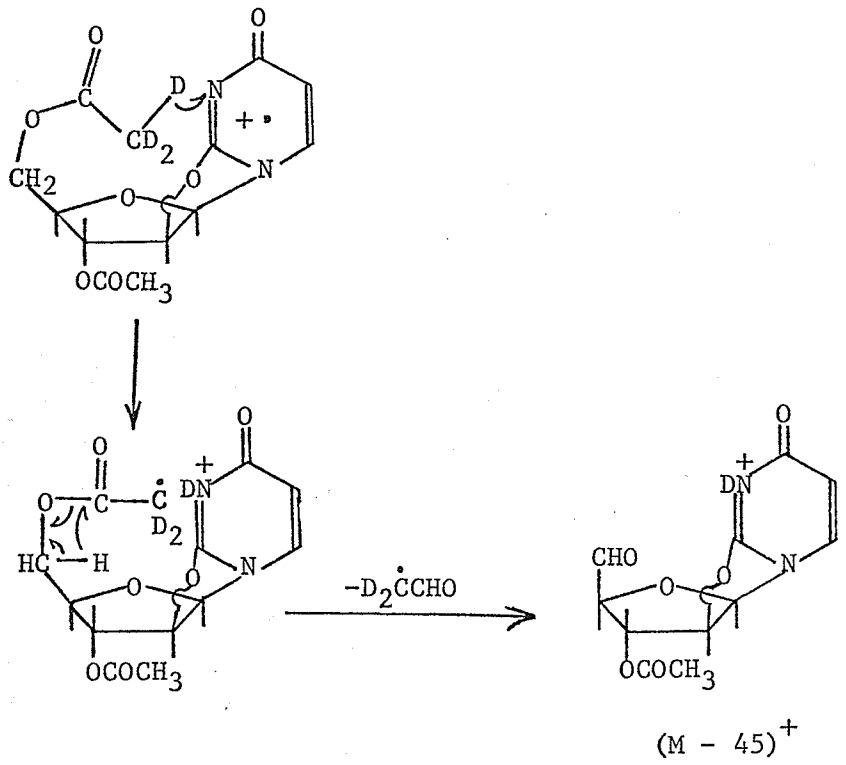
$(M-CH_3CO_2)^+$

The spectra of the deuterium-labelled compounds Ib'' and Ib''' show that the CH_3CO_2 group lost arises nearly exclusively from the 3' position of the ribose ring. This is an important observation since it can be used as a diagnostic test for the presence of a 3'-O-acetyl group, a test which will be used in characterizing the isomeric pairs (To be discussed later).

$(M-C_2H_3O)^+$

This ion is of unexpected interest. The simplest way in which it could be formed is by a homolytic fission resulting in loss of either the 3' or 5' acetyl group. The following m/e values are to be expected for this ion: Ib, 267; Ib', 270; Ib'', 267 and 270; Ib''', 267 and 270; IIb, 281; IIb', 284. The spectra of Ib'' and Ib''' show that the homolytic fission involving loss of the 3'-acetyl group is of minor importance compared with the alternative process involving loss of the 5'-acetyl group. However, when a d_3-acetyl group is present on the 5' oxygen atom, loss of a fragment of m/e = 45 (C_2D_2HO) is more probable than the expected loss of a fragment of m/e = 46 (C_2D_3O), (compare the relative intensities of 271:270 in the spectrum of Ib', of 268:267 for Ib'' and 285:284 for IIb'). The peaks at m/e = 271, 268 and 285

could occur in the spectra of Ib', Ib'' and IIb' respectively if complete acetylation had failed when the samples were prepared, i.e. these peaks would be due to molecular ions of the monoacetyl derivatives. There was also the chance of hydrolysis by residual water vapor in the mass spectrometer. The first possibility was eliminated in two ways: (i) analysis of the products of the acetylation reaction by paper chromatography showed only the diacetyl derivatives to be present, (ii) the mass spectra of the diacetyl derivatives showed no significant differences whether the samples used were obtained directly from the reaction mixture or purified by paper chromatography. To test the possibility that the diacetyl derivatives underwent deacetylation reactions with water in the ion source the ionization chamber temperature was reduced from its normal 250°C to 90°C. The sample was moistened with d_6-acetic anhydride and d_6-acetic anhydride was also allowed to leak into the ion source. These procedures enhanced the relative intensities of the molecular ions but did not significantly change the ratio $(M-45)^+:(M-46)^+$. Additionally, the low abundance of m/e = 271 in the spectrum of Ib''' virtually eliminates the serious consideration of hydrolysis of the diacetyl compound as an explanation for the $(M-45)^+$ ion found for 5'-O-acetyl-d_3 derivatives. A suggested reaction sequence is:



Models support the feasibility of H transfer to N-3 from the 5'-O-acetyl group.

$\underline{(A+H)^+}$

This ion occurs at m/e = 137 for Ib, at 151 for IIb, and at 153 for IIIb, the same values as for I, II, and III respectively, and similar structures are assumed. For I and II the formation of this ion was shown to involve migration of the labile H atom from either of the OH groups on the ribose moiety into the ion product. When these labile hydrogens are replaced by acetyl functions the mass spectra clearly show that the hydrogen transferred into the ion product $(A+H)^+$

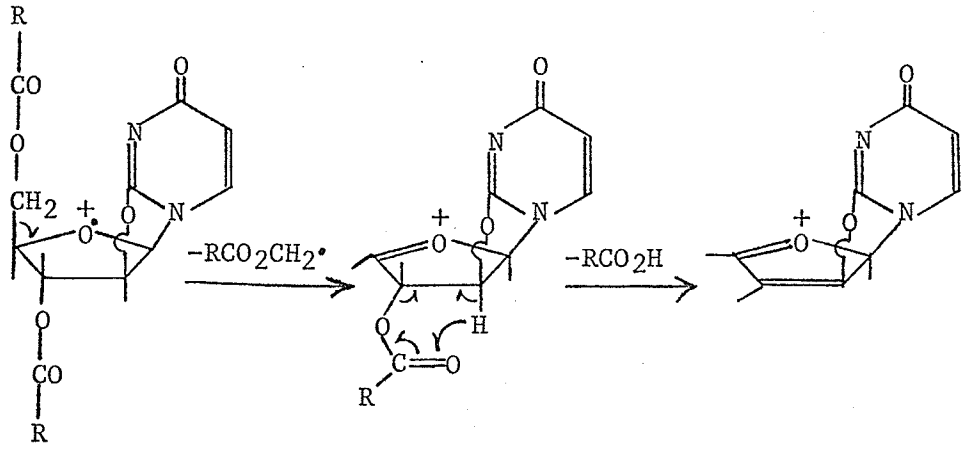
originates from the methyl of either acetyl group rather than from the ribose skeleton, i.e. $(A+H)^+$ occurs at m/e = 138 for Ib', 137 and 138 for both Ib'' and Ib''', and at 152 for IIb'.

$(B+H)^+$, $(B+2H)^+$

The $(B+2H)^+$ ion is prominent in its region of the spectrum. For I and II this ion incorporates hydrogens by transfer predominantly from the 3' and 5' OH groups. Consideration of the relative intensities of the peaks due to $(B+H)^+$ and $(B+2H)^+$ in the spectra of Ib, Ib', Ib'', Ib''', IIb and IIb' shows that hydrogen transfer now occurs from either methyl group or from the ribose skeleton, but more readily from the methyl groups.

C^+

This ion occurs at m/e = 177 and 191 for the 2'-AnhU and 2'-AnhT derivatives respectively. No deuterium is present in this ion for compounds Ib', Ib'', Ib''' or IIb'. The most likely route to its formation, illustrated for the anhydrouridine derivatives, is considered to be:

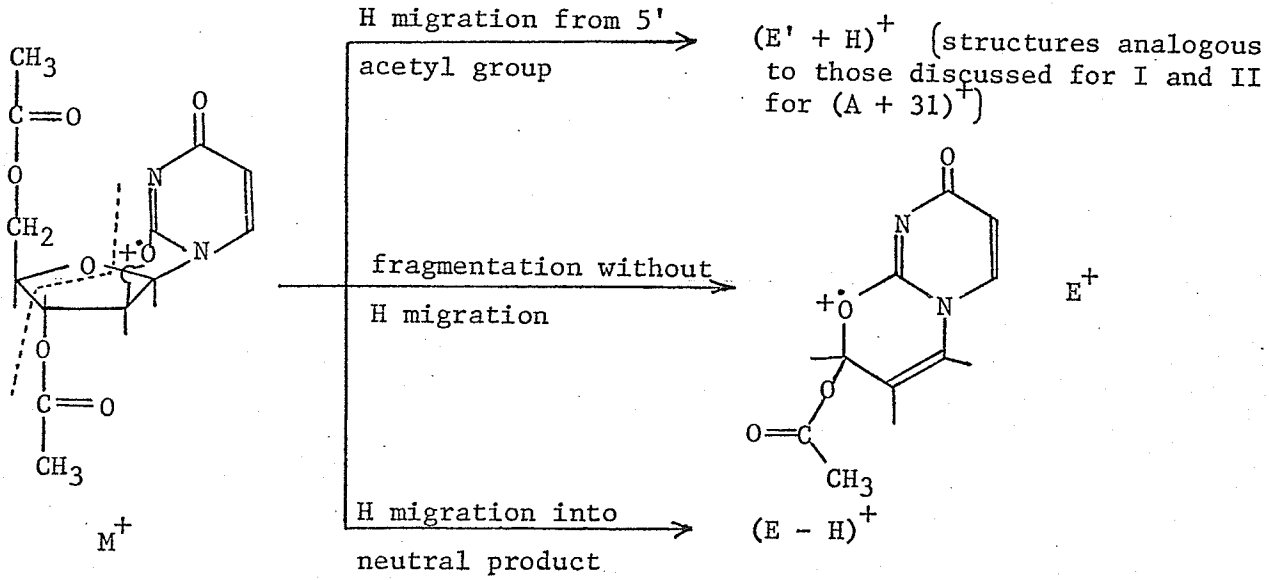


M^+ $(M - RCO_2CH_2)^+$ C^+

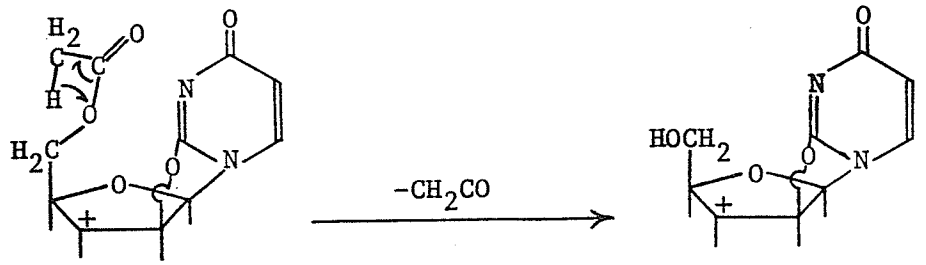
In the second step in this sequence elimination of a molecule of acetic acid occurs via hydrogen transfer involving a six-membered cyclic transition state.

$(E+H)^+$, E^+ and $(E-H)^+$

In the spectrum of Ib these ions appear at m/e values of 209, 208 and 207 respectively, with $(E+H)^+$ being the most abundant. Corresponding ions are of low abundance in the spectra of IIb and IIIb. Probable routes to their formation are considered to be:



or



$(M - CH_3CO_2)^+$ $(E''+H)^+$

A decision as to the relative importance of these processes can be made by noting that the m/e values for these ions in the deuterated derivatives should be as follows:

$(E'+H)^+$: Ib, 209; Ib', 213; Ib'', 210; Ib''', 212

E^+ : Ib, 208; Ib', 211; Ib'', 208; Ib''', 211

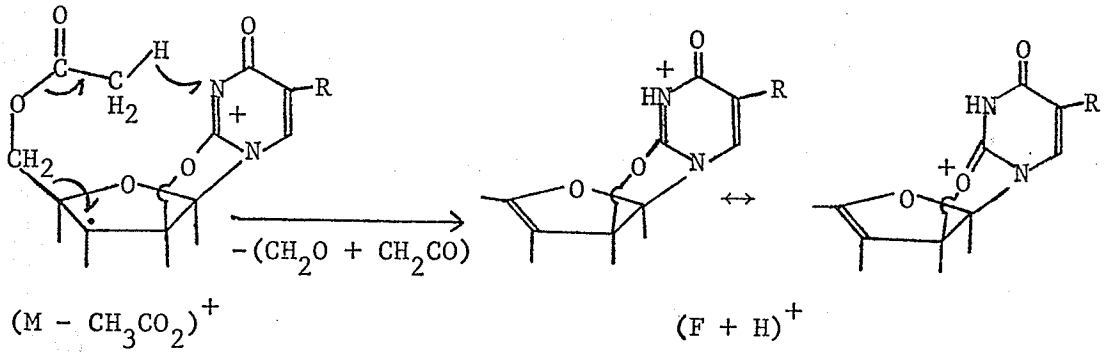
$(E-H)^+$: Ib, 207; Ib', 209 or 210; Ib'', 207; Ib''', 209 or 210

$(E''+H)^+$: Ib, 209; Ib', 210; Ib'', 210; Ib''', 209

The spectra of Ib' and Ib'' (Figure 2) show m/e = 210 to be the biggest peak in this region of their spectra, clearly indicating that the formation of $(E''+H)^+$ is the preferred pathway. However, other peaks, especially m/e = 211 for Ib', have enhanced intensity indicating some contribution from other structures, especially E^+. The ion $(E'+H)^+$ which is analogous to the prominent ion $(A+31)^+$ in the spectrum of I and II, is of low intensity, because the labile hydrogen of the 5' OH group has been replaced by the acetyl group, and alternative fragmentations become preferred, e.g. formation of $(M-C_2H_3O)^+$ discussed earlier.

$\underline{(F+H)^+}$

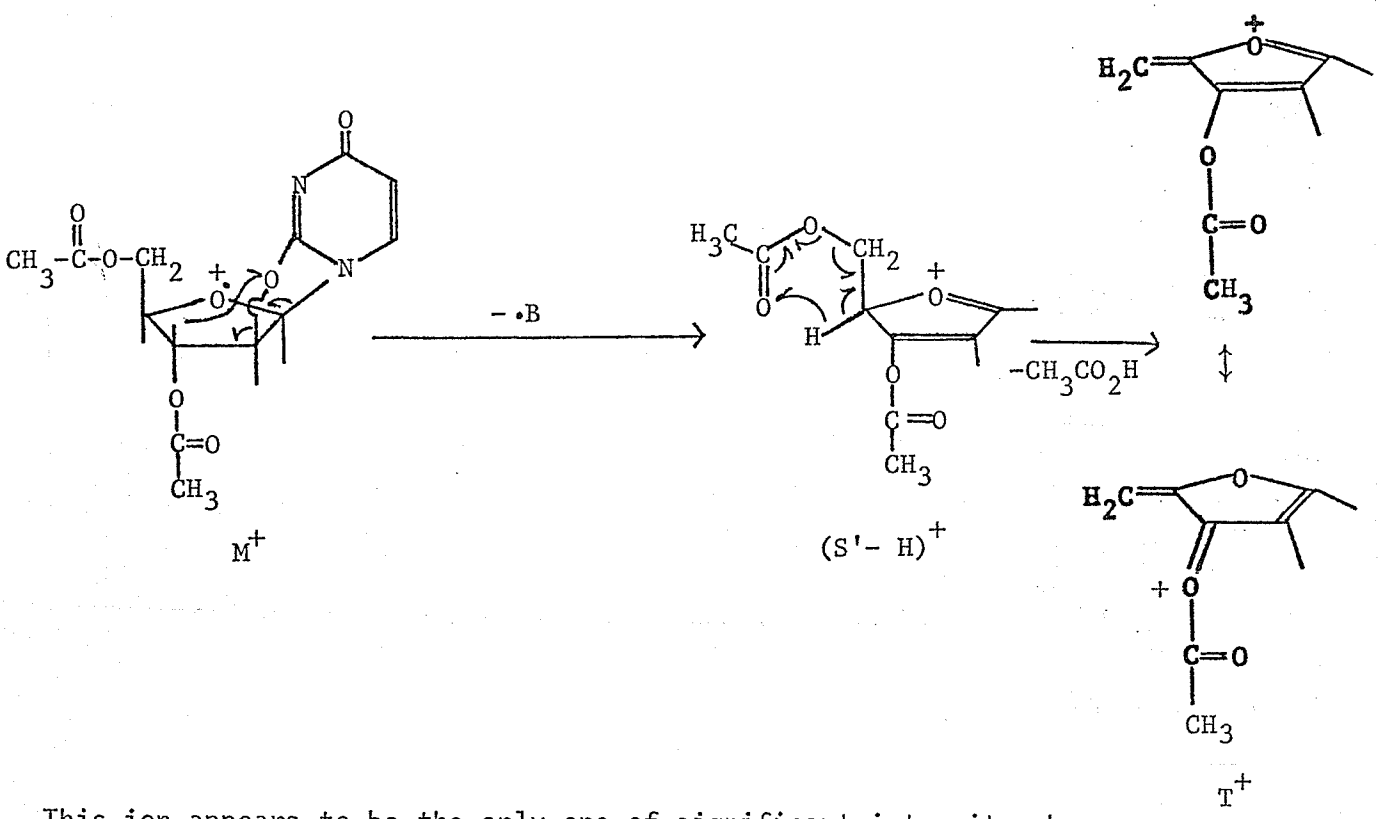
This ion occurs at m/e = 179 in the mass spectrum of Ib, shifts to 180 in the spectra of Ib' and Ib'', and to 193 and 194 in the spectra of IIb and IIb' respectively. These results suggest that the probable route to the formation of this ion is:



Molecular models indicate the feasibility of H transfer from the 5'acetyl group to the base moiety. This is further supported by its presence in the spectrum of 3'-TFAc-5'-Ac-2'AnhU (Ip) but not in that of 3'-Ac-5'-TFAc-2'AnhU (Io). (To be shown later; see Figure 16.)

$\underline{T^+}$

This ion has m/e = 139 in the spectra of Ib and Ib'' and 142 in the spectra of Ib' and Ib'''. It is of negligible magnitude in the spectra of IIb and IIIb in which fragment ions containing the base moiety are of relatively greater importance. These observations suggest that this ion contains the ribose moiety, formed possibly as follows:

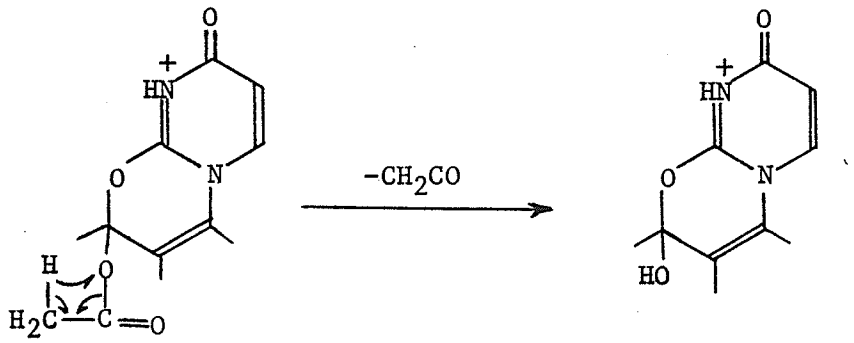


This ion appears to be the only one of significant intensity above m/e = 100 which is characteristic of the sugar moiety. Its assumed precursor $(S'-H)^+$ is of negligible intensity, possibly because the resonance stabilized ion T^+ can be formed by elimination of acetic acid from $(S'-H)^+$ via a six-membered cyclic transition state, in a reaction of low activation energy. (The structure suggested for $(S'-H)^+$ differs from that suggested earlier for the analogous ion in the spectra of I and II where the H lost from the ion product originated from one of the two OH groups. These labile H atoms are not present in Ib and alternative reactions can be considered.)

$\underline{(A+31)^+}$

This ion appears at m/e = 167 in the spectrum of Ib and is not significant in the spectra of IIb and IIIb in which it should appear

at m/e = 181 and 183 respectively. It occurs at m/e = 168 in the spectra of Ib'' and Ib''' and at 169 in the spectrum of Ib'. It thus contains one hydrogen from the methyl group of each acetyl function and probably has the same structure as the corresponding ion in the spectrum of I . A possible precursor of this ion is $(E'+H)^+$ but not $(E"+H)^+$ though $(E'+H)^+$ is of low intensity:



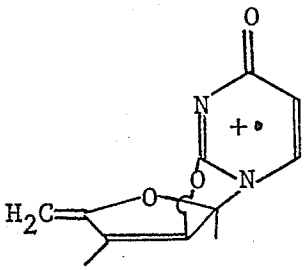
$(E' + H)^+$ $(A + 31)^+$

It can be noted that $(A+31)^+$ is also present in the mass spectrum of 3'-Ac-5'-piv-2'AnhU (Iq) but not in that of 3'-piv-5'-Ac-2'AnhU (Ir) (spectra to be discussed later).

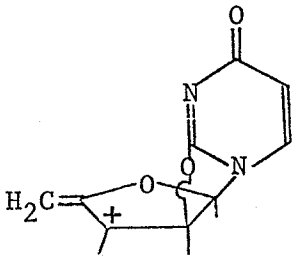
$\underline{(A+54)^+, (A+55)^+, (A+56)^+}$

A group of ions appears at m/e = 190, 191 and 192 in the spectrum of Ib. Their relative intensities are quite variable and the appearance of the group changes from spectrum to spectrum. In the spectrum of IIb, only m/e = 204, $(A+55)^+$, is prominent. Comparison of the spectra of Ib and IIb with those of Ib', Ib'', Ib''' and IIb' shows

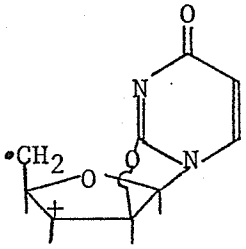
that no deuterium is incorporated into these ions. They thus correspond to loss of 2 CH_3CO_2H, $CH_3CO_2^{\bullet}$ + CH_3CO_2H, and 2 $CH_3CO_2^{\bullet}$ from the parent species (possibly by thermal effects in addition to ionic fragmentations). The following structures are assigned:



$(A+54)^+$



$(A+55)^+$



$(A+56)^+$

These ions are assumed to be formed in reactions similar to those already discussed.

3',5'-di-O-trifluoroacetyl-2,2'-anhydropyrimidine nucleosides

The mass spectra of 3',5'-di-TFAc-2'AnhU (Ic) and 3',5'-di-TFAc-2'AnhT (IIc) are shown in Figure 3. These derivatives were studied because of their anticipated enhanced volatility compared to that of their parent compounds (I & II) and indeed such is the case; see their respective sample temperatures in TABLE I. Many of the peaks can be readily explained by homolytic fission of single bonds, or have their counterparts in the mass spectra of the di-O-acetyl derivatives. For Ic many of the fragmentations are illustrated in Figure 3.

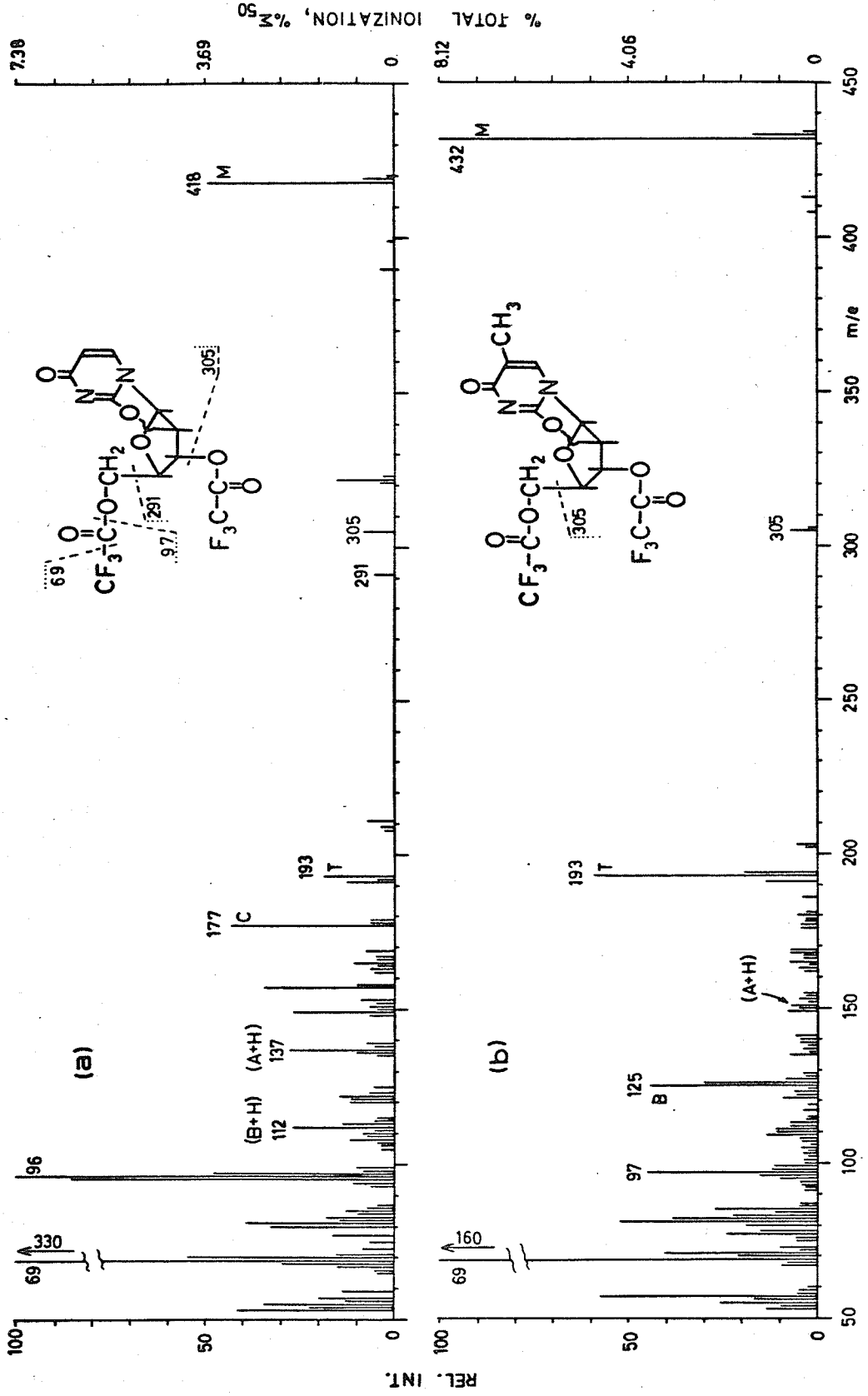


Figure 3. Mass Spectra of (a) 3',5'-di-TFAc-2'AnhU, (Ic); and (b) 3',5'-di-TFAc-2'AnhT, (IIc).

Note that the formation of ion $(M-CF_3CO_2)^+$ is depicted as an exclusive loss of the 3'-CF_3CO_2 group similar to the formation of $(M-CH_3CO_2)^+$ in di-Ac-2'AnhU. This is supported by the evidence from the spectra of 3'-Ac-5'-TFAc-2'AnhU (Io), 3'-TFAc-5'-Ac-2'AnhU (Ip), 3'-TFAc-5'-Piv-2'AnhU (Ix) and 3'-Piv-3'-TFAc-2'AnhU (Iy); loss of the CF_3CO_2 group occurs only in the spectra of Ip and Ix. (These isomeric pairs will be discussed later.) The molecular ion peaks of Ic and IIc are well defined. The peaks at m/e = 322 and 211 in the spectrum of Ic may be due to the mono-trifluoroacetyl derivative. It was not possible to check the completeness of the acetylation reaction by paper chromatography because the trifluoroacetyl derivatives hydrolyse very readily. Probably some hydrolysis occurs in the ion source itself.

$(A+H)^+$ and $(B+2H)^+$

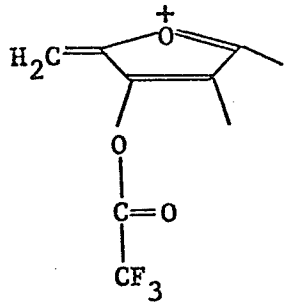
These ions are assumed to have the same structure as the corresponding ions discussed previously for anhydropyrimidine nucleosides and their acetyl derivatives. In this case the hydrogen transferred into the ion products originates from the ribose skeleton.

C^+ and $(A+55)^+$

These ions are assumed to form by similar pathways to the analogous ions in the spectra of the acetyl derivatives.

T^+

This is thought to be the sugar fragment formed in a similar way



m/e = 193

to the corresponding ion in the spectra of acetyl derivatives. However, its assumed precursor $(S'\text{-}H)^+$, expected at m/e = 307 could not be detected in the spectra. This ion is of special interest because it requires the presence of the 3'-TFAc group. It will be shown in the discussion for Io, Ip, Ix and Iy later that its presence indicates a 3'TFAc substituent.

The mass spectra of trifluoroacetyl derivatives of a number of nucleosides have been reported[61]. An ion corresponding to T^+ is present in the spectra of the appropriately substituted parent compound. In agreement with the above proposal its precursor was assumed to be an analogue of the $(S'\text{-}H)^+$ ion.

3',5'-di-O-trityl-2,2'-anydrouridine

The mass spectrum of Id is shown in Figure 4a. This compound was studied because the trityl group is an acid-labile protecting group used in synthetic procedures. Since the mass spectrum is dominated by peaks due to the trityl fragments the peaks characteristic of the

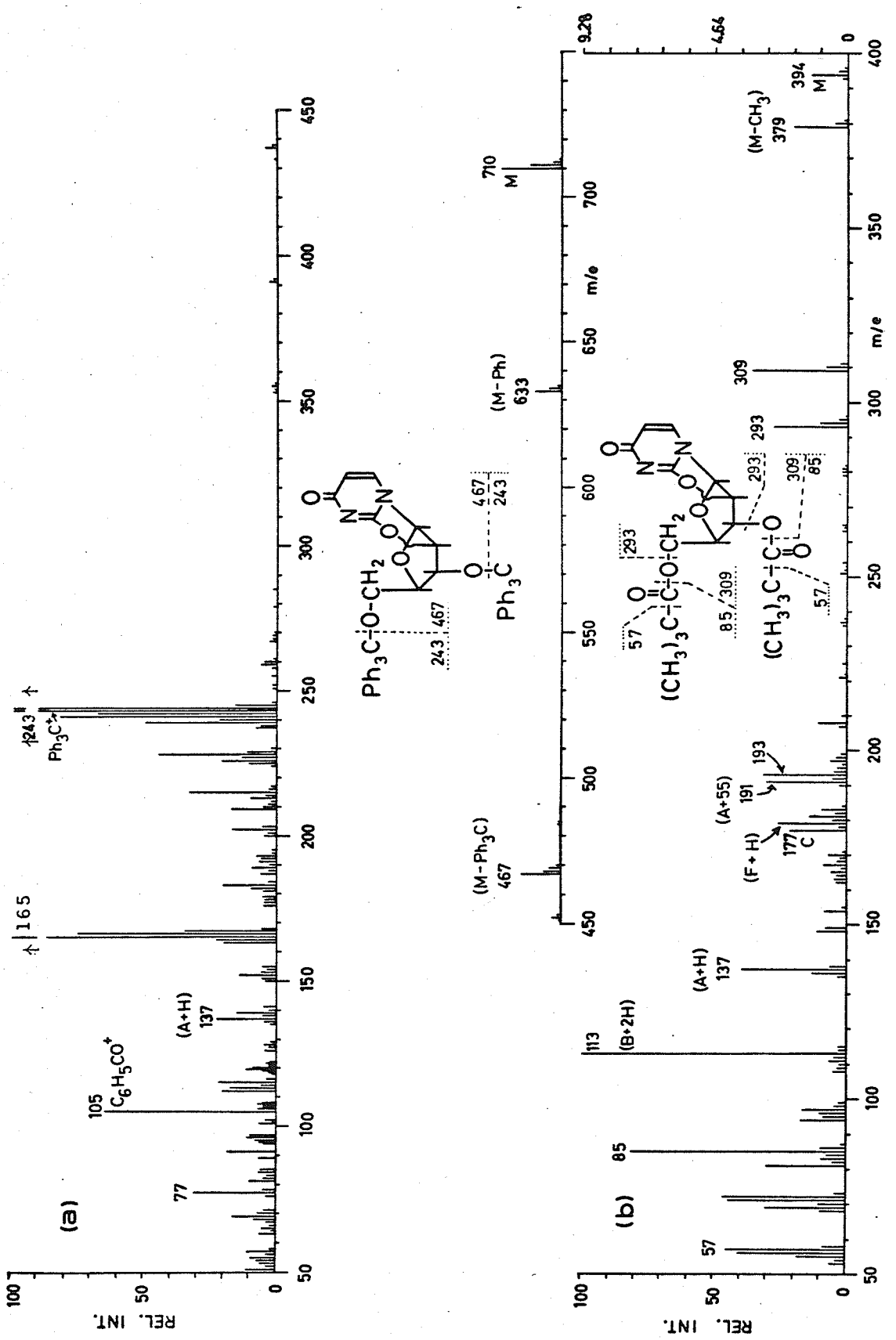


Figure 4. Mass Spectra of (a) 3',5'-di-Tri-2'AnhU, (Id) and (b) 3',5'-di-Piv-2'AnhU, (Ie).

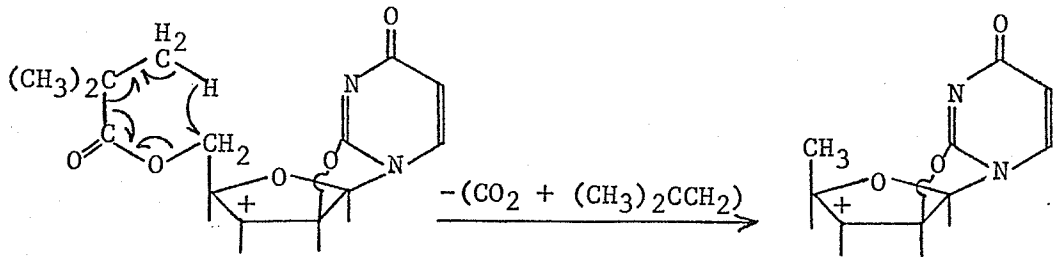
nucleoside are much less prominent. However, the molecular ion and other ions at high mass are easily of sufficient intensity for purposes of identifying the compound. The $(B+H)^+$ and $(B+2H)^+$ peaks are not readily distinguishable from neighbouring peaks. The $(A+H)^+$ ion appears to be present, but other characteristic ions, e.g. C^+, $(E+H)^+$, $(S'-H)^+$, T^+ are of no significance. Important fragmentations are illustrated in Figure 4a. In addition to these peaks there are many ions arising from rearrangements and fragmentations involving the trityl moiety.

3',5'-di-O-pivaloyl-2,2'-anhydrouridine

The pivaloyl group is a base labile protecting group used in synthetic procedures. The mass spectrum of Ie is shown in Figure 4b. The characteristic $(B+2H)^+$ and $(A+H)^+$ peaks are present though other diagnostically useful peaks, such as $(A+31)^+$ and $(S'-H)^+$, even if present, do not stand out from neighbouring peaks. Most of the other prominent peaks in the spectrum can be readily explained by simple homolytic fragmentations as illustrated in Figure 4b.

From the study of 3'-TFAc-5'-Piv-2'AnhU (Ix) and 3'-Piv-5'-TFAc (Iy) (to be discussed later), it seems that the loss of the methyl group comes mainly from a 5'-pivaloyl group. The other ions at

m/e = 177, 179 and 191 are assumed to arise in the same way as the C^+, $(F+H)^+$ and $(A+55)^+$ ions respectively in the spectra of the acetyl derivatives. The remaining prominent peak at m/e = 193 appears to have no analogy. The simplest sequence in which it could be formed is:



m/e = 293 m/e = 193

The presence of the peak m/e = 193 in spectra of 3'-Ac-5'-Piv-2'AnhU (Iq) but not in its isomer, 3'-Piv-5'-Ac-2'AnhU (Ir) supports this proposal.

Trimethylsilyl and dimethylsilyl derivatives of 2,2'-anhydropyrimidine nucleosides

Trimethylsilylation, an alternate method to trifluoroacetylation to enhance compound volatility, appears to be an excellent procedure for this purpose (compare the sample temperatures in TABLE I), especially for anhydropurine nucleosides which tend to decompose rather than volatilize on heating. The mass spectra of 3',5'-di-TMS-2'AnhU (If), 3',5'-di-TMS-2'AnhT (IIf) and 3',5'-di-DMS-2'AnhU (Ig) are shown in

Fig. 5; those of 3',5'-di-dTMS-2'AnhU (If') in Figure 6 and 3',5'-di-dTMS-2'AnhT (IIf') in Figure 7. Attempts to prepare the TMS derivative of IIIa were unsucessful. IIIa is apparently unstable under the silylation conditions used.

In addition to the enhanced volatility of the TMS derivatives another advantage to their use is the increased relative abundance of peaks characteristic of the sugar moiety, especially $(S'-H)^+$. Observation of these peaks would add confidence to the recognition of the molecular ion for unknown anhydronucleoside TMS derivatives in which the molecular ion was of low intensity. Although the peaks $(B+H)^+$ and $(B+2H)^+$ are not very intense in these spectra, the characteristic ion, $(A+H)^+$, of 2,2'-anhydronucleosides and derivatives so far discussed, is still prominent though of somewhat reduced relative intensity. The prominent peak at m/e = 103 (112 for the TMS-d_9 derivatives) in the spectra of If and IIf, formed by the reaction $(CH_3)_3Si\text{-}O^{\bullet+}\text{-}CH_2\text{-}R \rightarrow (CH_3)_3Si\text{-}O^{+}{=}{=}CH_2 + R\bullet$, with the fission of the C-4', C-5' bond, is characteristic of TMS derivatives of nucleosides containing a 5'-OH group. It is not significant when the 5'-OTMS function is absent. Other prominent peaks in the spectra at m/e = 73, 75 and 147 are of little diagnostic value since they are commonly found in the mass spectra of poly-TMS derivatized compounds. The dimethylsilyl (DMS) derivative, Ig, of I (prepared as a less expensive alternative to the TMS-d_9 derivative to aid in the interpretation of the spectra of If) was not as useful as had been hoped, since its

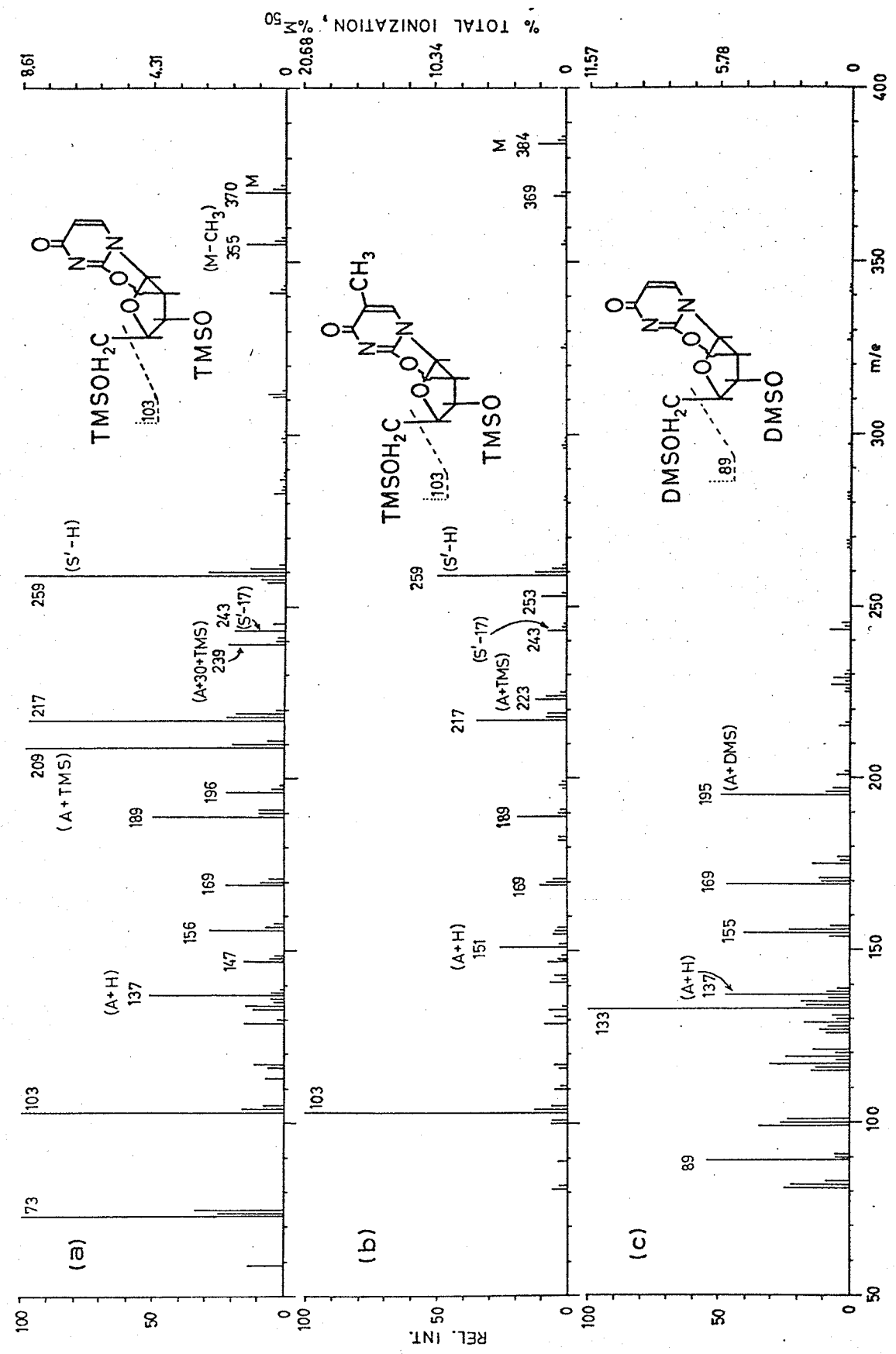


Figure 5. Mass Spectra of (a) 3',5'-di-TMS-2'AnhU, (If); (b) 3',5'-di-TMS-2'AnhT, (IIf) and (c) 3',5'-di-DMS-2'AnhU, (Ig).

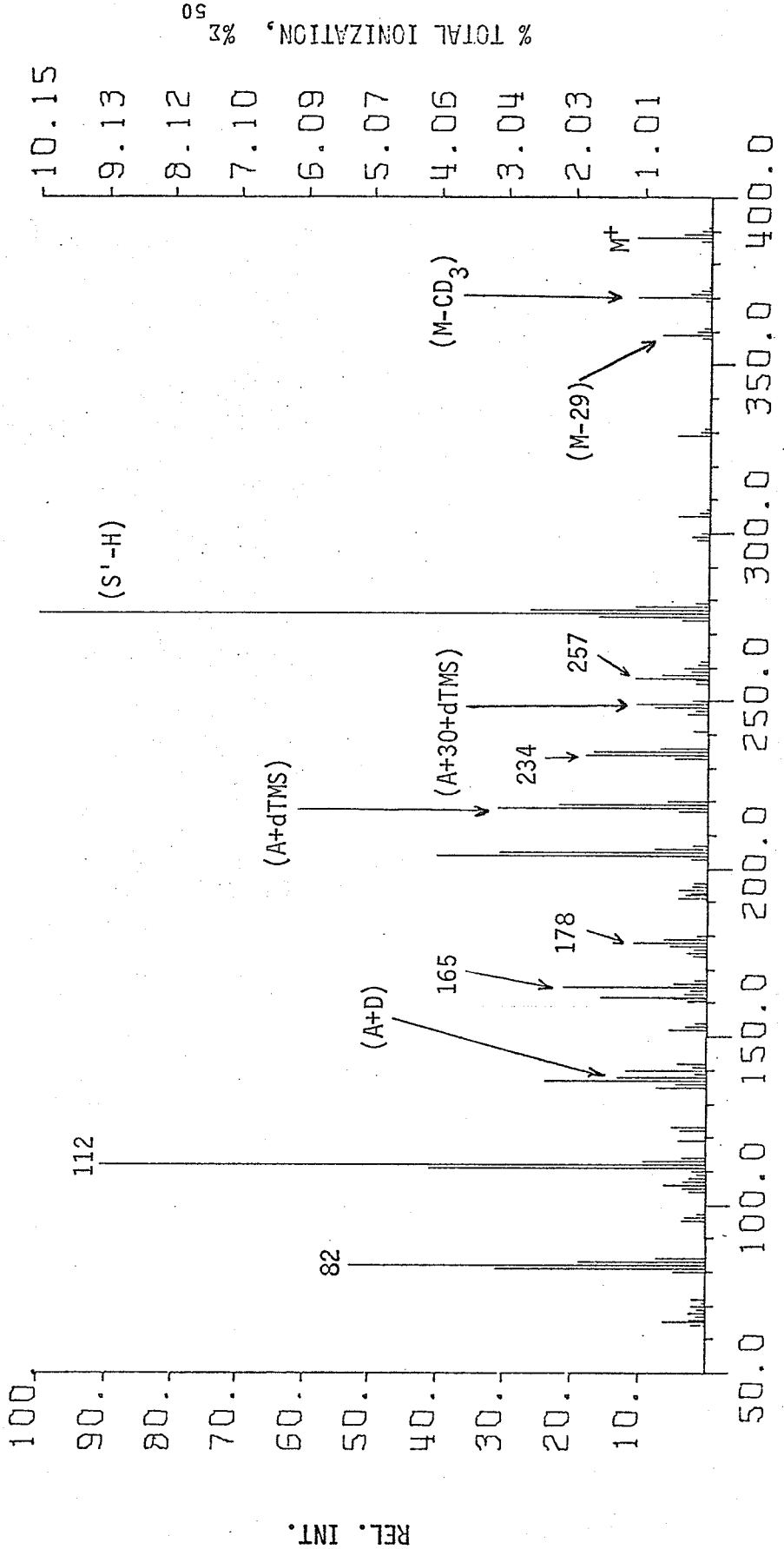


Figure 6. Mass Spectrum of 3',5'-di-dTMS-2'AnhU (If')

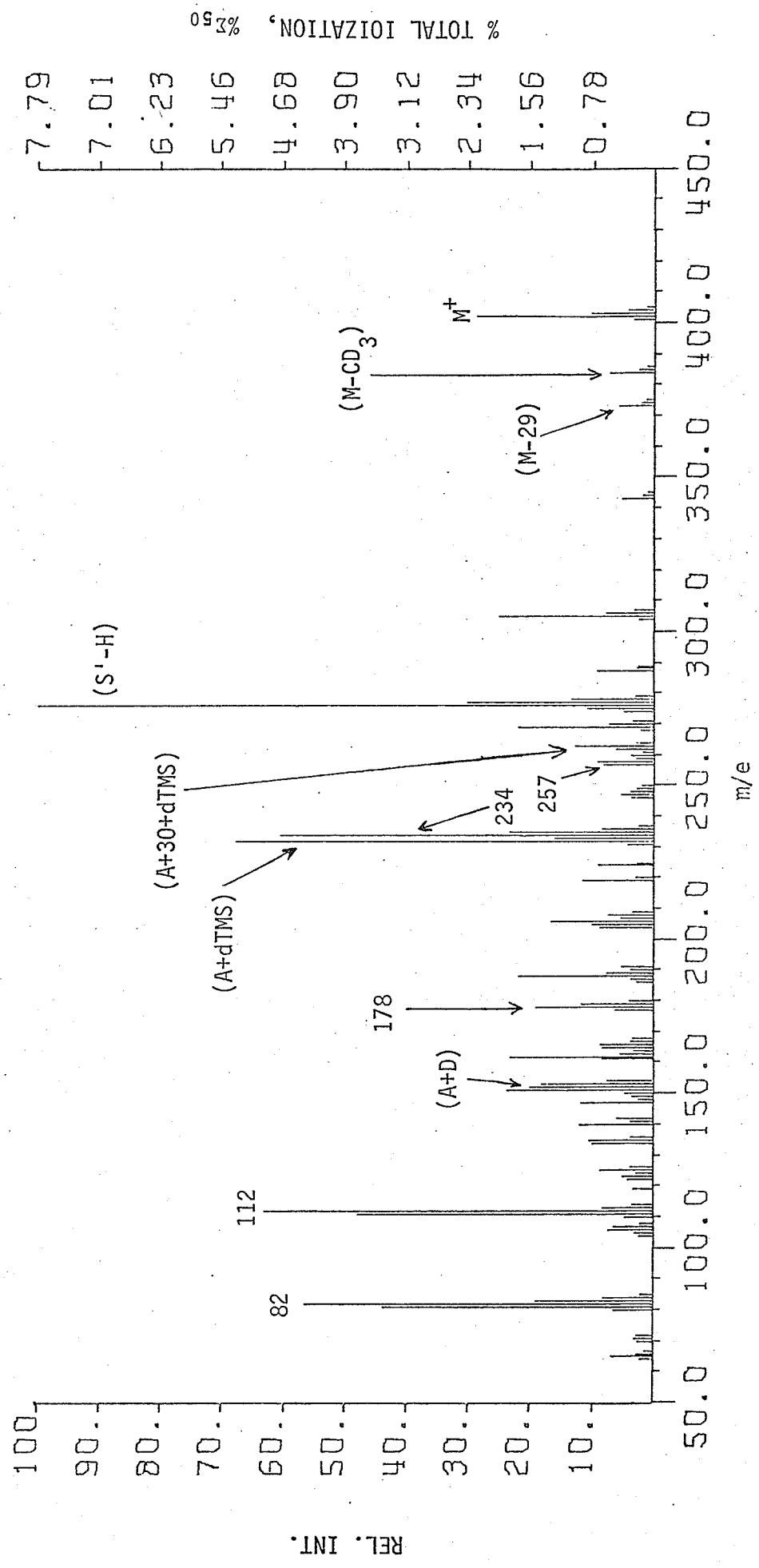


Figure 7. Mass Spectrum of 3',5'-di-dTMS-2'AnhT (IIf')

spectrum was not as similar to that of If as had been anticipated.

M^+ and $(M-CH_3)^+$

Loss of $\cdot CH_3$ from the molecular ion is a well-known reaction for trimethylsilyl ethers[93]:

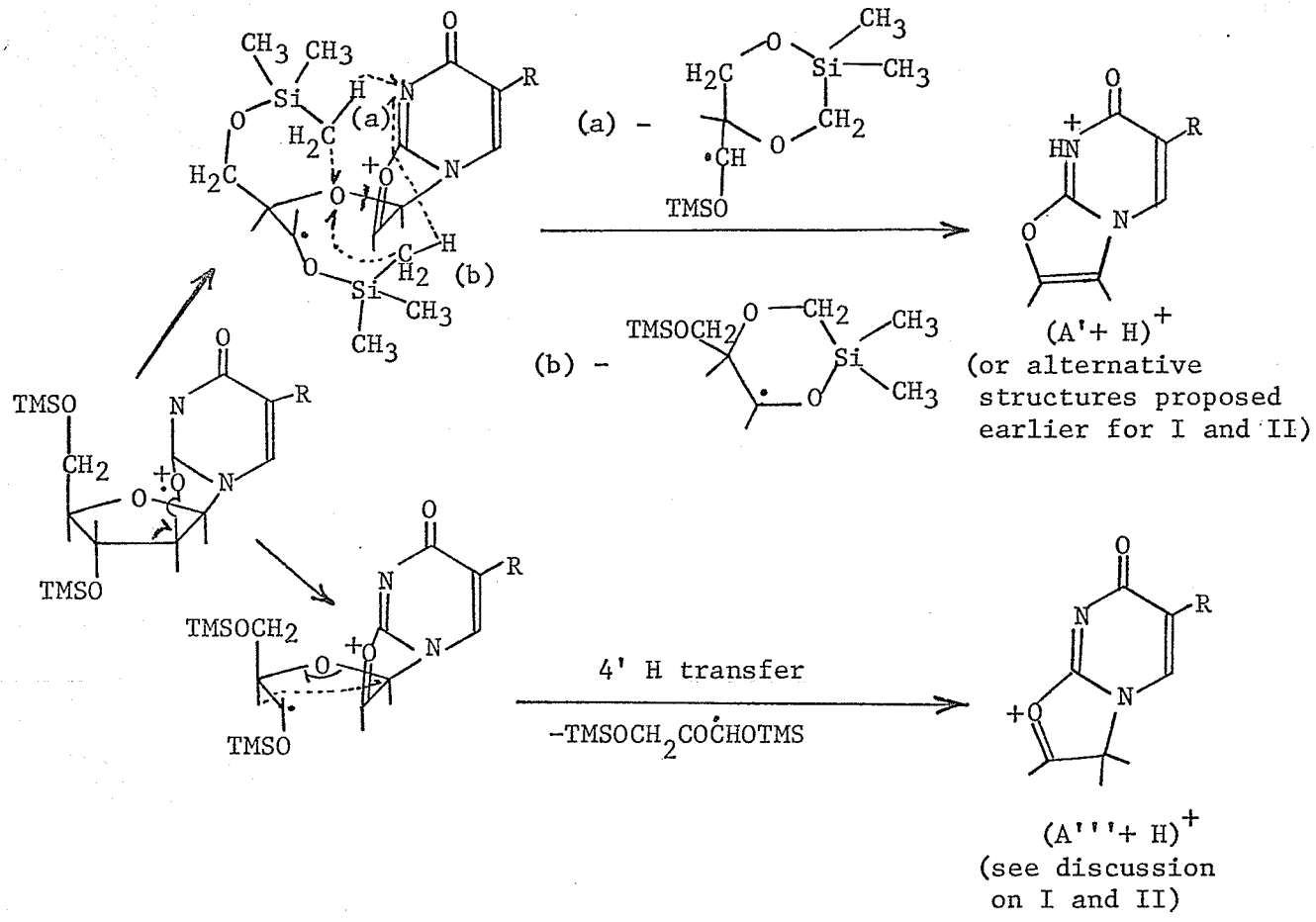
$$(CH_3)_3Si-\overset{\cdot}{\overset{+}{O}}-R \longrightarrow (CH_3)_2Si=\overset{+}{O}-R + \cdot CH_3$$

The presence of the two peaks enables the molecular ion to be identified with increased confidence. In doubtful cases comparison of spectra with those of dTMS derivatives will remove possible ambiguities in determining whether the peak with the highest m/e value is the M^+ or the $(M-CH_3)^+$ ion (if only one peak is present in the molecular ion m/e value region of the spectra).

$(A+H)^+$

This is the characteristic ion found in the mass spectra of 2,2'-anhydronucleosides. Comparison of the relative intensities of peaks at m/e = 137 and 138 for compounds If and If', and m/e = 151 and 152 for compounds IIf and IIf' shows an approximate 60:40 ratio for protium : deuterium incorporation into the ion, indicating that hydrogen transfer occurs from both the ribose skeleton and from the TMS groups. In contrast, for the acetyl derivatives Ib and IIb, hydrogen transfer occurred exclusively from the methyl groups of either acetyl function. As before, the process is envisaged to be initiated by cleavage of the C-2', C-3' bond. Molecular models

suggest that although a hydrogen atom of either TMS group can become suitably positioned in the transition state for transfer to the base moiety, the increased ring size leads to a lower probability for such conformations than for the acetylated compounds. Proposed reactions are:

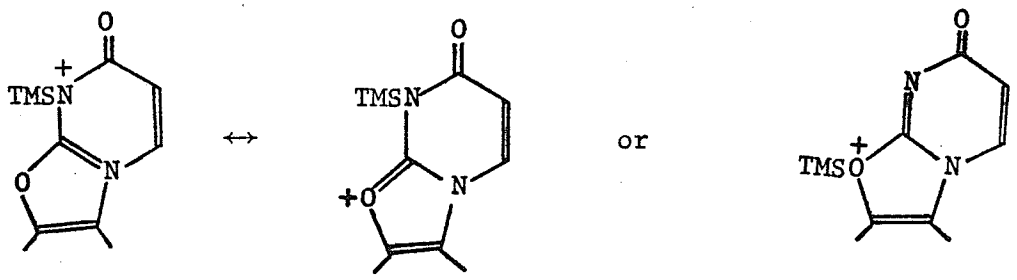


The 4'-H atom is considered to be the most likely ribose hydrogen to be transferred, since transfer of 3'- or 5'- H would lead to tri-radical or ring-strained neutral products. Since the ribose hydrogens

(3'-, 4'- or 5'-) are sterically inaccessible to N-3 it is proposed that transfer to C-1' occurs. McCloskey et al[35] suggested that the source of rearranged hydrogen was from the ribose skeleton only. They apparently did not detect any significant deuterium transfer.

$\underline{(A+TMS)^+}$

The appearance of this ion at m/e = 209 for If, at 218 for If', at 223 for IIf, and at 232 for IIf', indicates the presence of the base and also one TMS group. The corresponding $(A+DMS)^+$ ion is observed at m/e = 195 in the spectrum of Ig. It is known that the TMS group can undergo migration in ionic fragmentation reactions in a manner similar to the hydrogen atom [60,74,108-113]. Consequently a similar mechanism to that for formation of $(A+H)^+$ in the spectrum of I and II is proposed[35], to give, e.g.:

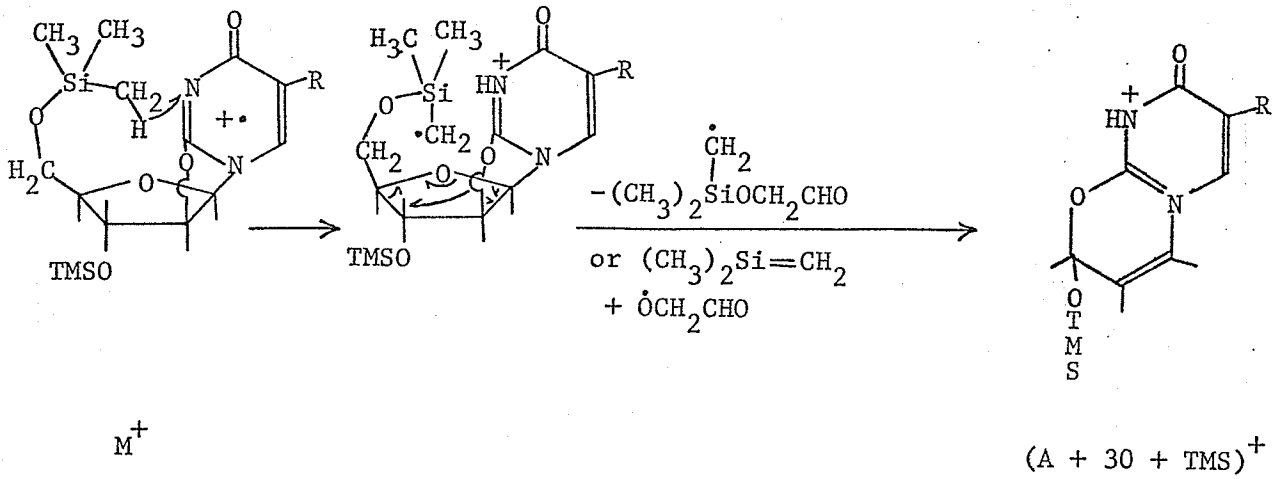


$(A'+TMS)^+$ $(A''+TMS)$

$(A+TMS)^+$ could possibly lose $(CH_3)_2Si{:}CH_2$ to give $(A'+H)^+$ though there is no evidence to support this.

$(A+30+TMS)^+$

This rather low intensity ion occurs at m/e = 239 in the mass spectrum of If, at 249 for If', at 253 for IIf, and at 263 for IIf'. It therefore contains the base, one intact TMS group and one hydrogen from the other TMS group. It is believed to correspond to the $(A+31)^+$ ion found in the spectra of I, II and III, and to the $(E'+H)^+$ ion in the spectrum of Ib.

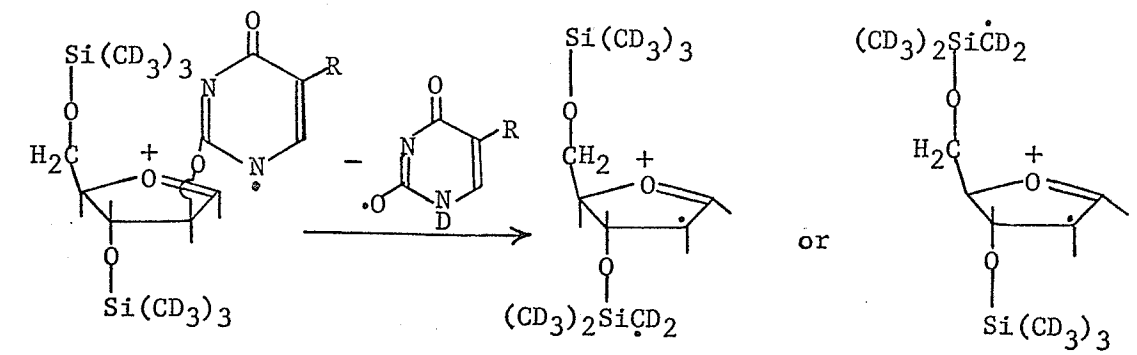


M^+ $(A + 30 + TMS)^+$

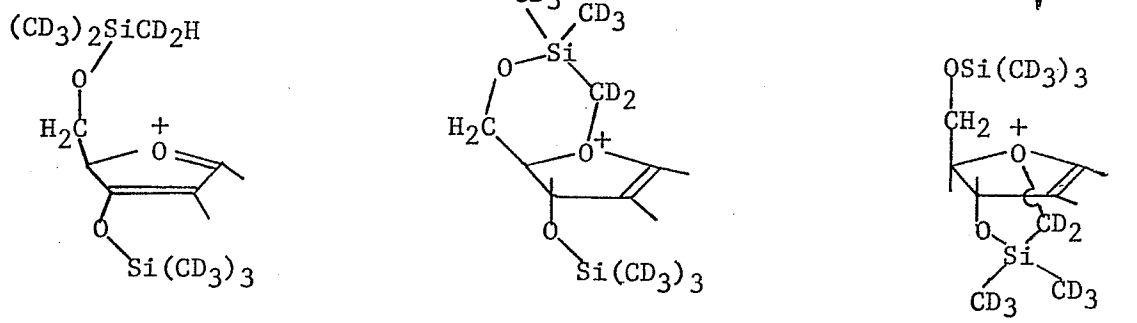
Models support the feasibility of H transfer from the 5' TMS group. The extent of incorporation of deuterium into this ion may be sensitive to instrumental and experimental parameters since Tsuboyama and McCloskey[35] obtained slightly different results and suggested hydrogen transfer from C-4' or C-5'. (This ion is of special interest in connection with the mass spectra of the TMS derivatives of purine 3'-anhydronucleosides.)

$\underline{(S'\text{-}H)^+}$

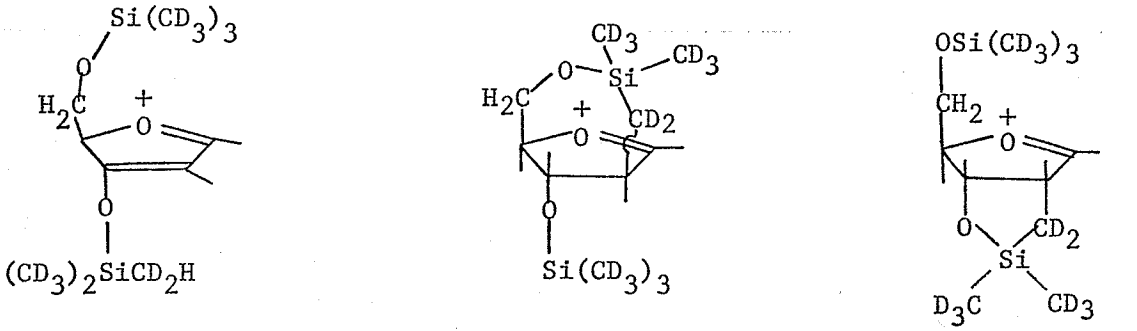
This ion appears at m/e = 259 in the mass spectra of If and IIf, and at 276 in the spectra of If' and IIf'. (The intensity of m/e = 277 can be wholly accounted for by the natural abundances of ^{13}C and ^{29}Si). The increase in m/e of 17 units for the deuterated compounds indicates that in this ion both the TMS groups are retained and that the hydrogen atom lost to the neutral products originates from one or either of the TMS groups. A metastable peak confirms that M^+ is the precursor of $(S'\text{-}H)^+$. (For If, 370 → 259, m* (calc) = 181.3, m* (obs) = 181.5; for If', 388 → 276, m* (calc) = 196.0, m* (obs) = 196.0.) This unimolecular reaction can be explained by initial localization of positive charge on O-4' and fission of the glycosidic bond (N-1, C-1' bond). Molecular models indicate that hydrogen transfer to the base from either the 3' or 5' TMS group is then sterically feasible. Fission of the O-2, C-2' bond can lead to several ion structures: (See following page.)



or



$(S_1' - H)^+$ $(S_3' - H)^+$ $(S_5' - H)^+$

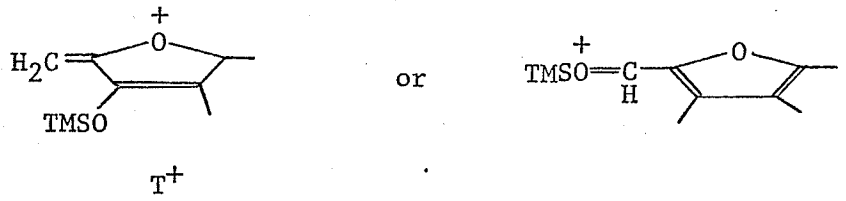


$(S_2' - H)^+$ $(S_4' - H)^+$ $(S_6' - H)^+$

The ion structures $(S_1'\text{-H})^+$ and $(S_2'\text{-H})^+$ should be well stabilized and can be formed by transfer of H-3'to the radical site on the TMS group. The remaining structures involve recombination of radical sites. Models suggest that ring strain is higher for structure $(S_3'\text{-H})^+$ than for $(S_4'\text{-H})^+$ but van der Waals interactions between non-bonded atoms are higher for $(S_4'\text{-H})^+$ than for $(S_3'\text{-H})^+$. Van der Waals interactions are small for $(S_5'\text{-H})^+$ and $(S_6'\text{-H})^+$, and ring strain is small in both cases, though higher for $(S_6'\text{-H})^+$ than for $(S_5'\text{-H})^+$. The preferred structures seem to be $(S_1'\text{-H})^+$, $(S_3'\text{-H})^+$ and $(S_4'\text{-H})^+$ from the evidence shown in the study of the TMS derivatives of monoacetyl and monopivaloyal 2'-anhydrouridine (to be discussed later). An alternate proposal by McCloskey et al[35] suggested an exchange of one TMS-hydrogen prior to the formation of this ion. Unexpectedly, the $(S'\text{-H})^+$ peak is not significant in the mass spectrum of Ig where it should appear at m/e = 231.

$(S'\text{-H-TMSOH})^+$

An ion appearing at m/e = 169 in the mass spectra of If and IIf shifts to m/e = 178 in the spectra of If' and IIf' and to m/e = 155 in the spectrum of Ig. It therefore contains one intact TMS group and the ribose ring. Although its atomic composition corresponds to removal of TMSOH from $(S'\text{-H})^+$ this latter ion is unlikely to be its precursor because multiple hydrogen transfers would be required. The mechamism of its formation remains doubtful because its precursor has not been identified. Possible structures for the ion are:

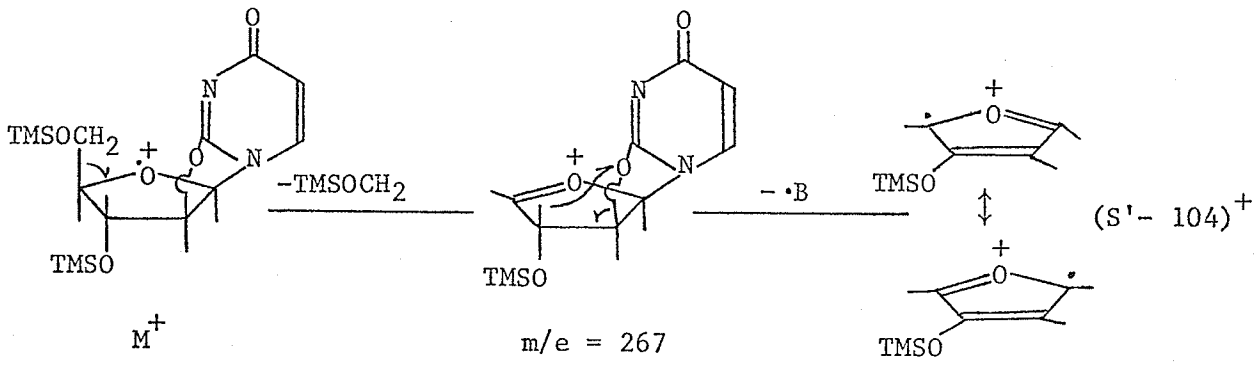


T^+

The first structure (labelled T^+) is analogous to corresponding T^+ ions found in the spectra of acetylated and trifluoroacetylated 2,2'-anhydronucleosides and to an ion in the spectra of the parent compounds I, II and III with m/e = 97 (H replacing TMS) which was not discussed because of the complexity in that region of the spectra. An ion with m/e = 169 is prominent in the spectra of TMS derivatives of mononucleotides[72] and of β-D-ribose 5-phosphate[109] and in each case the same structure was proposed.

$\underline{(S'\text{-}104)^+}$

This ion, occurring at m/e = 156 in the spectrum of If, shifts to m/e = 165 for If'. Neither it, nor its analogue are significant in the spectra of IIf and Ig. It may be formed by the reactions:

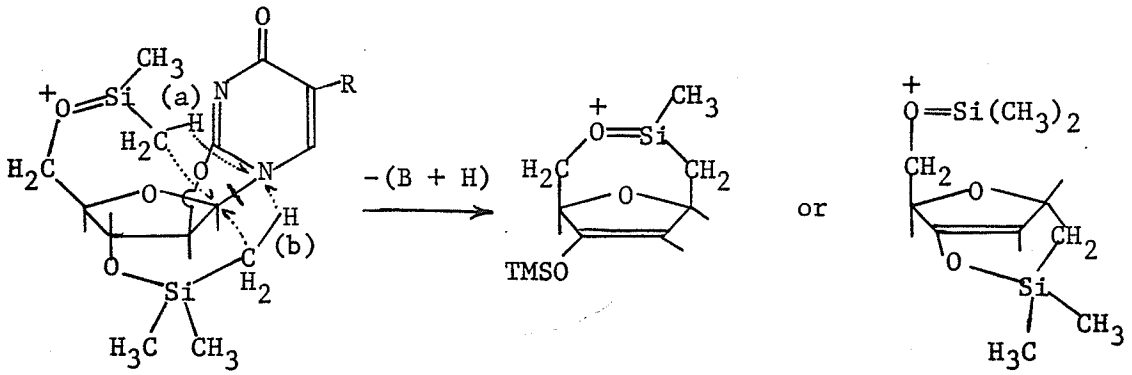


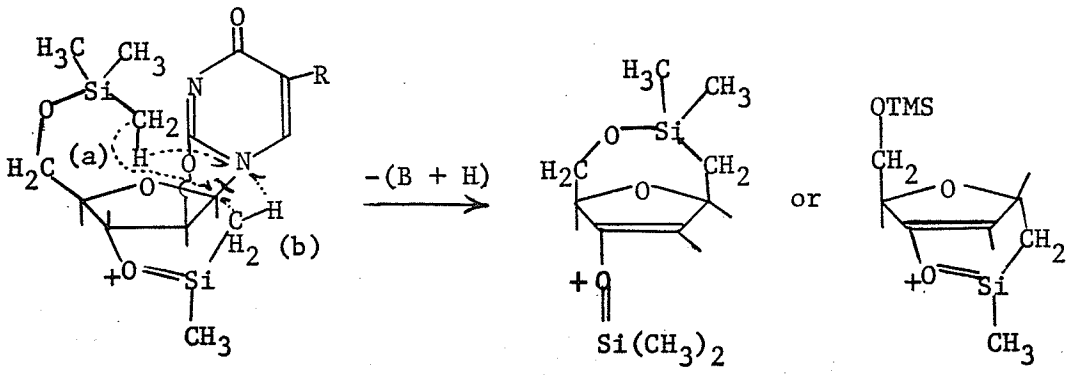
M^+ m/e = 267 $(S'\text{-}104)^+$

Its assumed precursor is not detectable. (Formation of this ion from $(S'-H)^+$ would require multiple H transfers to account for the observed mass shifts for the deuterated compound.)

$\underline{(S'-17)^+}$

This ion, found in the mass spectra of If and IIf at m/e = 243, shifts to m/e = 257 in the spectra of If' and IIf'[35]. No reasonable mechanism could be found for the loss of CD_3H from the $(S'-D)^+$ ion of If' and IIf' and the most likely precursor was assumed to be the $(M-CH_3)^+$ or $(M-CD_3)^+$ ions. On fragmentation, these latter ions then lose one more methyl hydrogen to the neutral species. Suggested reactions are:



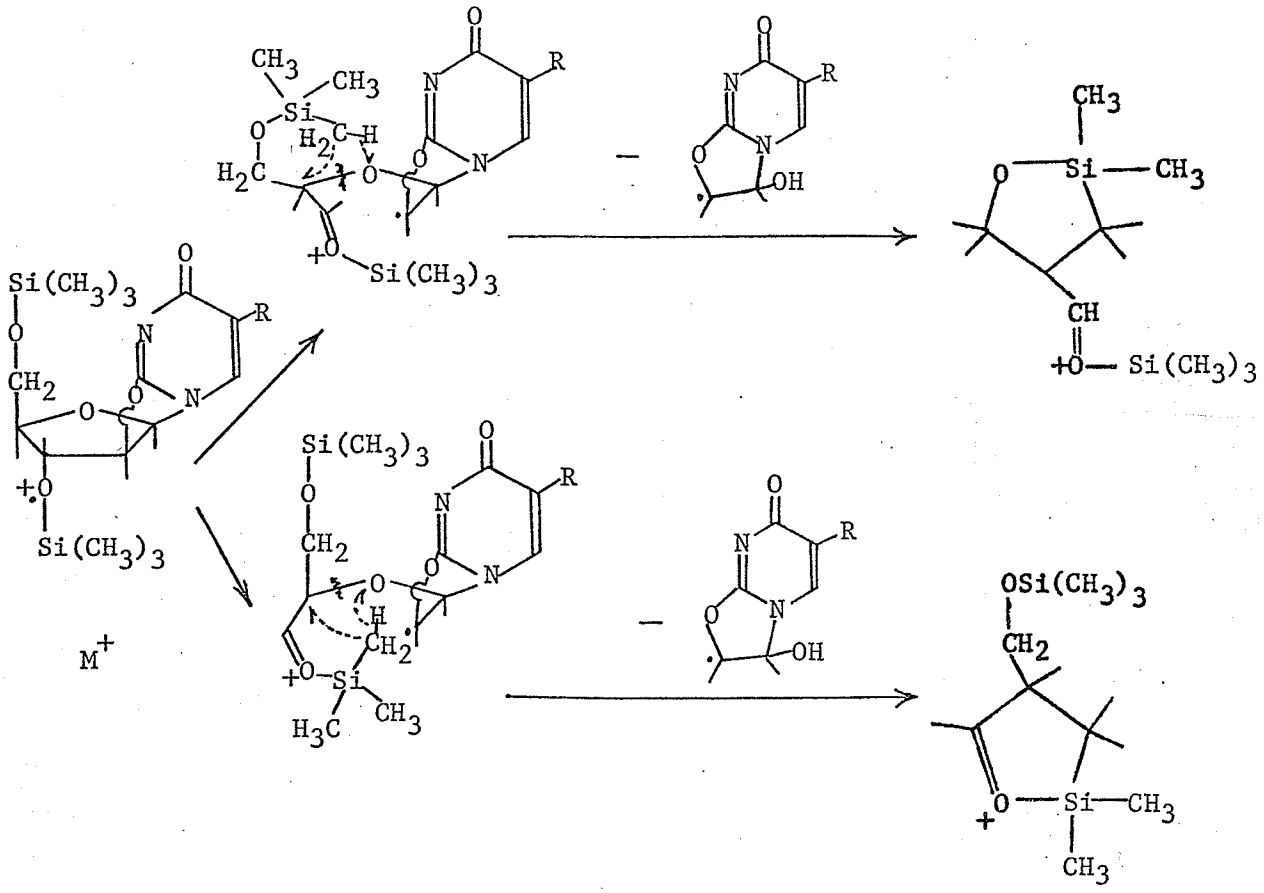


Models indicate that in all ion products the silicon containing rings are essentially strain-free and there are no large van der Waals inter-actions in the structures.

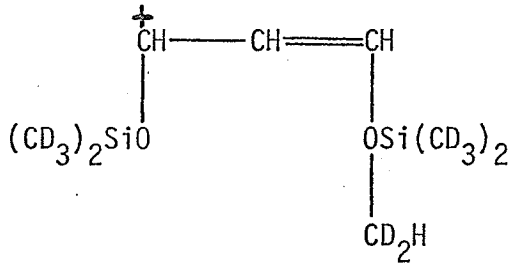
An ion with m/e = 243 and the same atomic composition has been detected in the mass spectra of derivatives of mononucleotides having 2'- and 3'- OTMS substituents[74]. It is, however, not structurally analogous to that described here, and is formed by a different pathway.

$\underline{(217)^+}$

This ion is found in the spectra of If and IIf and shifts to m/e = 234 in the spectra of If' and IIf'. It thus contains both TMS groups, but one of the methyl hydrogens has been lost to neutral products. Although an ion of this mass is commonly found in the mass spectra of TMS derivatives of certain sugars[108,109] and has also been found for nucleotide TMS derivatives[74] in these cases both TMS groups remained intact. Either, or both, of the following reactions are considered possible in the present case: (See following page)



Tsuboyama and McCloskey[35] suggested that hydrogen exchange as noted above for their $(S'\text{-}H)^+$ structure caused the deuterium shift to be 17 instead of 18 in the formation of this ion and the following structure for m/e = 234 was given:

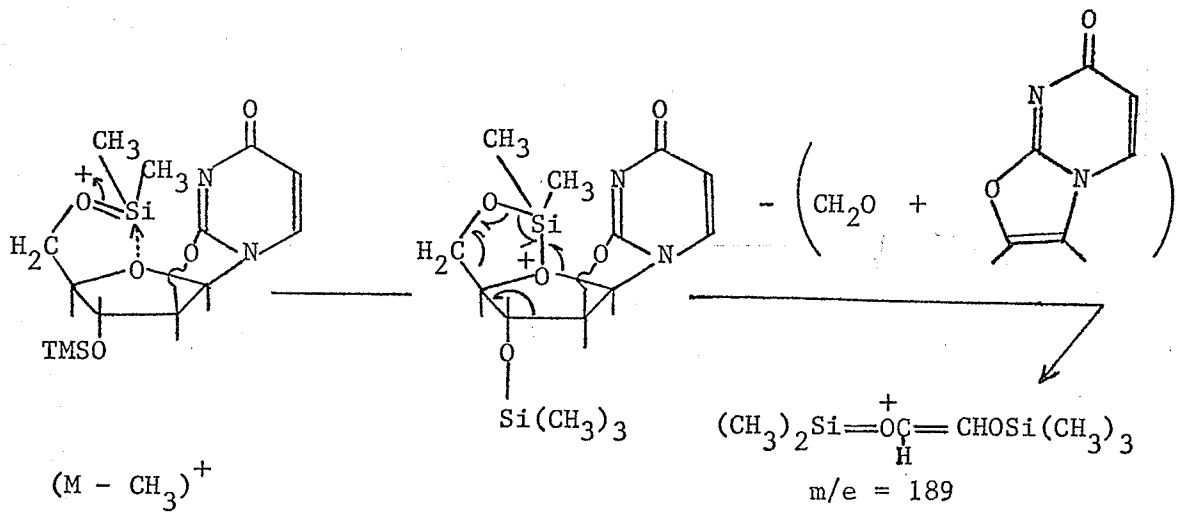


They cited the ion m/e = 259 as the precursor of the ion m/e = 217, and detected a metastable peak for such a transition (where $m^* = \frac{217^2}{259} = 181.8$).

A metastable peak at 181.5 was observed in this study but the corresponding metastable peak in the spectrum of the dTMS derivative, expected at 198.3 (276 → 234), was not observed. Instead, a metastable peak occurred at 196.0. The metastable peaks at 181.5 and 196.0 can be explained by the transitions $M^+ \rightarrow (S'-H)^+$ for the TMS and dTMS derivatives respectively, as discussed earlier under the formation of $(S'-H)^+$.

$\underline{(189)^+}$

This ion is found in the spectra of If and IIf, and shifts to m/e = 204 in the spectra of If' and IIf'. It therefore contains both TMS groups (less one methyl function) and part of the ribose ring. The simplest reaction which can be proposed is:



$\underline{(M-59)^+}$

This ion, which is of low intensity, occurs at m/e = 311 in the spectrum of If, at 329 in If', at 325 in IIf, and at 343 in IIf'. The

corresponding ion is also found in the spectra of I and II (described as $(A+31)^+$). Analogous structures are proposed for the ion. The TMS group is assumed to migrate to the base in much the same way as does the H atom of the 5' hydroxyl group.

$(M-29)^+$

This low intensity ion occurs at m/e = 341 in the spectrum of If, at 359 in If', at 355 in IIf, and at 373 in IIf'. It thus corresponds to the loss of ·CHO from M^+. Possible candidates involved in this loss are the C and O atoms from the 2', 3' or 5' positions (invoking migrations of TMS groups and H atoms as necessary). The available evidence is inadequate to decide between the possibilities[35].

Monoacetyl and Monopivaloyal -2,2'-anhydrouridine

The mass spectra of the compounds discussed thus far showed how their fragmentation patterns could be correlated with their structures. Furthermore, they show that mass spectrometry has the potential to distinguish between synthetically useful isomeric substituted derivatives of the parent compounds. The isomeric pairs of 2'AnhU derivatives were investigated.

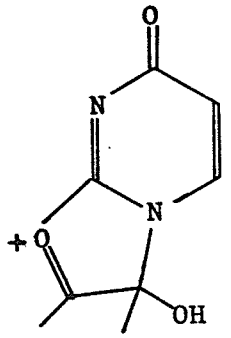
3'-Ac-2'AnhU (Ij), 5'-Ac-2'AnhU (Ik), 3'-Piv-2'AnhU (It) and 5'-Piv-2'AnhU (Is) are discussed first because the results of the mass spectral investigation were unexpected. For example, both

compounds Ij and Ik have a molecular weight of 268. When mass spectral analysis was attempted, in addition to an ion at m/e = 268 there were also prominent ions at m/e = 226 and 310, m/e values for the molecular ions of 2'AnhU (I) and 3',5'-di-Ac-2'AnhU (Ib) respectively. Thermal rearrangements in the sample probe of the mass spectrometer were suspected. When the unvaporized residues from the sample probe were analysed by paper chromatography, four compounds I, Ib, Ij and Ik were identified[92] for both starting compounds. In spite of variations in experimental procedure to minimize the length of time the sample spent in the sample probe, the thermal reactions could not be prevented and the mass spectra of Ij and Ik free from the other compounds could not be obtained.

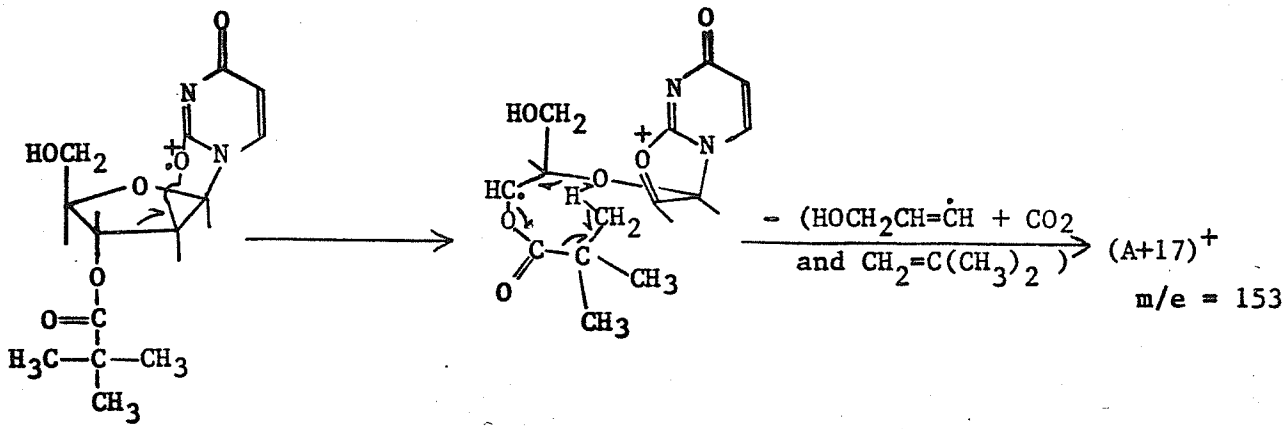
The thermal rearrangement also occurs to some extent for the monopivaloyl derivatives Is and It[92]. Their mass spectra are shown in Figure 8 (note small peaks at m/e = 394 and 379) from the dipivaloyl compound) along with some logical bond fissions. While prominent peaks in the spectra occur at the same m/e values for both compounds, there are quite large variations in relative intensities. Unless both isomers are available for direct comparison a distinction between isomers on the basis of individual spectra would be uncertain.

In addition to the now familiar characteristic ions of 2,2'-anhydronucleosides, as labelled in Figure 8, an ion, labelled as

$(A+17)^+$, appears in the spectra at m/e = 153. This is likely to be the species



Its formation would be analogous to an ion $(B+60)^+$ occurring in certain derivatives of natural nucleosides described by McCloskey and co-workers[69] (also see Introduction). The formation of $(B+60)^+$ seemed to require the presence of 3'-OH groups. The $(A+17)^+$ ion is not structurally identical to $(B+60)^+$ but its presence in both spectra of Figure 8 may indicate thermal isomerism. However, it also seems feasible that H-transfer from a 3'-O-pivaloyl group to the 4'-O atom could occur:



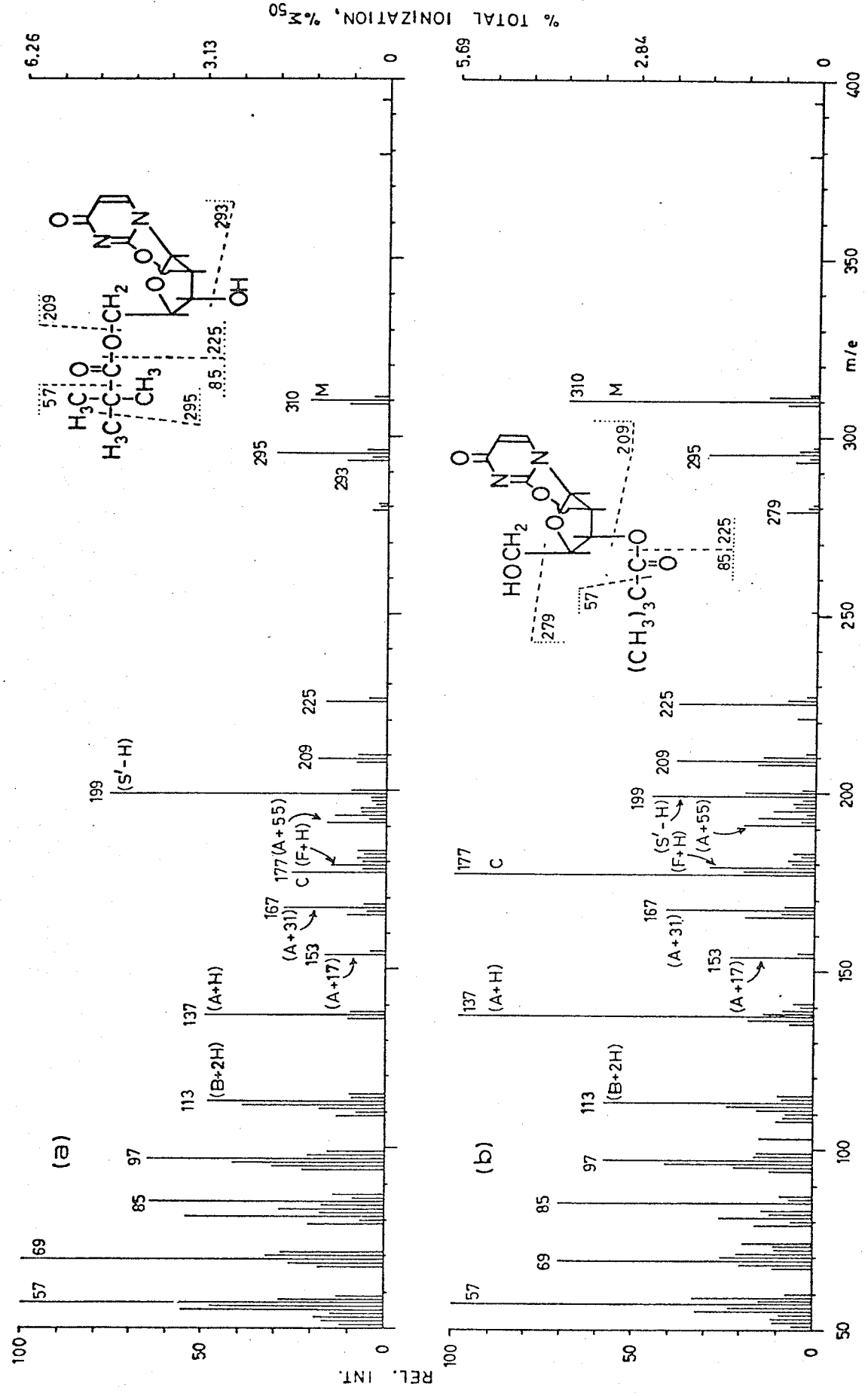


Figure 8. Mass Spectra of (a) 5'-Piv-2'AnhU, (Is) and (b) 3'-Piv-2'AnhU (It).

3'-O-acetyl-5'-O-pivaloyl-2,2'-anhydrouridine (Iq) and
3'-O-pivaloyl-5'-O-acetyl-2,2'-anhydrouridine (Ir)

The mass spectra of this pair of isomers are shown in Figure 9. Both show prominent molecular ions. Many peaks in the spectra can be used to both characterize the isomers and to distinguish between them. Even peaks of the same m/e value which can arise by simple bond fissions in either isomer often show significant differences in relative intensity. The preferred bond fissions, shown in Figure 9, are consistent with observations on the mass spectra of diacetyl- and dipivaloyl-2,2'-anhydrouridine (Ib & Ie). Thus, elimination of CH_3CO_2 occurs more readily from the 3'- than from the 5'-O-acetyl substituted isomer. Elimination of C_2H_3O occurs more readily from the 5'- than from the 3'-O-acetyl substituted isomer. (This is not really a simple bond fission as shown earlier, but a reciprocal hydrogen transfer occurs between the acetyl group and elsewhere in the molecule.) Elimination of $\cdot CH_3$ from acetyl substituents has been seen to be insignificant. Comparison of the spectra in Figure 9 shows that loss of $\cdot CH_3$ occurs more readily from a 5'- than from a 3'-O-pivaloyl group. Elimination of the pivaloyl group itself also occurs more readily from the 5' position.

An ion was found in the mass spectrum of 3',5'-di-Piv-2'AnhU (Ie) at m/e = 193 and its interpretation required the presence of the 5'-O-pivaloyl group. This ion is also present in the spectrum of Iq and supports the view that it is characteristic of a 5'-O-pivaloyl group.

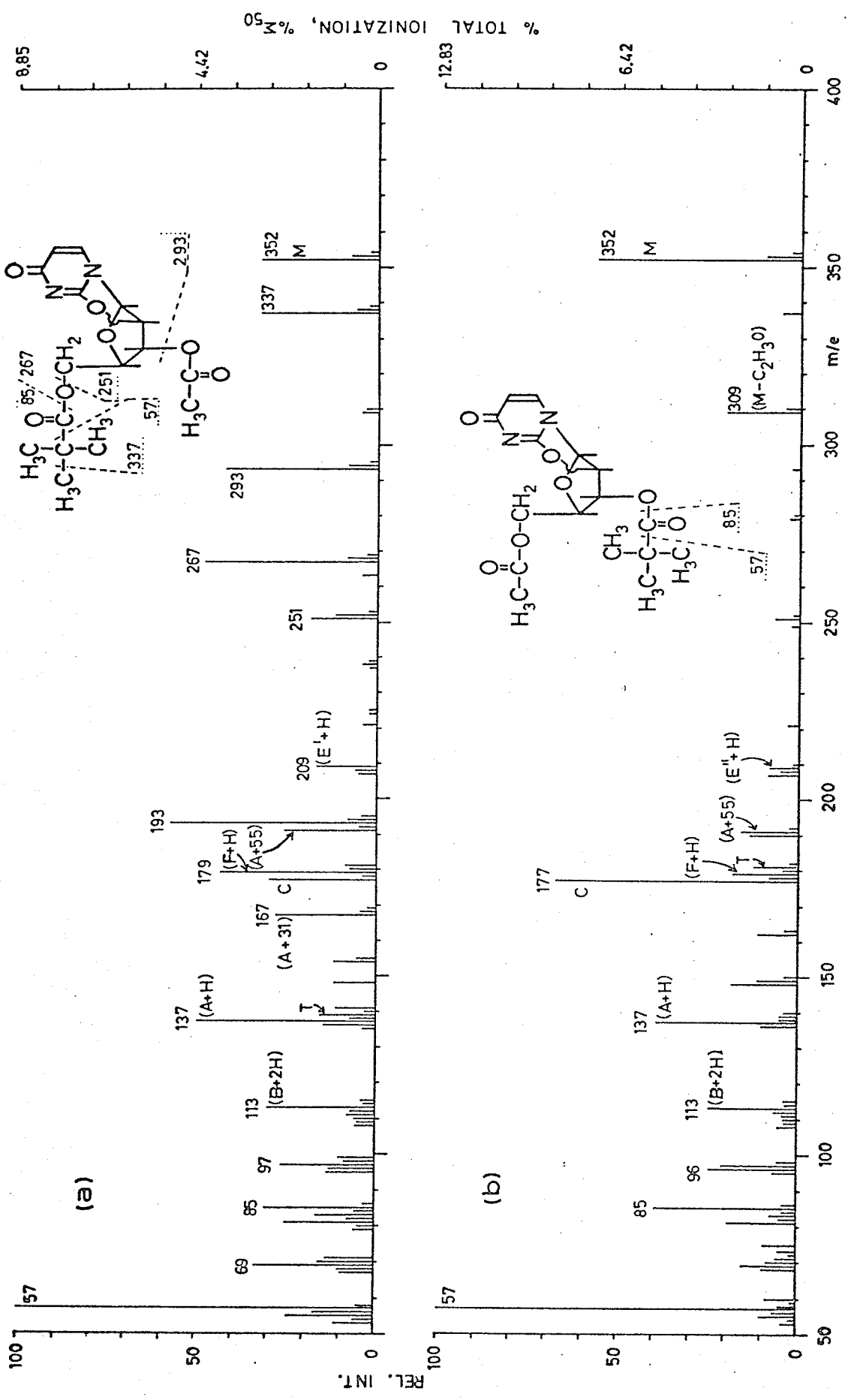
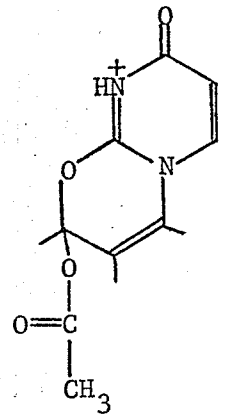
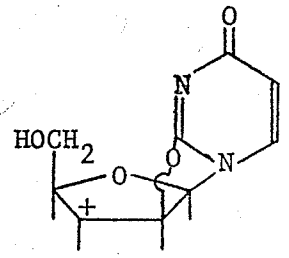


Figure 9. Mass Spectra of (a) 3'-Ac-5'-Piv-2'AnhU, (Iq) and (b) 3'-Piv-5'-Ac-2'AnhU, (Ir).

The ions occurring in the two spectra at m/e = 209 are of interest. Possible structures for these ions have been discussed earlier. Alternative structures seem appropriate for this ion arising from the two isomers:



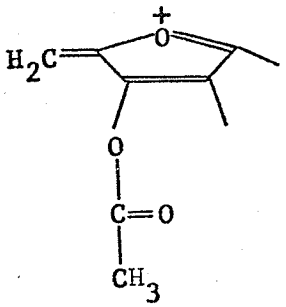
$(E'+H)^+$ from Iq



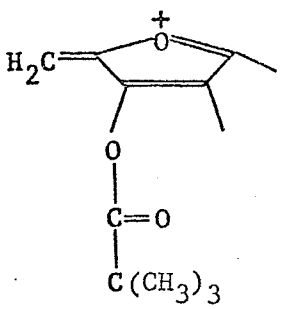
$(E''+H)^+$
from Ir

In the discussion of the mass spectra of 3',5'-di-Ac-2'AnhU (Ib) earlier on, $(E'+H)^+$ was suggested as the most logical precursor of $(A+31)^+$ (m/e = 167). In agreement with this, m/e = 167 is present in the spectrum of Iq but is not significant in the spectrum of Ir.

Another ion expected to differentiate between Iq and Ir is the ion T^+:



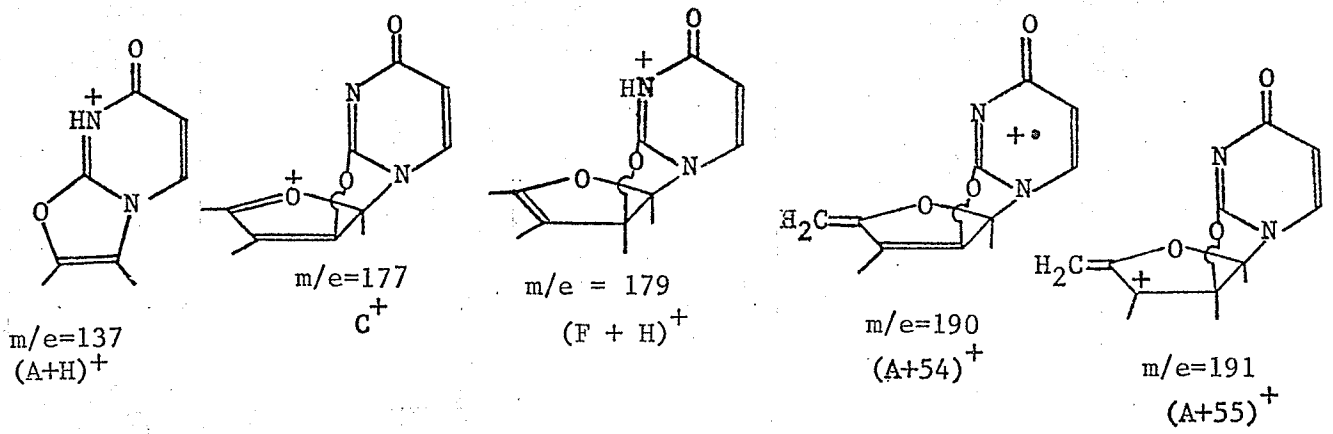
T^+ (m/e = 139 from Iq)



T^+ (m/e = 181 from Ir)

However, these ions are not of sufficient prominence to be useful in these spectra.

Most of the remaining prominent ions in the spectra can logically arise from either isomer by means of fragmentation reactions described previously. These ions are shown below:



Trimethylsilyl derivatives of monoacetyl- and monopivaloyl-2,2'-anhydrouridine

It has been shown earlier that a clear distinction between the mono-substituted acetyl or pivaloyl 2'AnhU could not be made because of thermal rearrangements. The following TMS and dTMS derivatives were prepared: 3'-Ac-5'-TMS-2'AnhU (Il), 3'-Ac-5'-dTMS-2'AnhU (Il'), 3'-TMS-5'-Ac-2'AnhU (Im), 3'-dTMS-5'-Ac-2'AnhU (Im'), 3'-TMS-5'-Piv-2'AnhU (Iu), 3'-dTMS-5'-Piv-2'AnhU (Iu'), 3'-Piv-5'-TMS-2'AnhU (Iv) and 3'-Piv-5'-dTMS-2'AnhU (Iv'). Their enhanced volatility and fully

substituted character minimizes the possibility of thermal reactions and allows fragmentations characteristic of the 3'- or 5'- substituents to be recognized.

The spectra of Il and Im are shown in Figure 10; Iu and Iv in Figure 11; Il' in Figure 12; Im' in Figure 13; Iu' in Figure 14 and Iv' in Figure 15. For each compound the molecular ion is of useful intensity. It is seen that the single bond fissions illustrated in Figures 10 & 11 often lead to significant intensity differences which support and extend the observations made earlier. In particular: (i) Figure 10 and the spectra of the TMS-d_9 analogues, show that elimination of $\cdot CH_3$ occurs only from the TMS group, and more readily from a 5'- than from a 3'-O-TMS group; (ii) Figure 10 confirms that elimination of m/e = 43 (i.e. C_2H_3O) occurs more readily when the acetyl group is in the 5'- rather than the 3'- position; (iii) the spectra of the TMS-d_9 labelled isomers Iu' and Iv' show that elimination of $\cdot CH_3$ occurs more readily from the 5' substituent independently of whether it is a pivaloyl or TMS group; (iv) Figure 11 shows that elimination of 85 mass units (i.e. $(CH_3)_3CCO$) occurs more readily when the pivaloyl group is in the 5' rather than the 3' position.

$\underline{(A+H)^+}$

A hydrogen transfer occurs in the formation of this ion. It has been shown that hydrogen transfer from acetyl groups is a favorable process whereas hydrogen transfer from TMS groups occurs no more

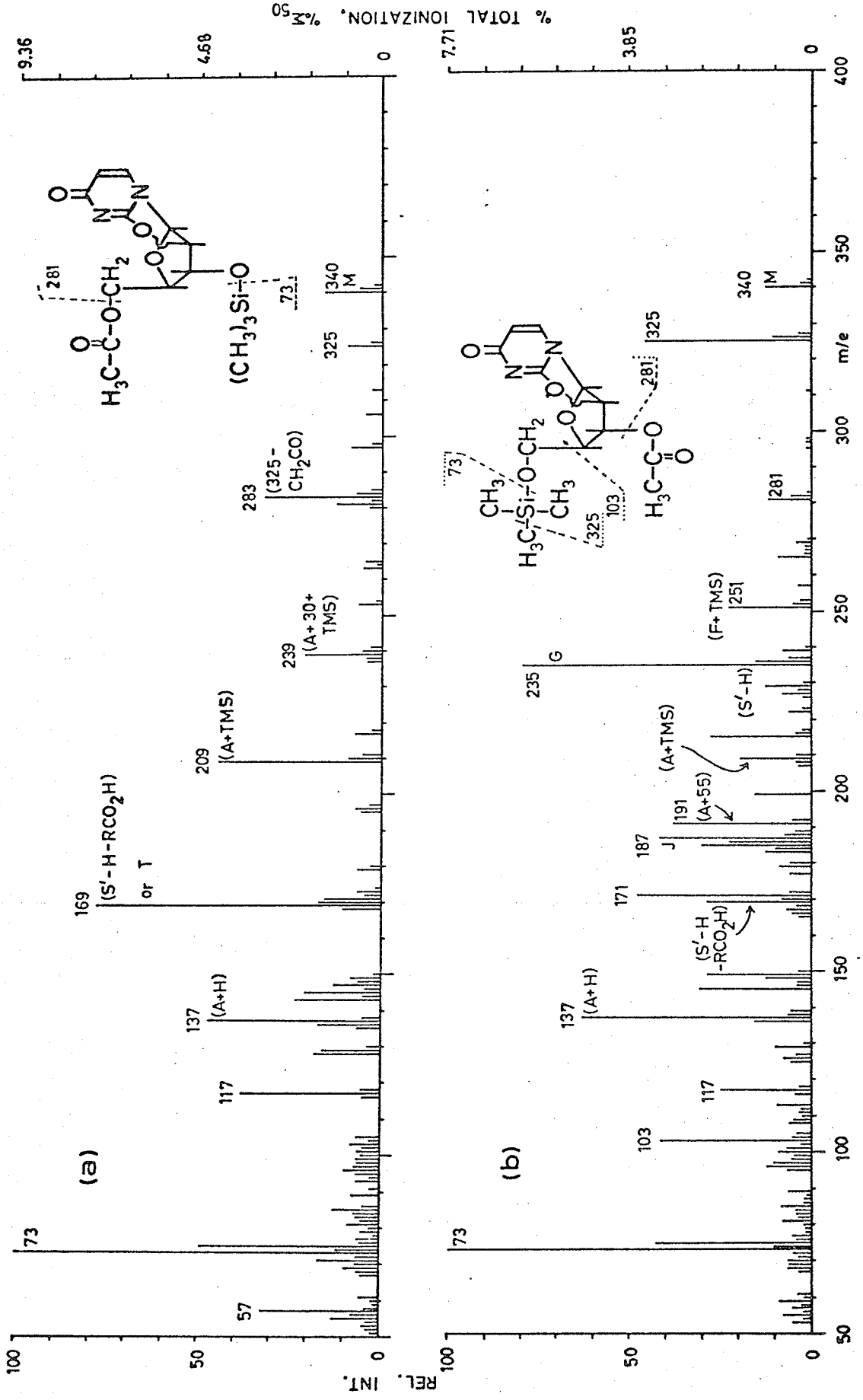


Figure 10. Mass Spectra of (a) 3'-TMS-5'-Ac-2'AnhU, (Im) and (b) 3'-Ac-5'-TMS-2'AnhU, (II).

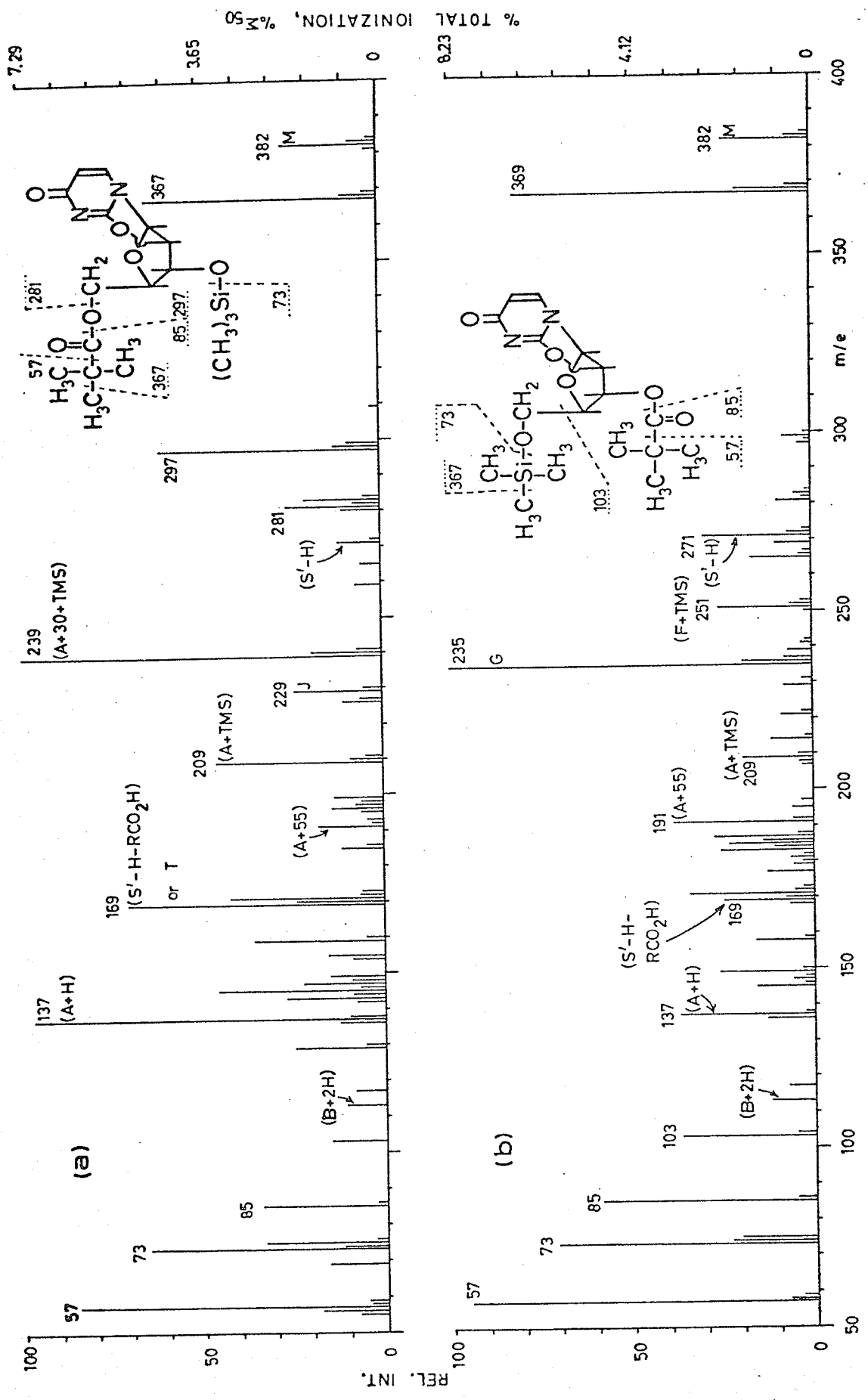


Figure 11. Mass Spectra of (a) 3'-TMS-5'-Piv-2'AnhU, (Iu) and (b) 3'-Piv-5'-TMS-2'AnhU, (Iv).

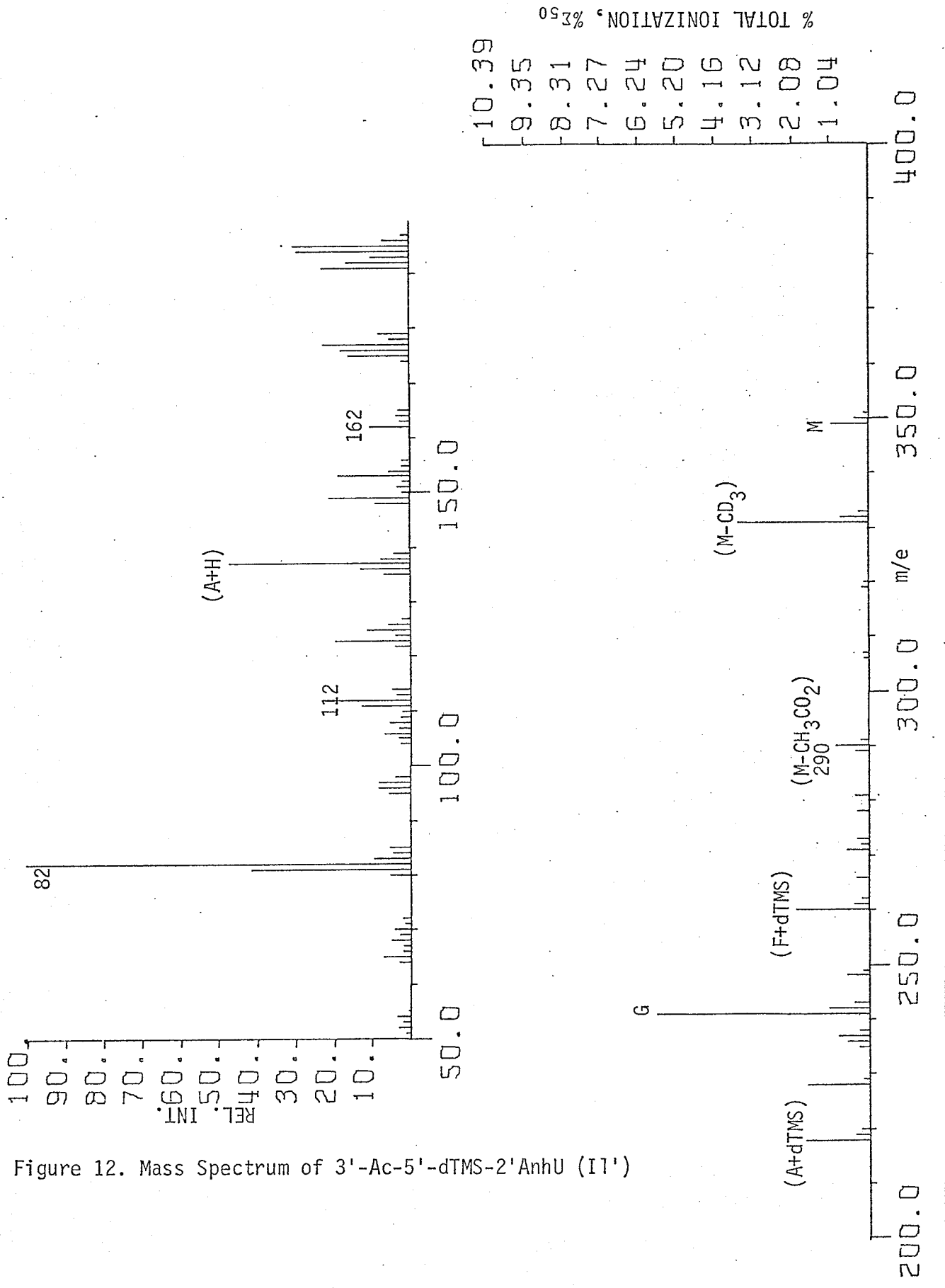


Figure 12. Mass Spectrum of 3'-Ac-5'-dTMS-2'AnhU (I1')

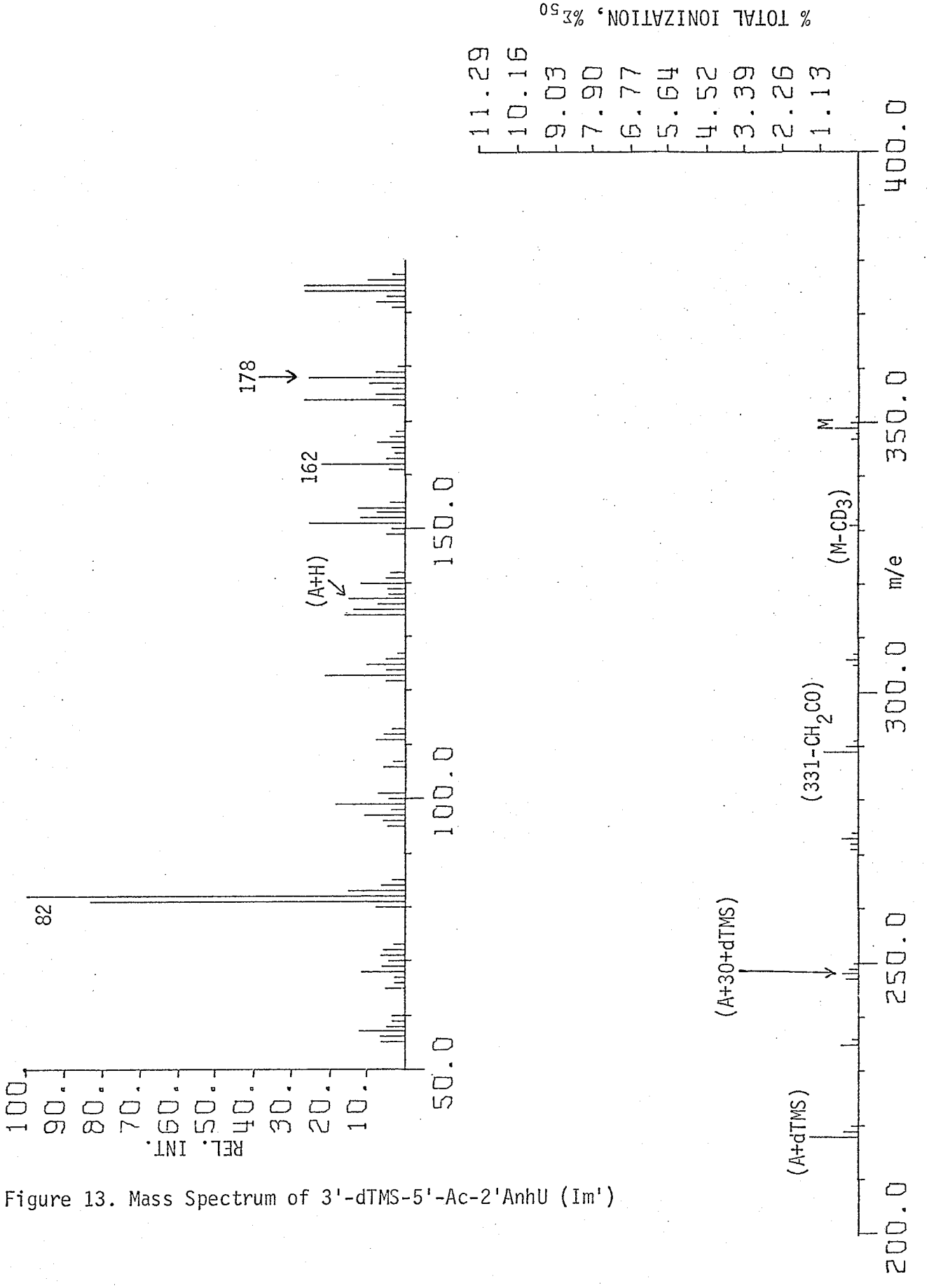


Figure 13. Mass Spectrum of 3'-dTMS-5'-Ac-2'AnhU (Im')

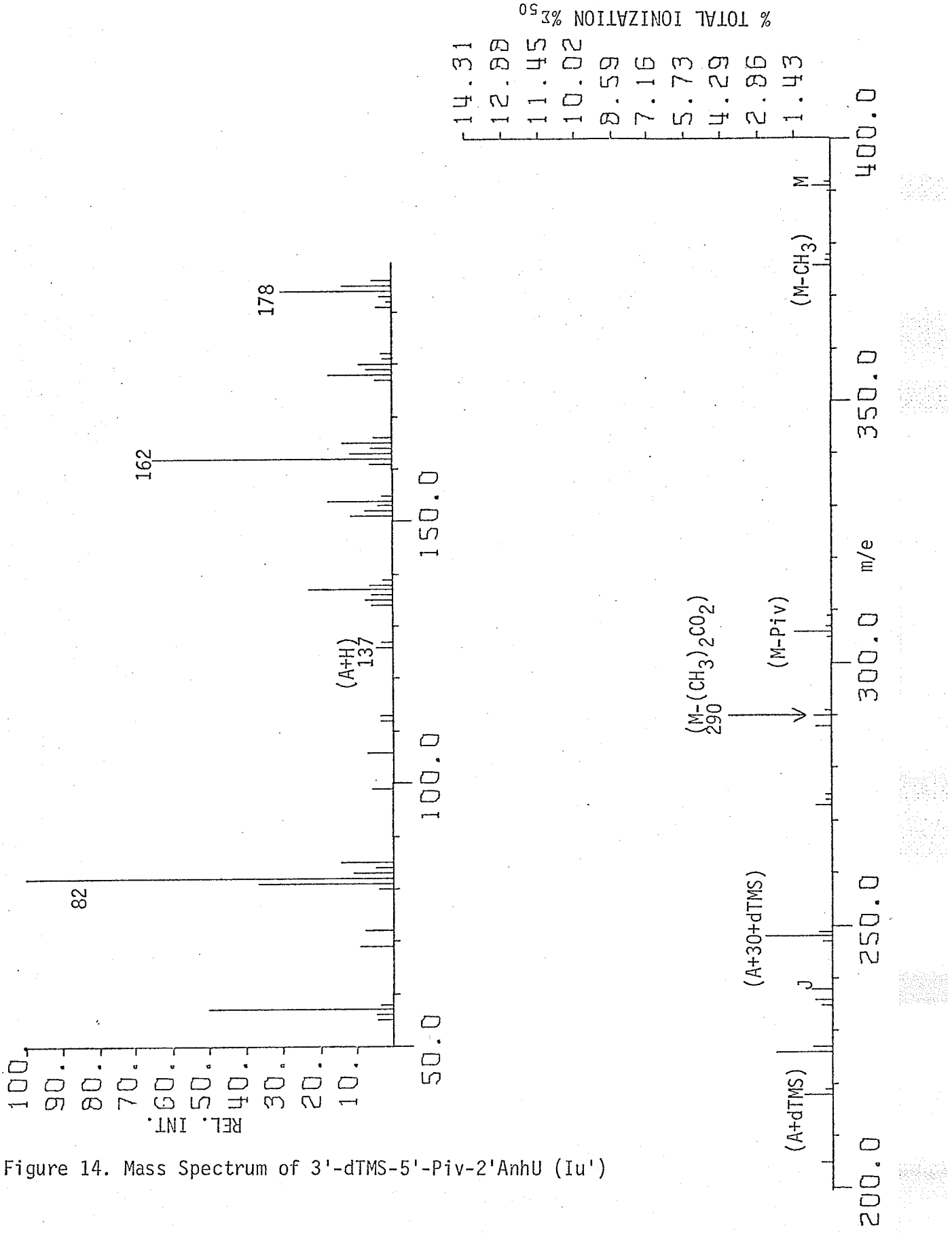


Figure 14. Mass Spectrum of 3'-dTMS-5'-Piv-2'AnhU (Iu')

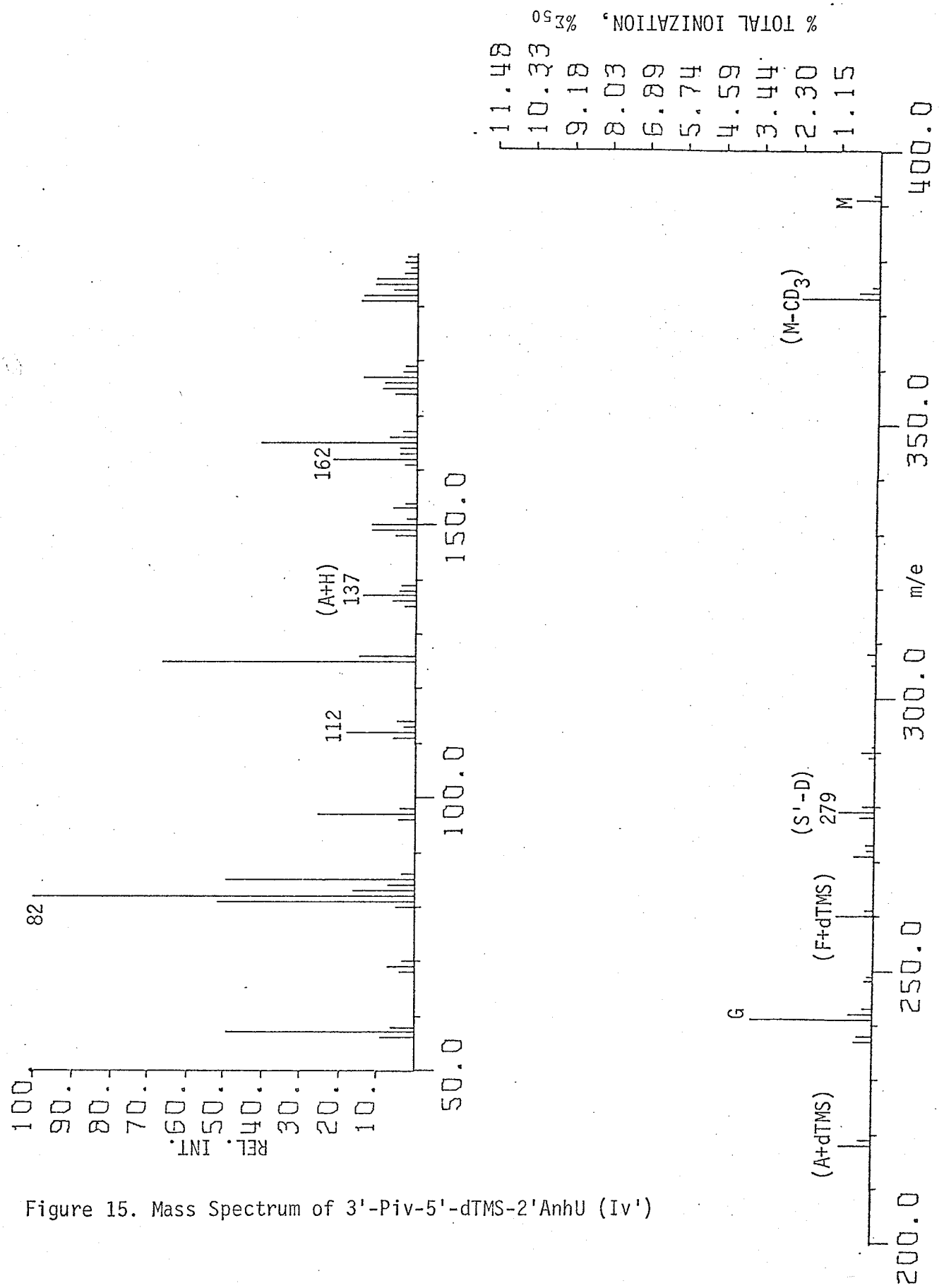


Figure 15. Mass Spectrum of 3'-Piv-5'-dTMS-2'AnhU (Iv')

readily than from the ribose skeleton. In support of this observation the $(A+H)^+$ ion appears at m/e = 137 in all spectra, including those of the compounds containing the TMS-d_9 groups. Thus, hydrogen transfer from the acyl groups is indicated. (It is also noted from discussions later on that hydrogen transfer from pivaloyl groups is also a favorable process).

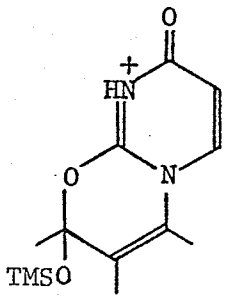
$(S'-H)^+$

This is a prominent ion in the mass spectra of bis(tri-methylsilyl) derivatives of 2,2'-anhydrouridine (If) and 2,2'-anhydrothymidine (IIf), but insignificant in the spectra of their diacetyl and dipivaloyl derivatives (Ib, Ie & IIb). It is expected to occur at m/e = 229 in the spectra of Il and Im (Figure 10) and at m/e = 271 in the spectra of Iu and Iv (Figure 11). It is of lower abundance for the isomers having the 3'-O-TMS group. Only the spectrum of compound Iv' gave an unequivocal answer as to the identity of the hydrogen atom lost to the neutral products. For the Iv' isomer this ion occurs at m/e = 279 indicating loss of a deuterium atom from the 5'-O-TMS-d_9 group. Thus, of the structures proposed in the earlier discussion of If and IIf for $(S'-H)^+$ those labelled $(S_1'-H)^+$, $(S_3'-H)^+$, $(S_4'-H)^+$ are apparently preferred.

$(A+30+TMS)^+$

This ion occurs at m/e = 239 in the mass spectra of Im (Figure 10a) and Iv (Figure 11a) and at m/e = 248 for Im' and Iv', i.e. this ion

contains the intact 3'-O-TMS group. This ion also appears in the mass spectra of the bis(trimethylsilyl) derivatives of 2,2'-anhydrouridine (If) and 2,2'-anhydrothymidine (IIf) and its appearance here is consistent with the structure proposed:



$(A+30+TMS)^+$

The greater intensity of the ion in these spectra may reflect the more facile hydrogen transfer to the base moiety from a 5'-O-acetyl or 5'-O-pivaloyl group than from a 5'-O-TMS group and may also explain the low intensity of the acetyl and pivaloyl analogues of this ion in the mass spectra of the 5'-O-TMS isomers. (These analogues should occur at m/e = 209 and 251 respectively. Although ions of this mass do occur in the spectra of Il and Iv, they are absent in the spectra of Il' and Iv', and are not therefore structurally related.)

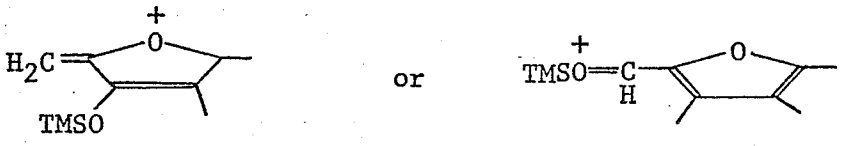
$\underline{(A+TMS)^+}$

This ion occurs at m/e = 209 in all spectra of Figures 10 and 11 and shifts to m/e = 218 in the spectra of the TMS-d_9 analogues. As discussed in the spectra of If and IIf it is formed by a process

involving migration of a TMS group. These spectra show that migration of either TMS group can occur.

$(S'\text{-}H\text{-}RCO_2H)^+$

This ion occurs at m/e = 169 in all spectra of Figures 10 and 11. Alternative structures are proposed depending upon whether a 3'- or 5'-O-TMS group is present:

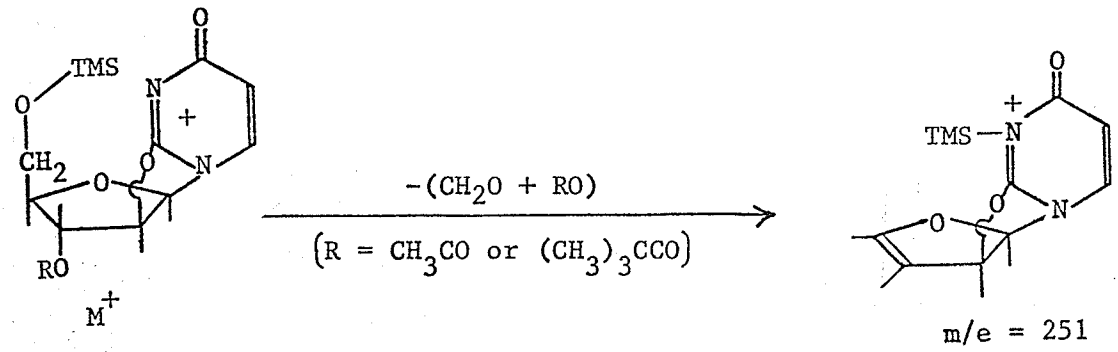


(also labelled T^+)

Both these structures have been suggested for analogues of this ion formed from bis(TMS) derivatives of pyrimidine 2,2'-anhydronucleosides.

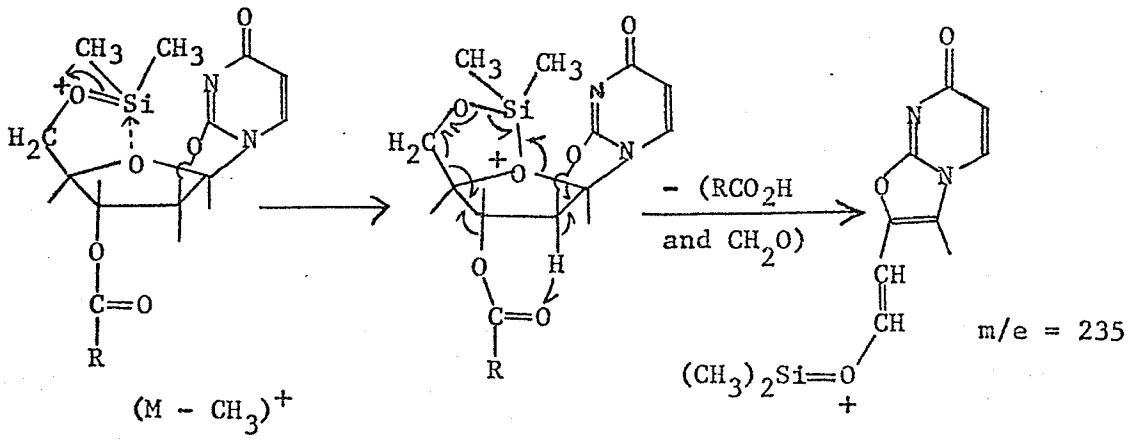
$(F+TMS)^+$

This ion occurs at m/e = 251 in the spectra of Il (Figure 10b) and Iv (Figure 11b) and at m/e = 260 for their TMS-d_9 analogues. It therefore retains the intact 5'-O-TMS group but has lost the 3'-substituent. It appears to be structurally analogous to the $(F+H)^+$ ion observed in the spectra of diacyl-2,2'-anhydropyrimidine nucleosides and is logically formed by a process involving migration of the 5'-TMS group:



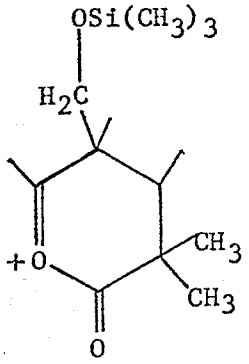
G^+

This ion occurs at m/e = 235 in the spectra of Il (Figure 10) and Iv (Figure 11) and shifts to m/e = 241 for Il' and Iv'. It therefore has lost one methyl group from the 5'-O-TMS group and has also lost the substituent from the 3'-position. A possible route to its formation is:

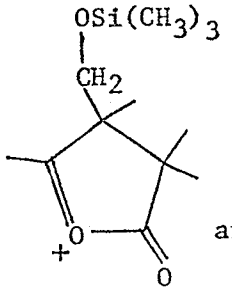


J^+

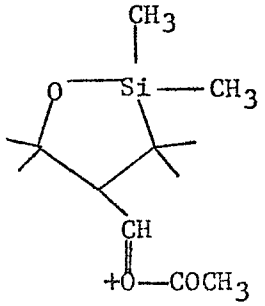
This ion is an analogue of an ion of m/e = 217 in the mass spectra of bis(trimethylsilyl)-pyrimidine-2,2'-anhydronucleosides and occurs in the mass spectrum of Il (Figure 10) at m/e = 187 and for Iu (Figure 11) at m/e = 229. It shifts to 238 for Iu', but for Il' a number of peaks in the region m/e = 191-196 confuse the determination of the mass shift, where m/e = 195 is about three times as abundant as 196. Possible structures, by analogy to those proposed before are:



m/e = 229



and/or



m/e = 187

Trifluoroacetyl derivatives of monoacetyl- and monopivaloyl-2,2'-anhydrouridine

The mass spectra of 3'-Ac-5'-TFAc-2'AnhU (Io) and 3'-TFAc-5'-Ac-2'AnhU (Ip) are shown in Figure 16 and those of 3'-Piv-5'-TFAc-2'AnhU (Ix) and 3'-TFAc-5'-Piv-2'AnhU (Iy) in Figure 17. Trifluoroacetylation of monoacetyl- and monopivaloyl-2'AnhU allow distinction to be made between the isomers Io and Ip and between Ix and Iy. Many of the

prominent peaks are readily interpreted to show the presence of a specific 3' or 5' substituent. The more important of these are briefly discussed. The preferred simple bond fissions are illustrated in Figures 16 and 17.

Loss of 113 mass units shows the presence of the 3'-O-trifluoroacetyl substituent in Figures 16b and 17a. This loss of the CF_3CO_2 group from M^+ occurs only from the 3' position, similar to the loss of CH_3CO_2 from 3'-O-Ac substituted 2'AnhU. The peaks equivalent to the loss of the CF_3CO_2 group from M^+ are absent in the spectra of Io and Ip.

Loss of 43 mass units enables the presence of the 5'-O-acetyl group to be recognized in Figure 16b.

The preferred loss of 15 and 85 mass units confirms the presence of a 5'-O-pivaloyl group in Figure 17a.

The ion T^+ (formed by elimination of the substituent from the 5' position, and the base moiety) occurring at m/e = 193 in Figure 16b enables the 3'-O-trifluoroacetyl substituent to be recognized. The high intensity of peak m/e = 193 in Ix, Figure 17a, probably arises from the ion T^+ and also the ion of m/e = 193, characteristic of the 5'-pivaloyl group in 2'AnhU as discussed earlier.

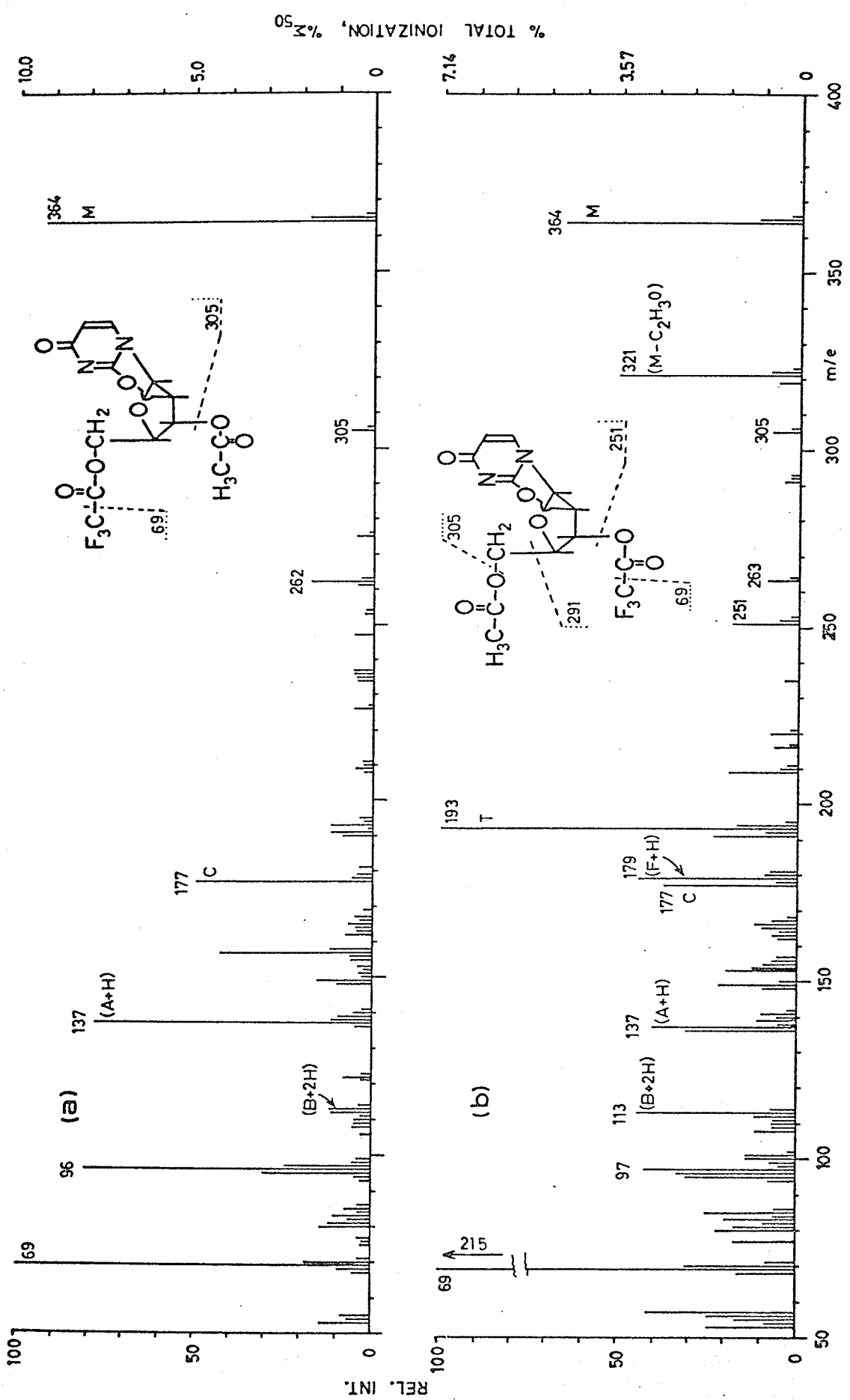


Figure 16. Mass Spectra of (a) 3'-Ac-5'-TFAc-2'AnhU, (Io) and (b) 3'-TFAc-5'-Ac-2'AnhU, (Ip).

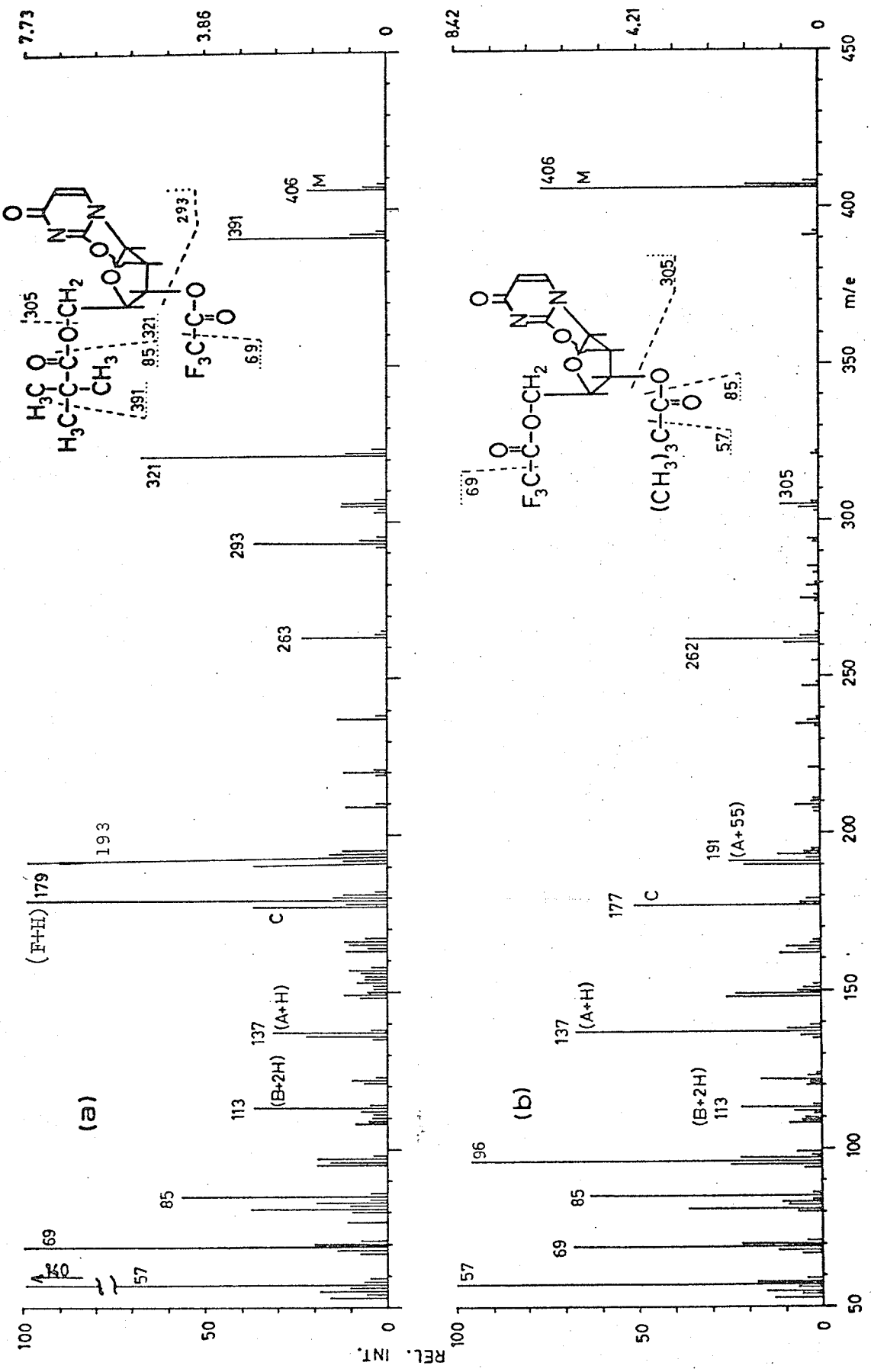
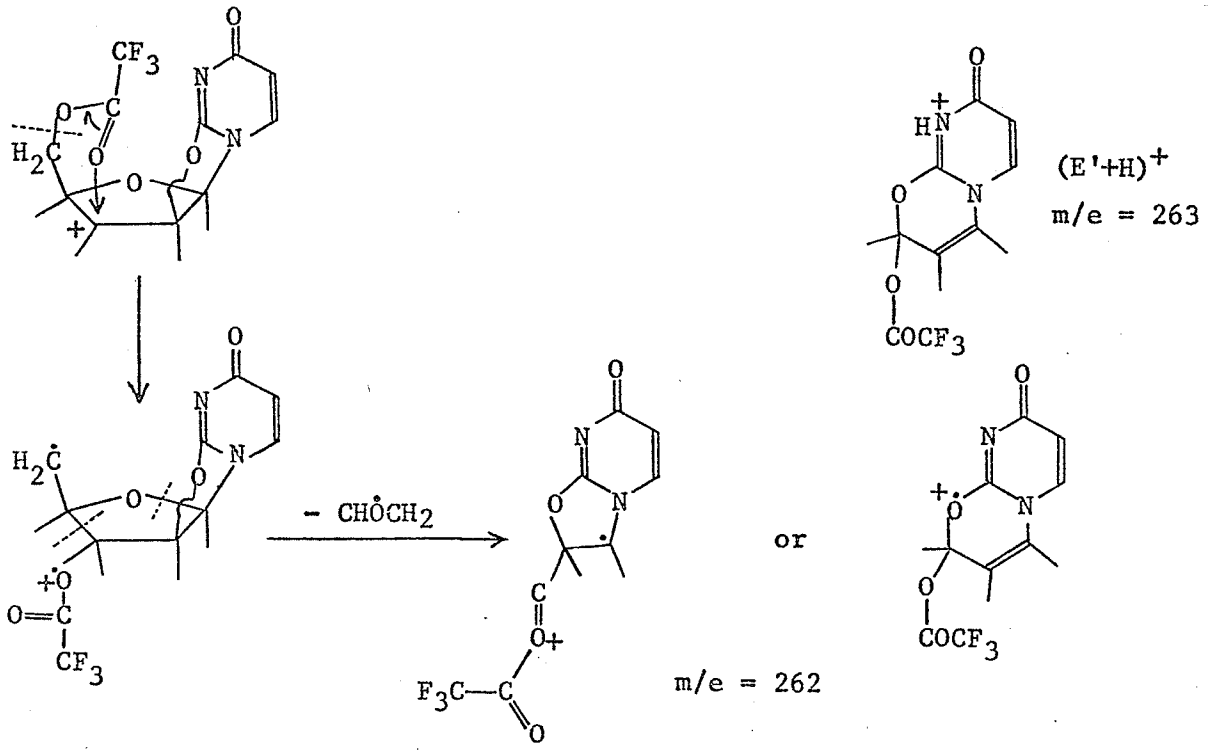


Figure 17. Mass Spectra of (a) 3'-TFAc-5'-Piv-2'AnhU, (Ix); and (b) 3'-Piv-5'-TFAc-2'AnhU, (Iy).

The formation of ion $(F+H)^+$ (m/e = 179) requires H transfer from the 5' substituent and reveals that this is the location of the acetyl and pivaloyl groups in Figures 16b and 17a.

The ion $(A+H)^+$ (m/e = 137) can be formed in a number of ways, e.g. by H transfer from the 3' or 5' substituents or from the ribose skeleton. Figures 16 and 17 suggest that this transfer occurs most readily from 3'- rather than 5'-O-acetyl or O-pivaloyl substituents.

Isomers can also be distinguished by peaks at m/e = 263 and 262. When a 3'-O-trifluoroacetyl group is present a peak occurs at m/e = 263. When a 5'-O-trifluoroacetyl group is present a peak occurs at m/e = 262. The former peak is $(E'+H)^+$ formed by hydrogen transfer from the 5' acyl group to the base moiety, followed by elimination of the 4' C and O, and 5' C atoms and their substituents. A similar H transfer cannot occur when a 5'-O-trifluoroacetyl substituent is present. The peak at m/e = 262 can be explained by elimination of the 3'-O-acyl substituent followed by the rearrangement (involving a six-membered cyclic transition state) below: (See next page)



5'-O-trityl-2,2'-anhydrouridine and 3'-O-acetyl-5'-O-trityl-2,2'-anhydrouridine

It was not possible to compare the mass spectra of the 5'- and 3'-O-trityl isomers because the 3'-O-trityl isomer is not readily prepared. While this is unfortunate from the viewpoint of assistance in emphasizing mass spectral differences between the isomers the

failure to obtain a mass spectrum of the 3'-O-trityl isomer is of little practical consequence because monotritylation occurs exclusively at the 5' position for 2'-anhydronucleosides.

The mass spectra of 5'-O-trityl-2,2'-anhydrouridine, Ih, and 3'-O-acetyl-5'-O-trityl-2,2'-anhydrouridine, Ii, are shown in Figures 18 and 19 respectively. They are dominated by peaks from the trityl group and fragmentations therein. Although of relatively low intensity the M^+, $(M-C_6H_5)^+$ and $(M-Tri)^+$ ions can be used to identify the compound. The high volatility of compound Ii is noteworthy. It does not appear that there are any prominent peaks in either spectrum which are obviously characteristic of the 5'-O-trityl isomers.

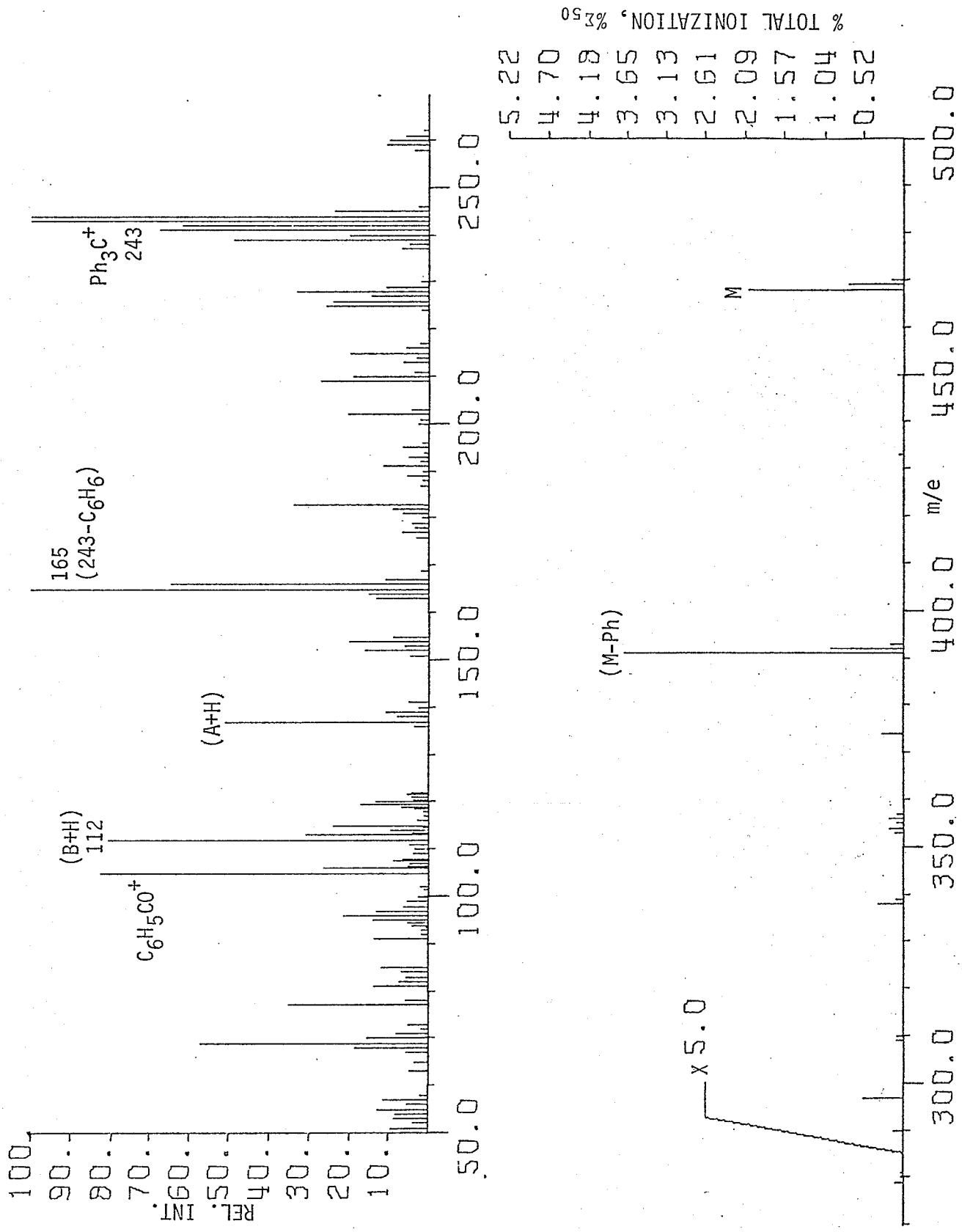


Figure 18. Mass Spectrum of 5'-Tri-2'AnhU (Ih)

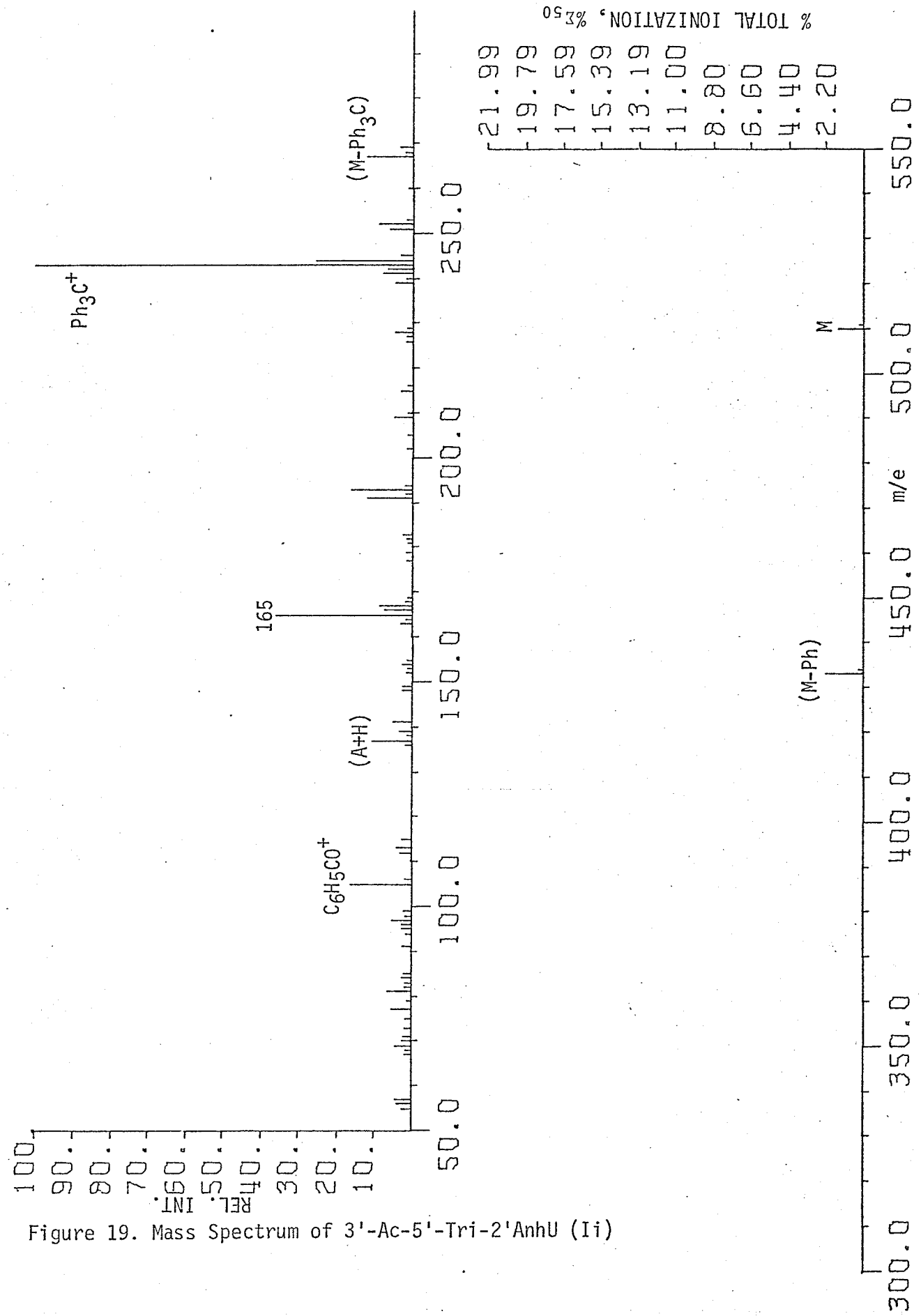


Figure 19. Mass Spectrum of 3'-Ac-5'-Tri-2'AnhU (Ii)

CHAPTER FOUR: RESULTS AND DISCUSSION (PART II)

Purine 8,2'-anhydronucleosides, 8,3'-anhydronucleosides and their TMS Derivatives

Most of the purine anhydronucleosides, except those derived from adenine, were not volatile enough to be studied by mass spectrometry. They decomposed when their melting points were taken and also when they were heated on the sample probe (see TABLE I). To circumvent this problem trimethylsilyl derivatives were prepared and found to be most suitable for mass spectrometric study. Generally the mass spectra of the purine anhydronucleoside TMS-derivatives differ from those of the pyrimidines in that they show much less extensive fragmentation. However, the characteristic ions, even though they are of low abundance, are of sufficient intensity to provide structural information.

8,2'-O-, 8,2'-S- and 8,2'-N-anhydroadenosine

The mass spectra of 8,2'-O-AnhA (IV), 8,2'-S-AnhA (V) and 8,2'-N-AnhA (VI) are shown in Figures 20, 21 and 22 respectively. Partial spectra of these compounds have been reported[87-89] but no discussions on the structural significance of the fragment ions were presented. The general features of the present spectra agree with those in the literature.

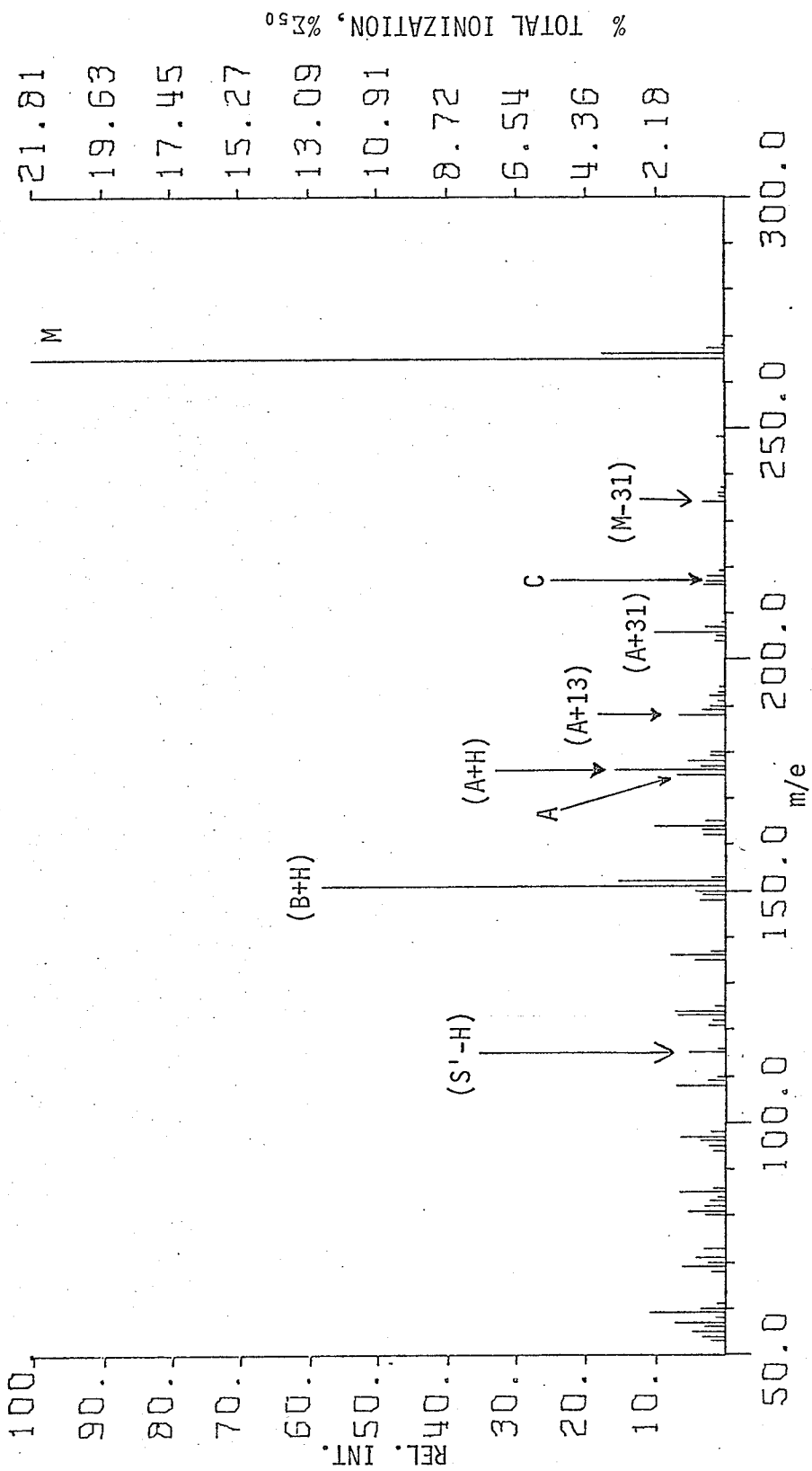


Figure 20. Mass Spectrum of 8,2'-O-AnhA (IV)

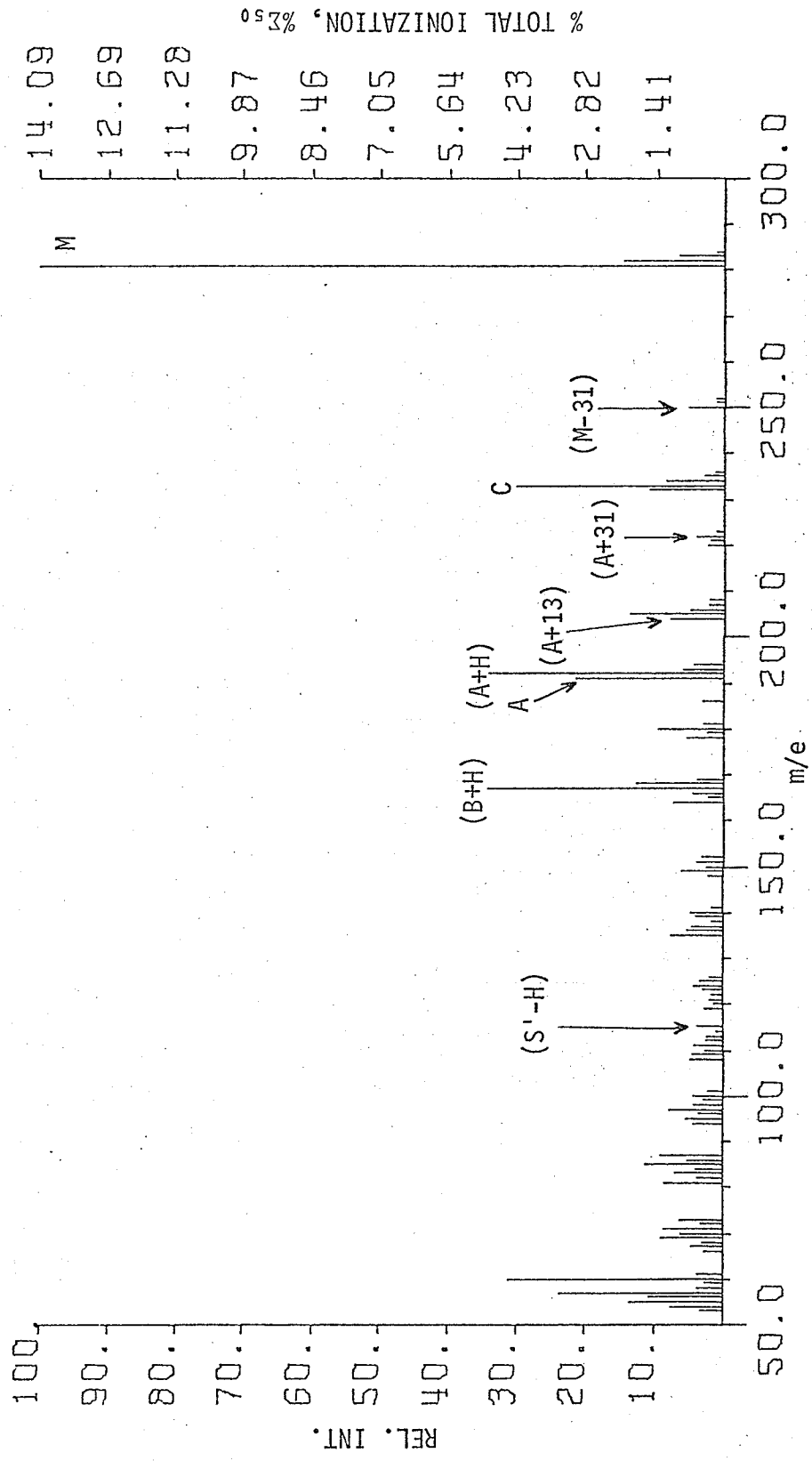


Figure 21. Mass Spectrum of 8,2'-S-AnhA (V)

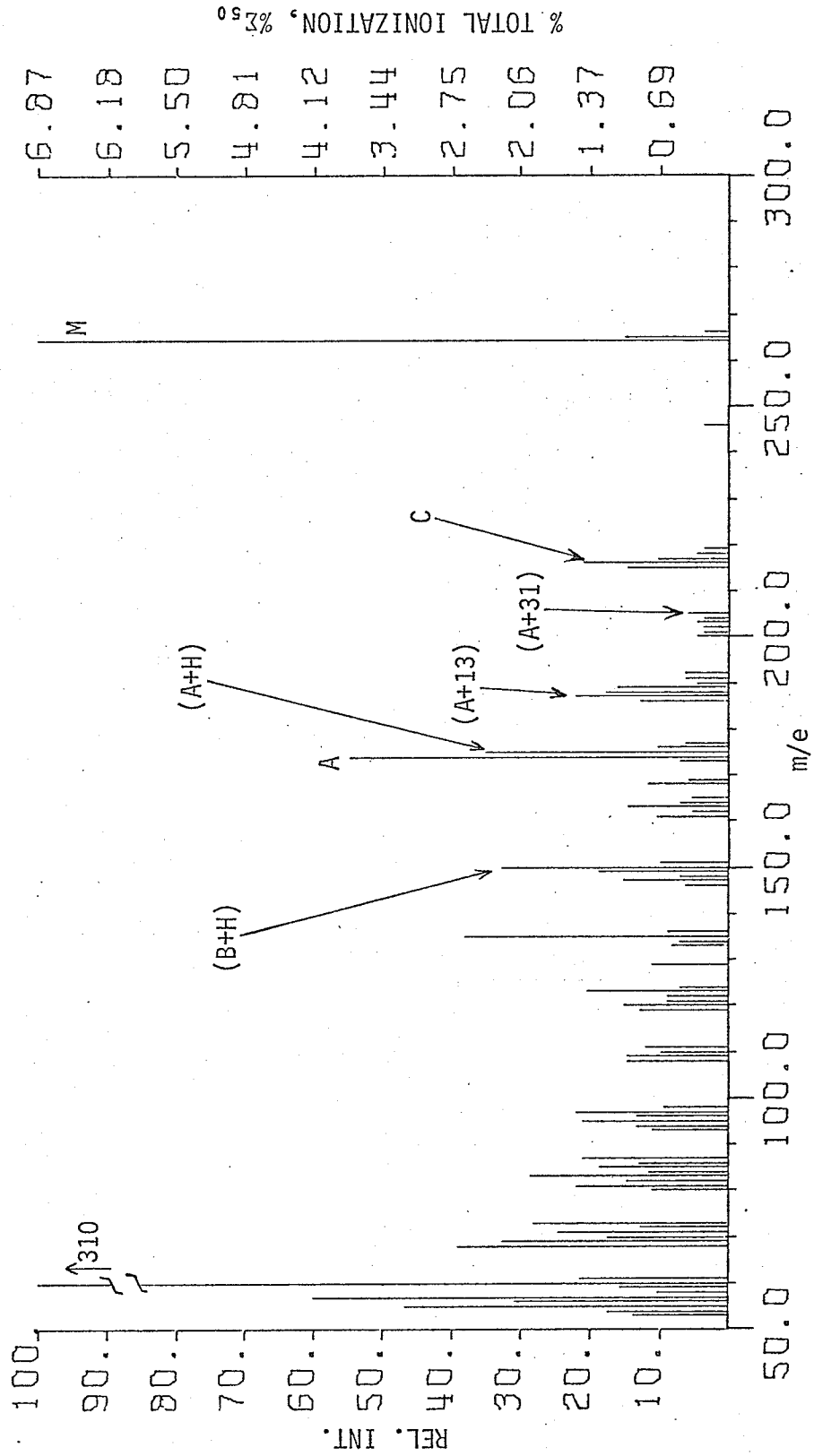
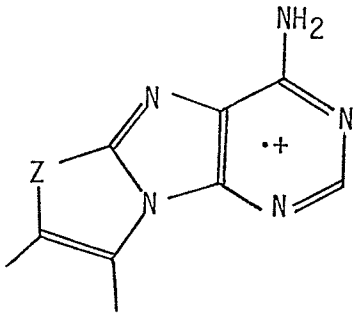


Figure 22. Mass Spectrum of 8,2'-N-AnhA (VI)

For IV, V and VI, except for two peaks, the fragmentation patterns resemble those of the pyrimidine 2,2'-anhydronucleosides. Ions M^+, $(M-31)^+$, $(A+H)^+$, $(A+31)^+$, B^+, $(B+H)^+$, $(B+2H)^+$, $(S'-H)^+$ and C^+ are present in their spectra and probably have analogous structures and fragmentation pathways to the ones proposed earlier for the pyrimidine 2,2'-anhydronucleosides. In these cases 3' or 5' hydroxyl hydrogen may transfer to N-7 of the purine bases instead of N-3 of the pyrimidine bases, or to the heteroatom of the anhydro-linkage. It is interesting to note the low intensity of the ion $(S'-H)^+$ compared to that of the $(B+H)^+$ ion in these spectra and to the same ion in 2'AnhU (I) and 2'AnhT (II). It shows in IV, V and VI that the probability of localization of the positive charge on the sugar moiety is much lower than that on the purine base which is consistent with the trend established for natural nucleosides[47].

An ion, $(M-90)^+$, was reported to be characteristic to 8,2'-N-cycloadenosine[88]. This ion, here labelled as A^+, is present in the spectra of IV and V as well as VI. Its relatively high intensity is most likely due to the high probability of localization of the positive charge on the base moiety and its ability to stabilize the ion radical, making the following ion stable:

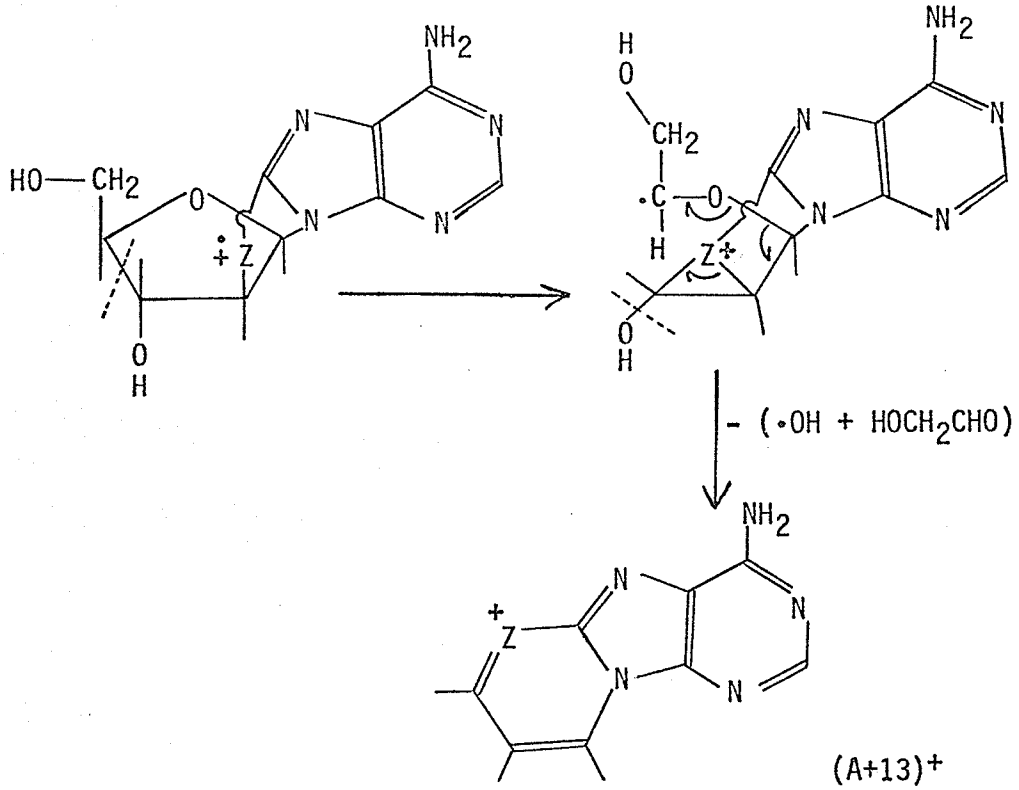


A^+

Z = O, S or NH

The neutral product accompanying the formation of A^+ would have a relatively stable epoxide structure. The formation of A^+ will undoubtedly compete with that of $(A+H)^+$ which, according to earlier discussions, is initiated by charge localization on the heteroatom atom of the anhydro-linkage. The abundance of A^+ ion in the spectrum of VI seems to indicate that the probability of charge localization on the base is higher than that of the nitrogen atom of the anhydro-linkage.

Another ion, labelled as $(A+13)^+$, is present in the spectra IV, V and VI. It is also observed in the mass spectra of most of the TMS derivatives of the purine 8,2'-anhydronucleosides but not in those of the pyrimidine anhydronucleosides. Comparison of the spectra shows that this ion must contain the base moiety and the anhydro-linkage heteroatom or group. The only logical route to its formation therefore involves fission of the C-3', C-4' bond and incorporation of the C-3' and H-3' atoms into the ion product:



Trimethylsilyl derivatives of purine 8,2'-anhydronucleosides

The mass spectra of the compounds studied can be found in the figures listed as follows:

IVf	8,2'-O-AnhA-$(TMS)_3$	(Figure 23)
IVf'	8,2'-O-AnhA-$(dTMS)_3$	(Figure 24)
Vf	8,2'-S-AnhA-$(TMS)_3$	(Figure 25)
Vf'	8,2'-S-AnhA-$(dTMS)_3$	(Figure 26)
VIf	8,2'-N-AnhA-$(TMS)_4$	(Figure 27)
VIf'	8,2'-N-AnhA-$(dTMS)_4$	(Figure 28)
VIIf	8,2'-O-AnhI-$(TMS)_3$	(Figure 29)

VIIIf	8,2'-S-AnhI-$(TMS)_3$	(Figure 30)
VIIIf'	8,2'-S-AnhI-$(dTMS)_3$	(Figure 31)
IXf	8,2'-N-AnhI-$(TMS)_4$	(Figure 32)
IXf'	8,2'-N-AnhI-$(dTMS)_4$	(Figure 33)
Xf	8,2'-O-AnhG-$(TMS)_4$	(Figure 34)
XIf	8,2'-S-AnhG-$(TMS)_4$	(Figure 35)
XIf'	8,2'-S-AnhG-$(dTMS)_4$	(Figure 36)
XIIf	8,2'-N-AnhG-$(TMS)_5$	(Figure 37)
XIIIf	8,2'-S-AnhX-$(TMS)_4$	(Figure 38)

These spectra exhibit several of the basic ion types which have so far been discussed. Individual ion types will now be discussed separately.

M^+ and $(M-CH_3)^+$

Both ions are of high abundance in all the spectra studied. Their high abundances, especially when compared to the fragment ions, indicate their high stability. This is helpful in that the molecular ion will be easily recognized for TMS derivatives of compounds of this structure type. As stated before, the dTMS derivatives will assist in identifying the M^+ ions in cases where their abundances are low.

$(A+H)^+$ and $(A+TMS)^+$

As shown previously these ions are characteristic in the mass spectra of the pyrimidine 2,2'-anhydronucleosides and the same phenomena are observed for the mass spectra of the TMS derivatives of

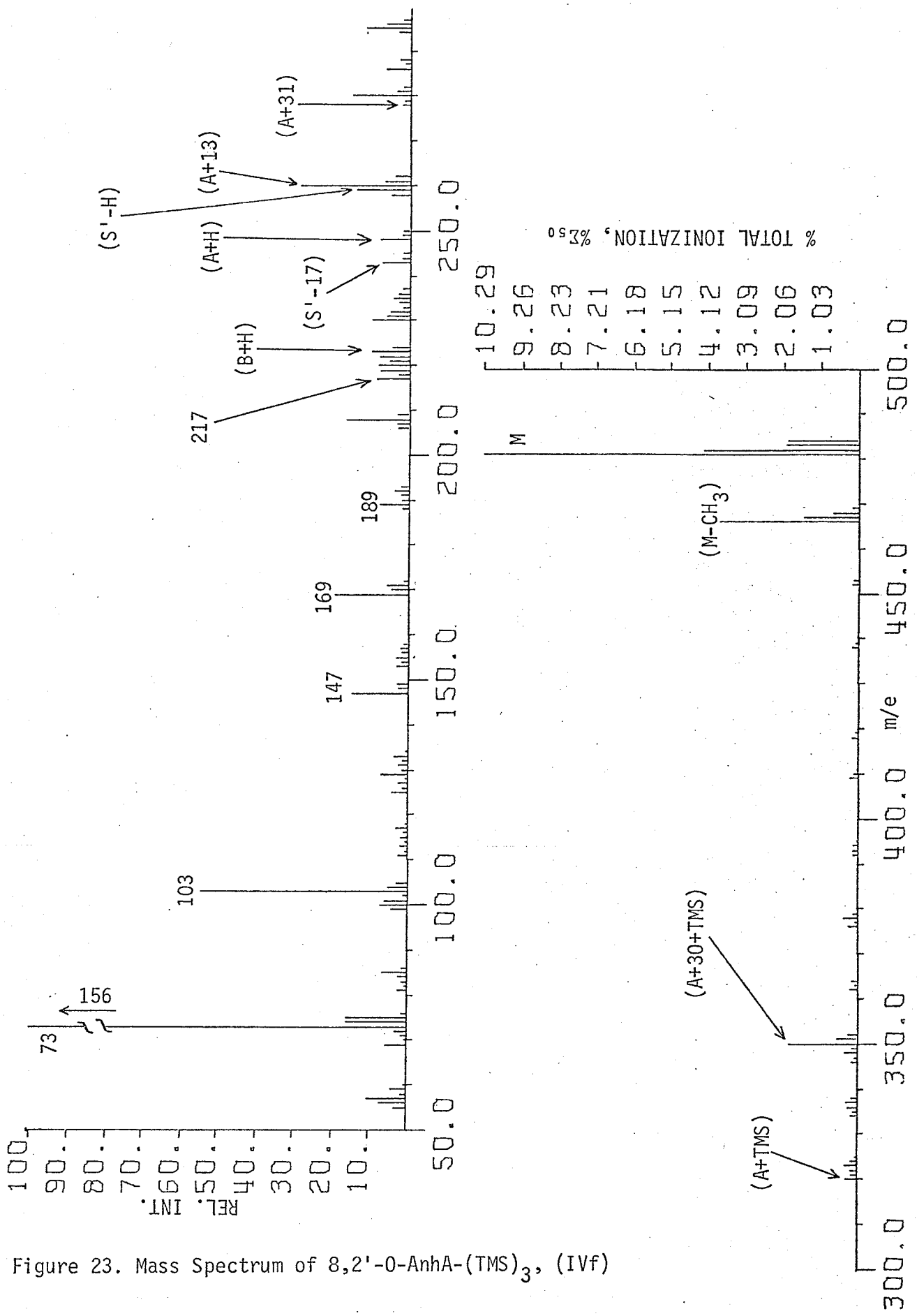


Figure 23. Mass Spectrum of 8,2'-O-AnhA-$(TMS)_3$, (IVf)

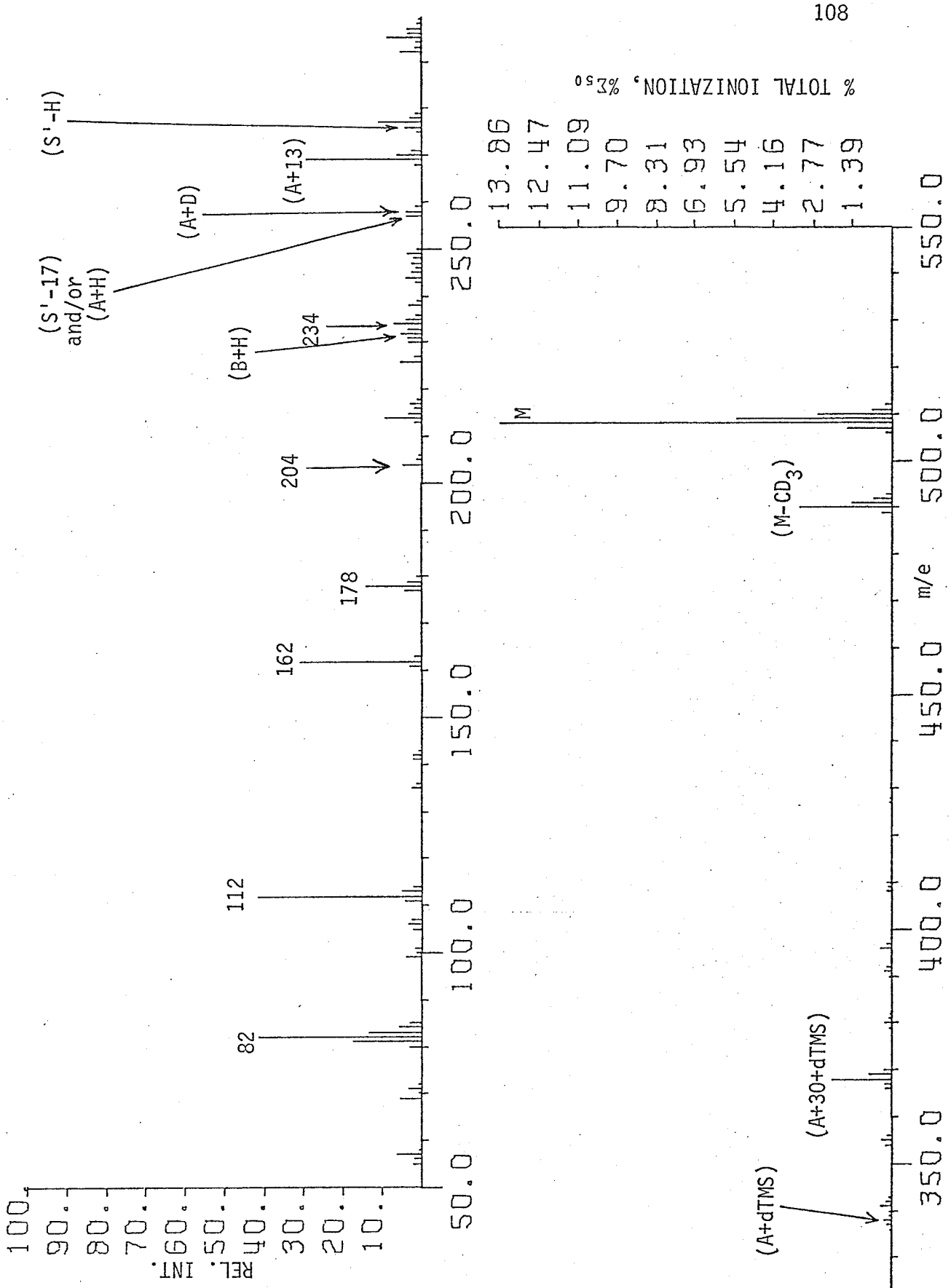


Figure 24. Mass Spectrum of 8,2'-O-AnhA-$(TMS)_3$, (IVf')

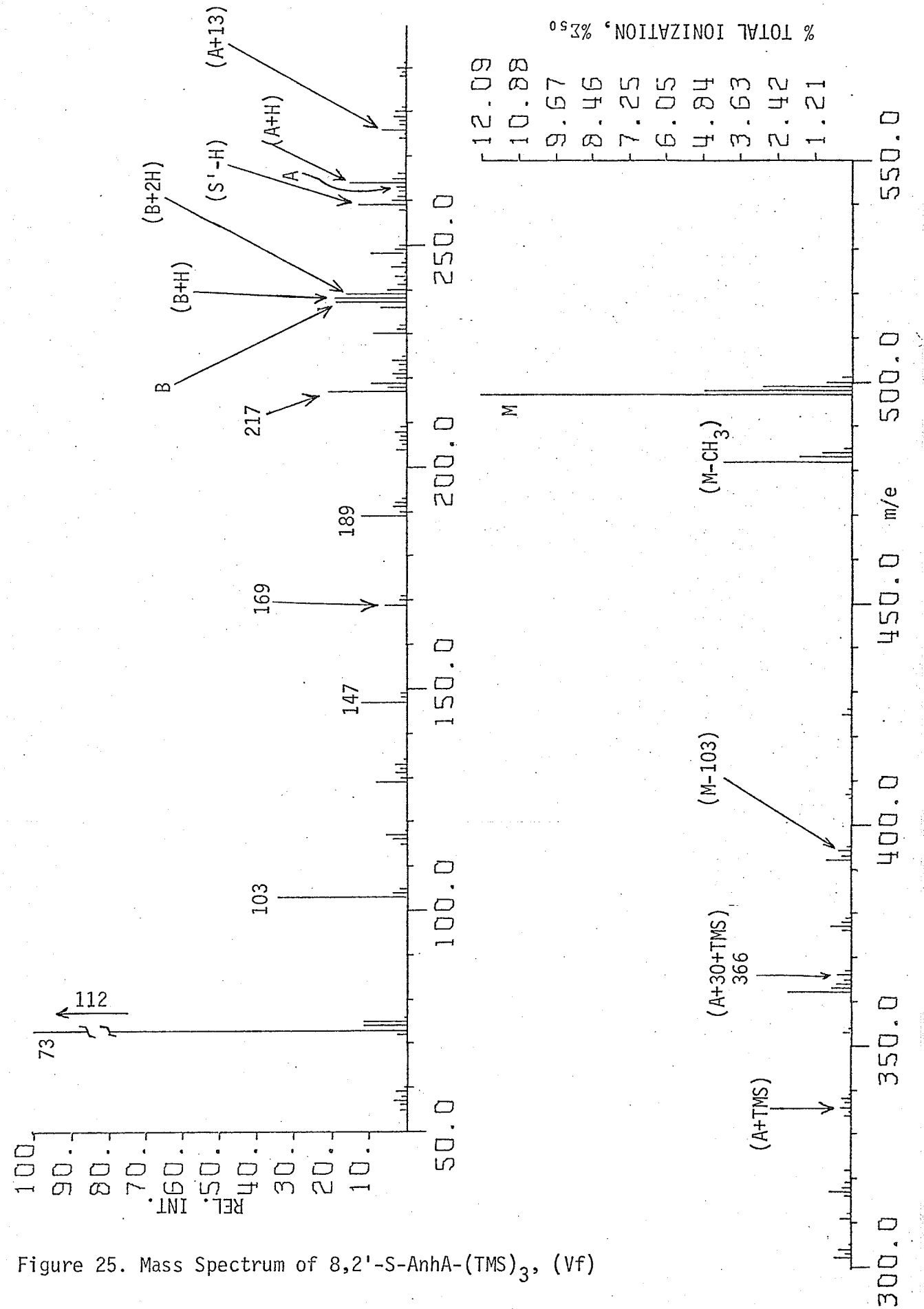


Figure 25. Mass Spectrum of 8,2'-S-AnhA-$(TMS)_3$, (Vf)

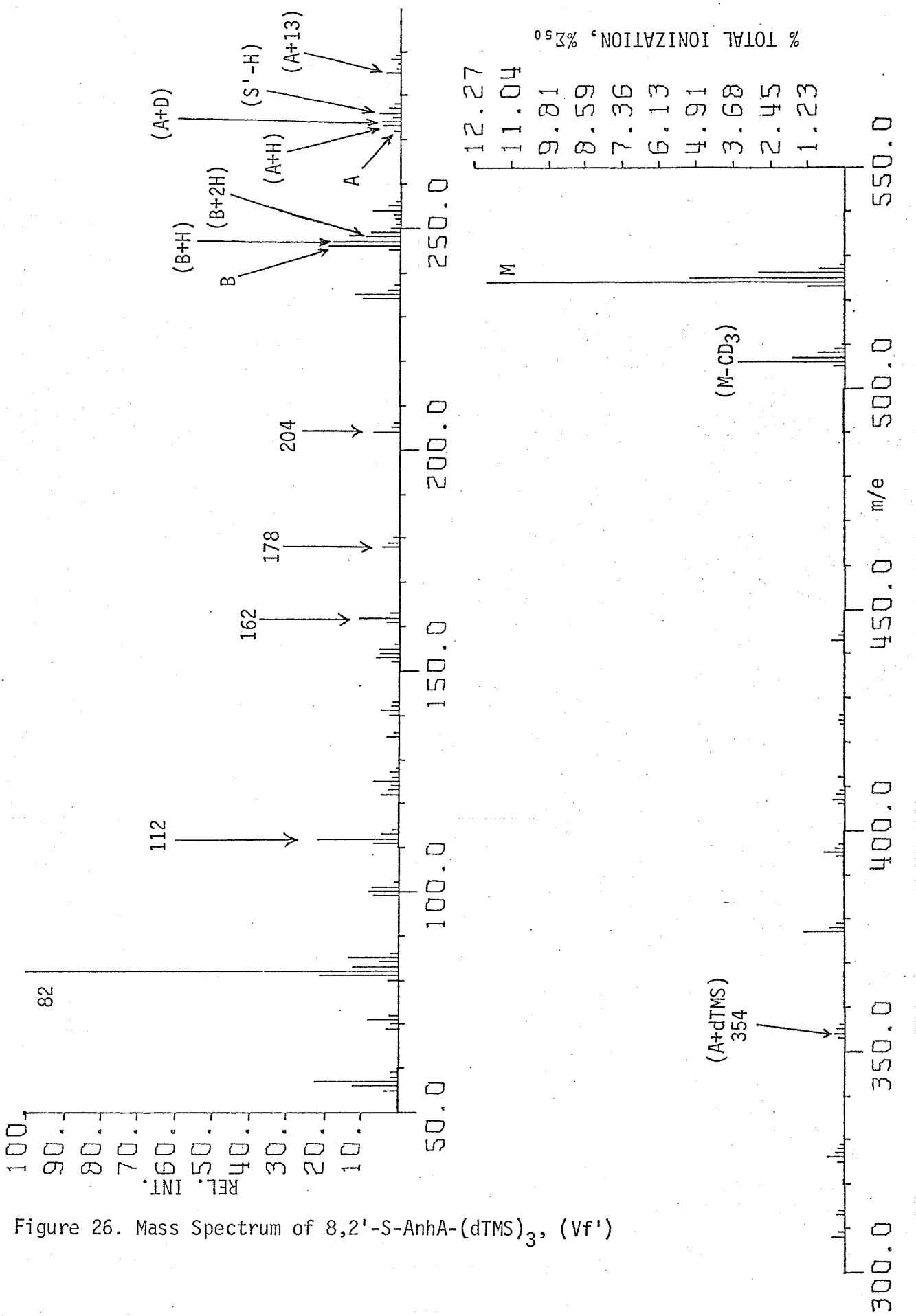


Figure 26. Mass Spectrum of 8,2'-S-AnhA-$(dTMS)_3$, (Vf')

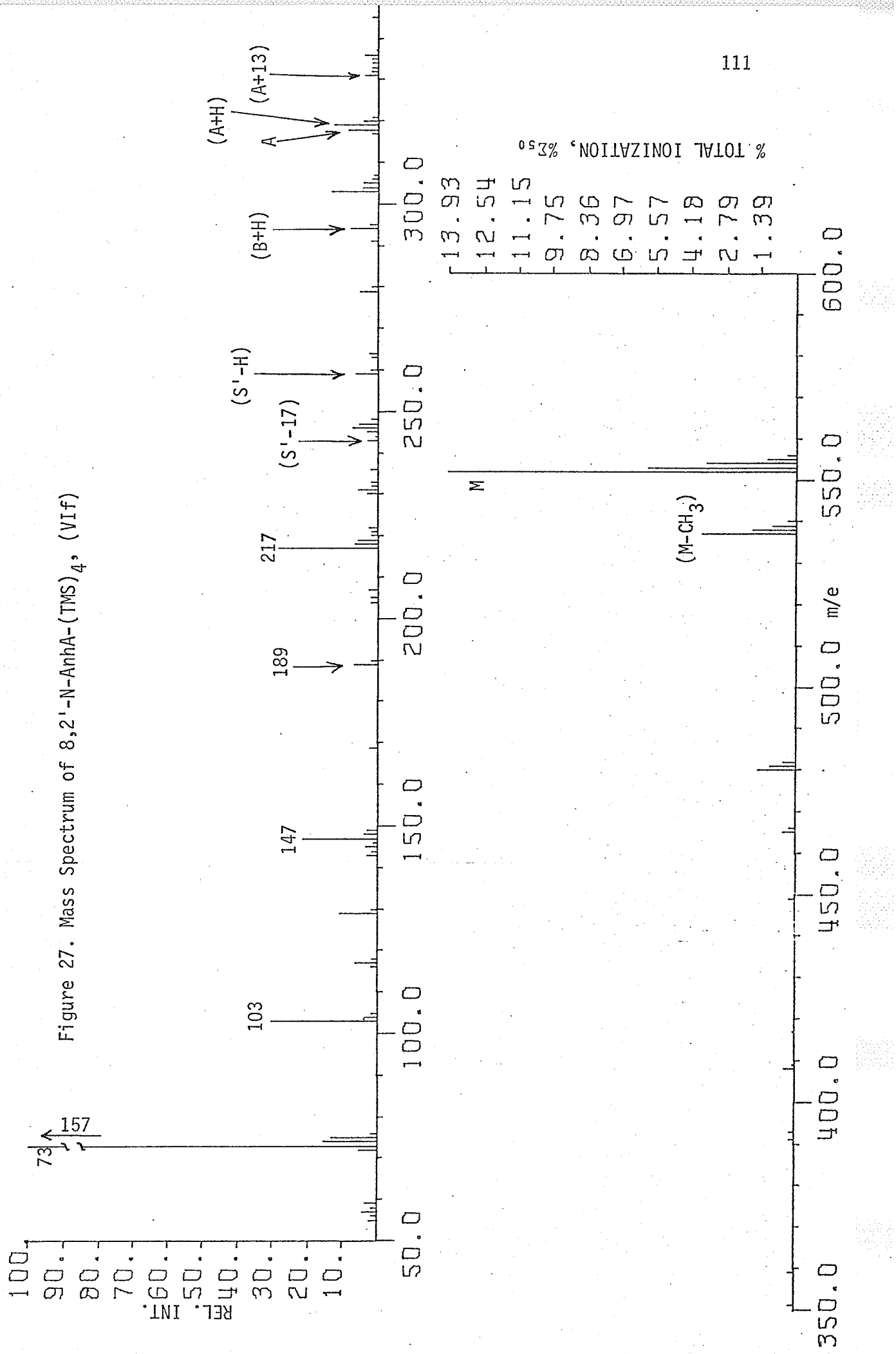
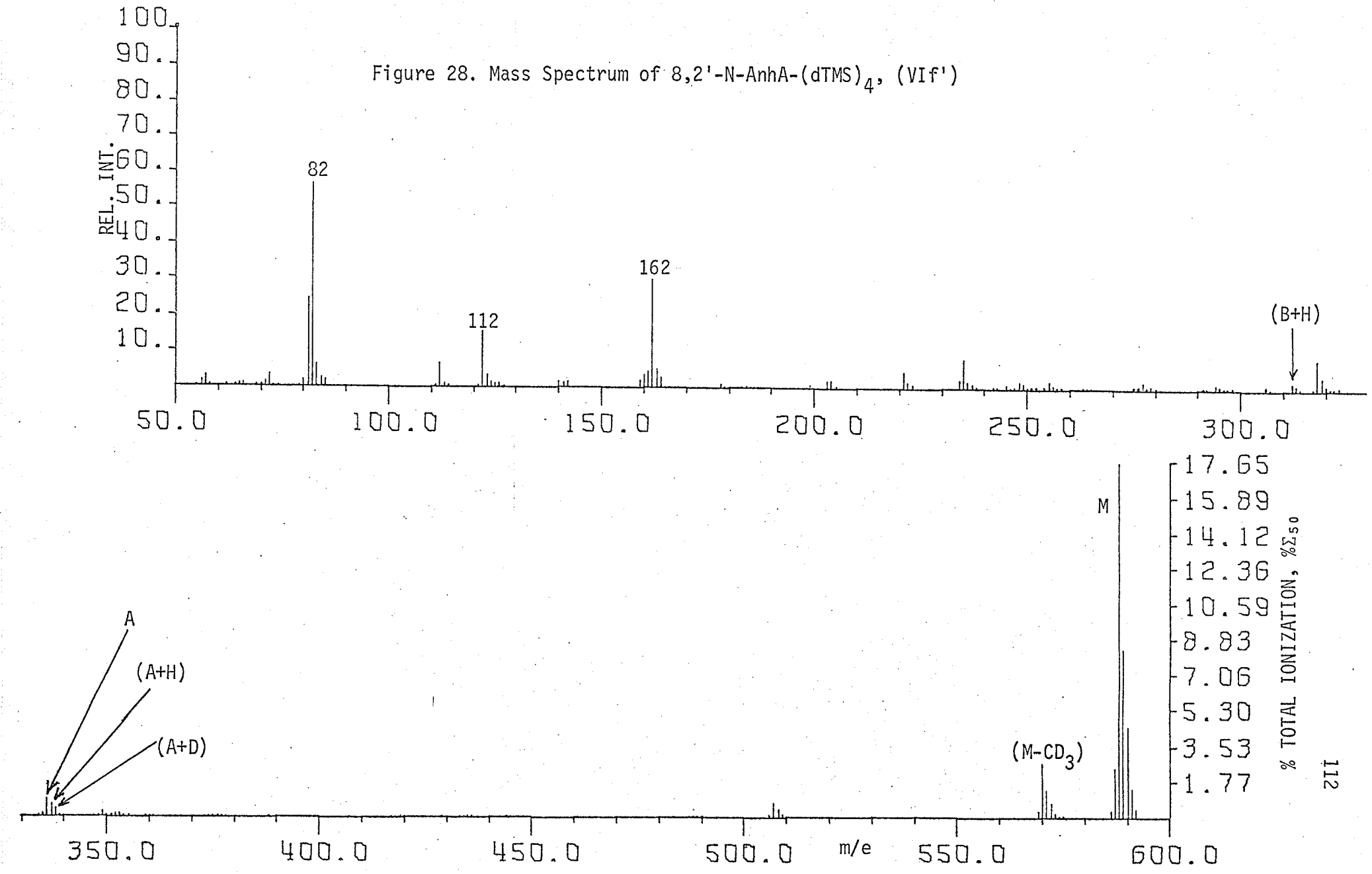


Figure 27. Mass Spectrum of 8,2'-N-AnhA-$(TMS)_4$, (VIf)

Figure 28. Mass Spectrum of 8,2'-N-AnhA-$(dTMS)_4$, (VIf')



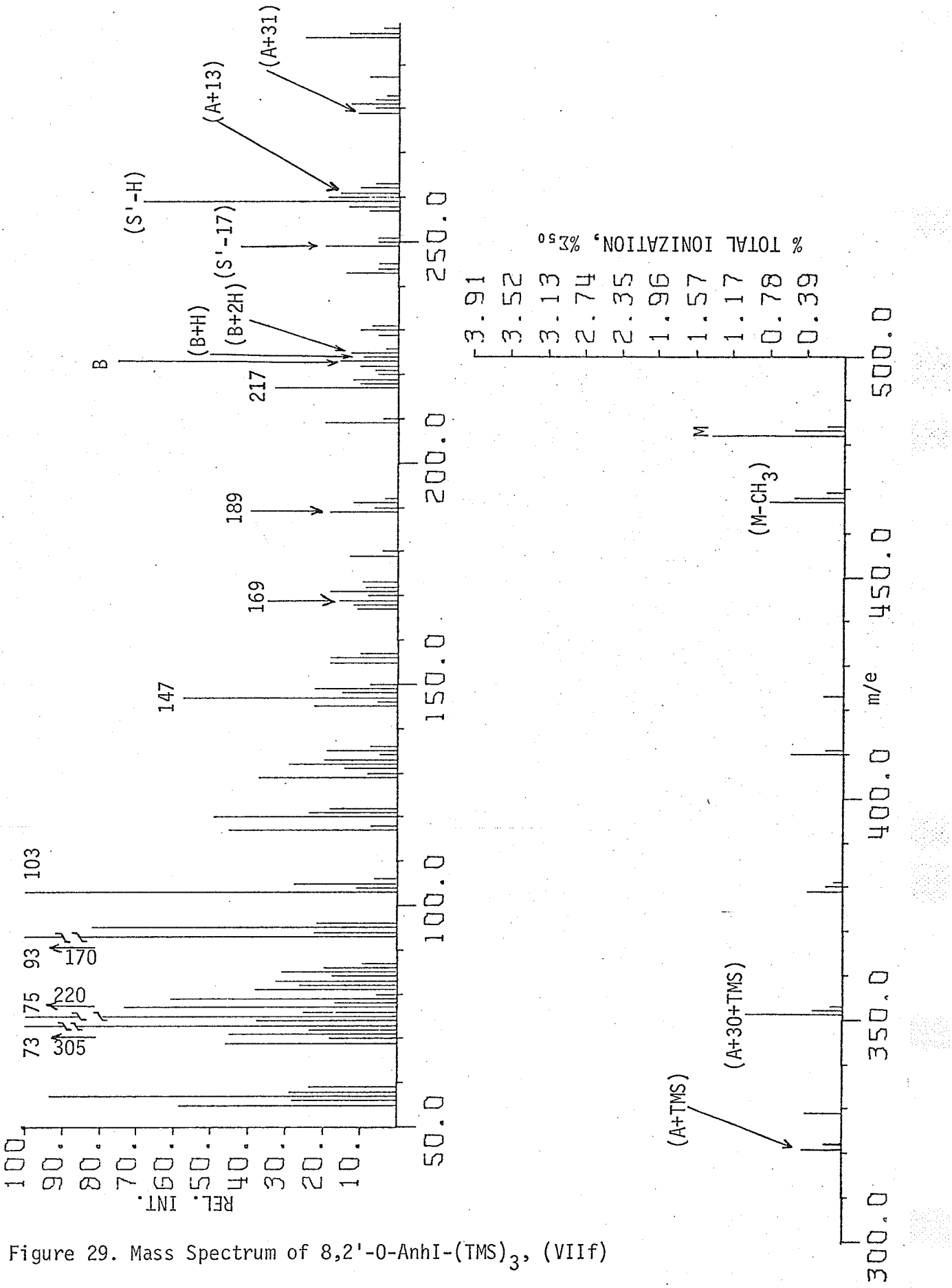


Figure 29. Mass Spectrum of 8,2'-O-AnhI-$(TMS)_3$, (VIIf)

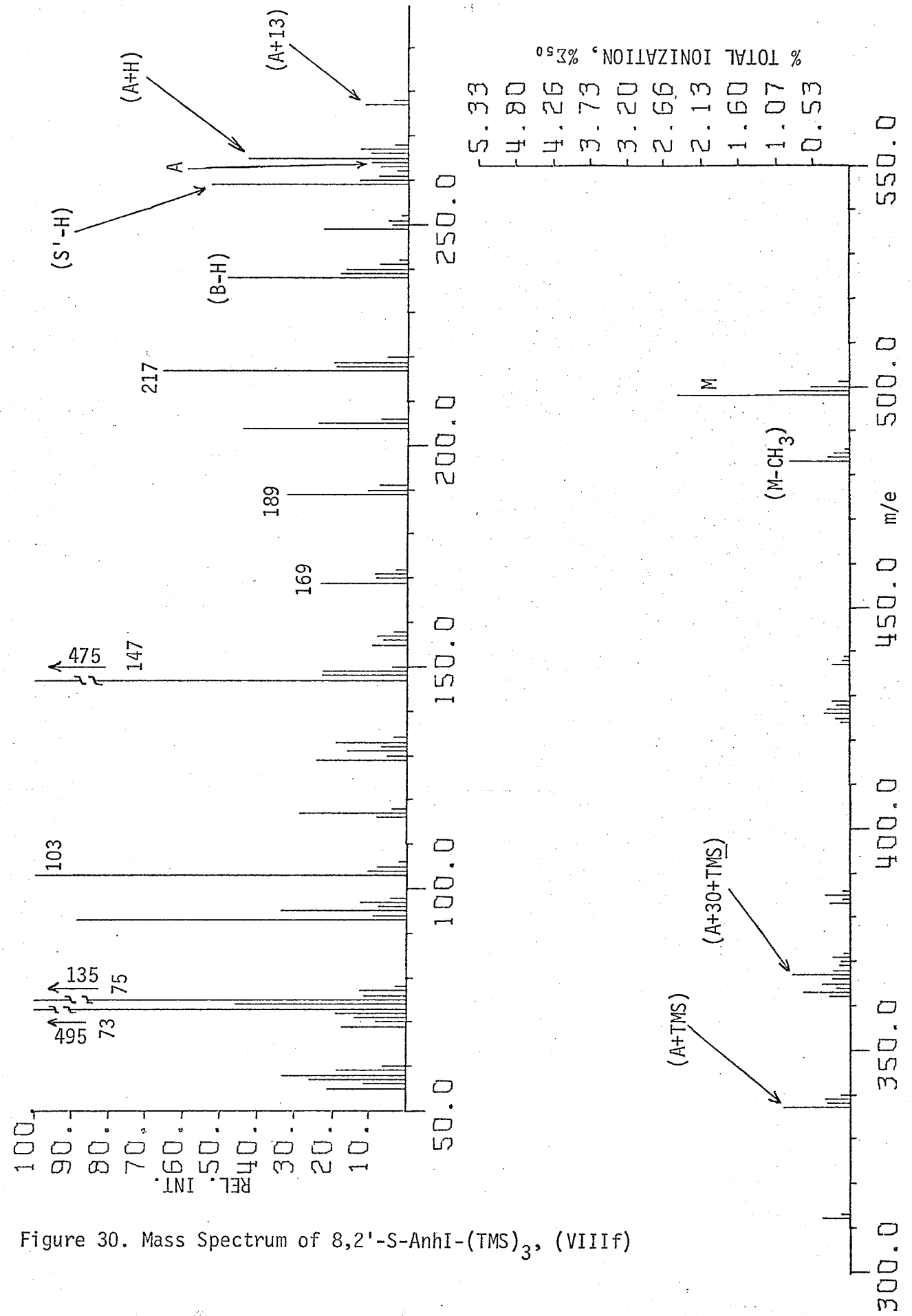


Figure 30. Mass Spectrum of 8,2'-S-AnhI-$(TMS)_3$, (VIIIf)

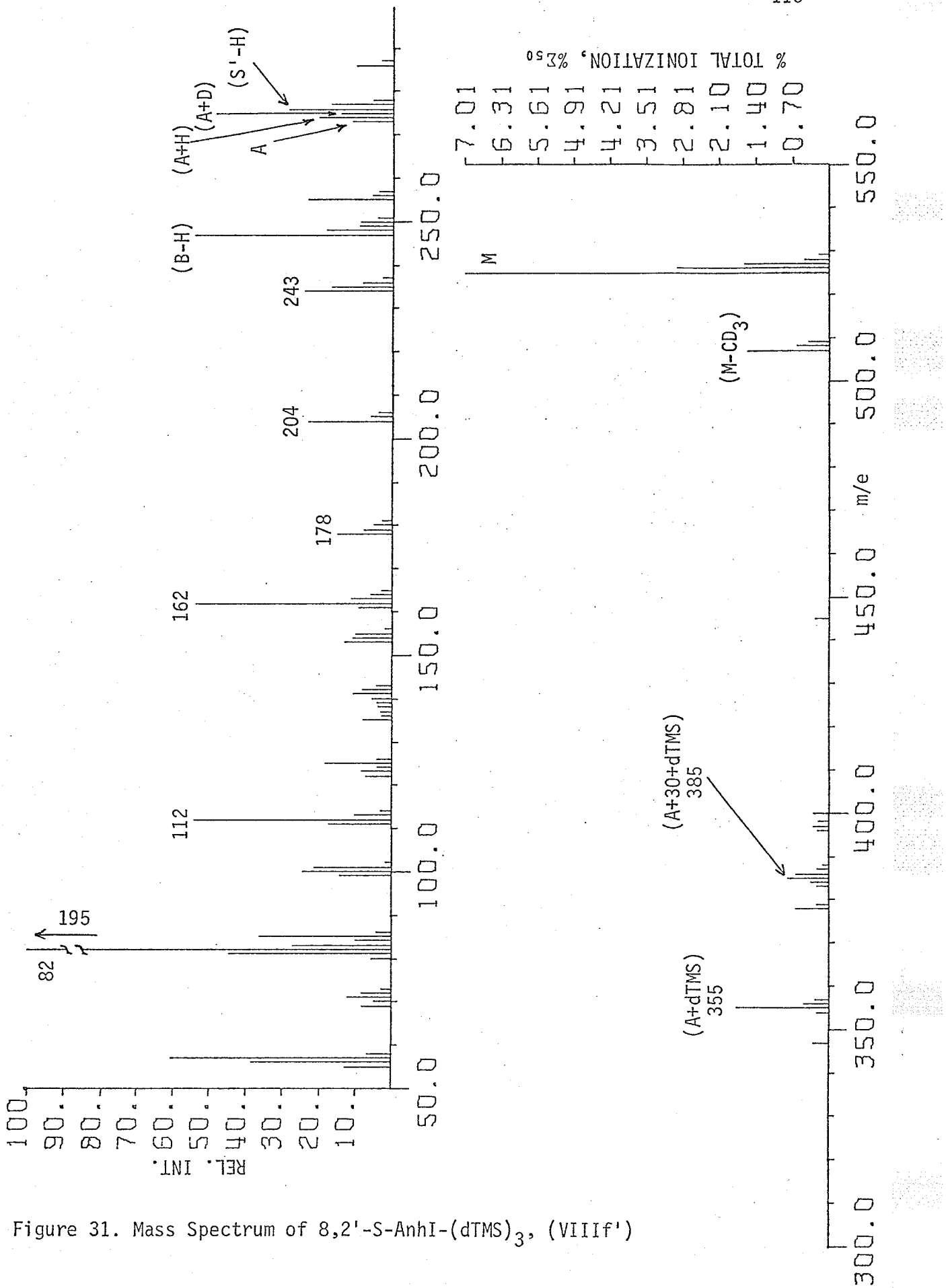


Figure 31. Mass Spectrum of 8,2'-S-AnhI-$(dTMS)_3$, (VIIIf')

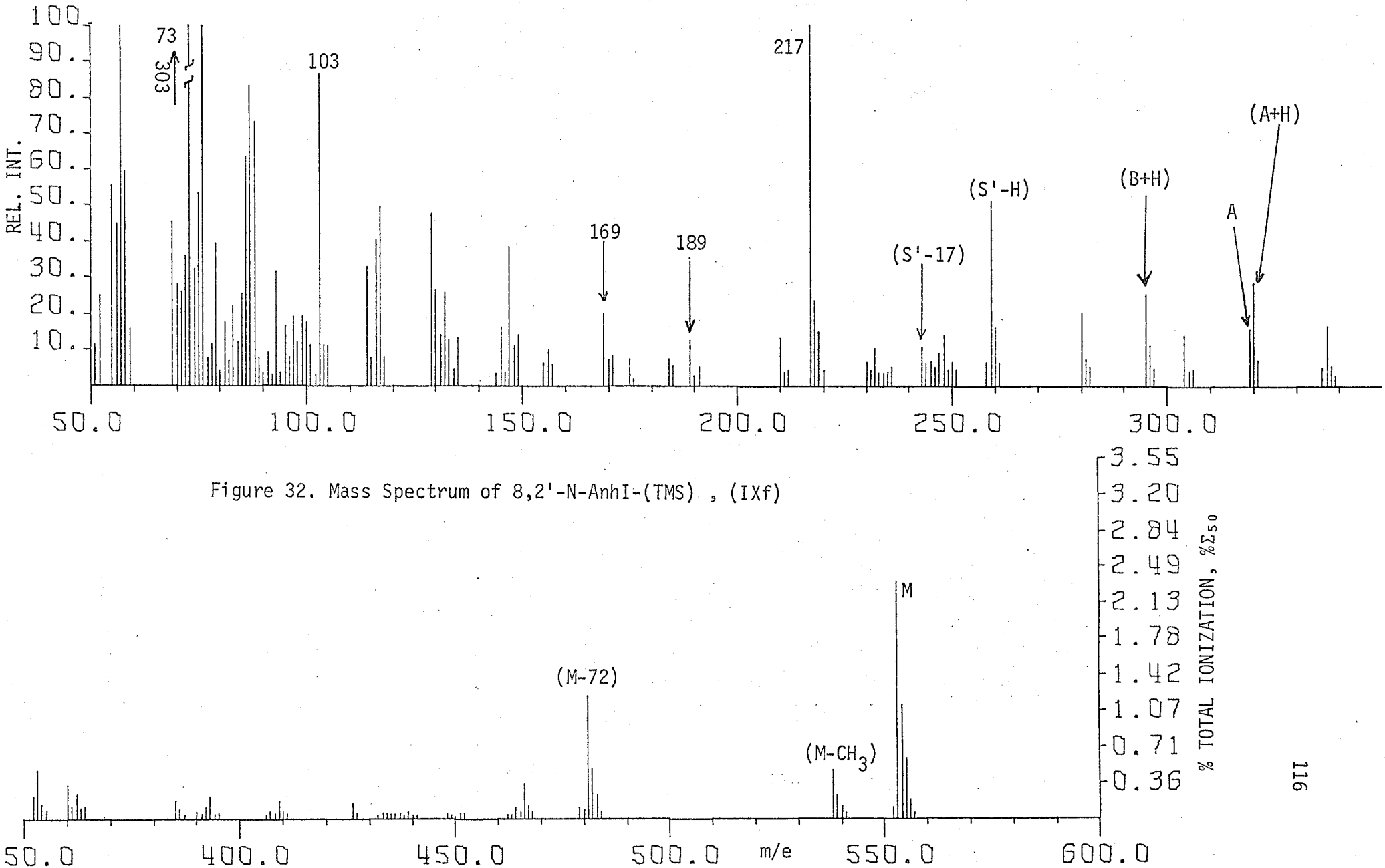


Figure 32. Mass Spectrum of 8,2'-N-AnhI-(TMS) , (IXf)

Figure 33. Mass Spectrum of 8,2'-N-AnhI-$(dTMS)_4$,(IXf')

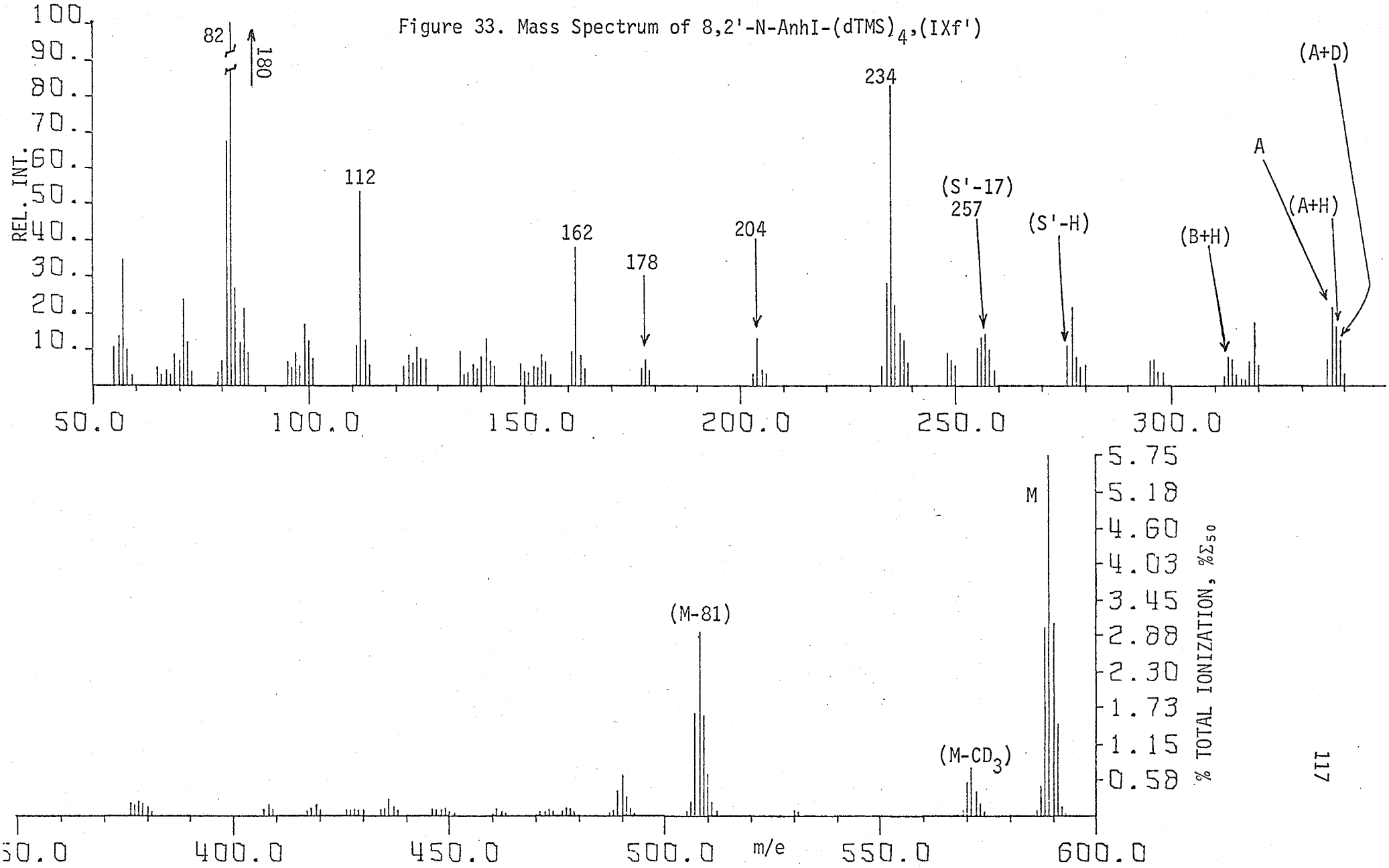


Figure 34. Mass Spectrum of 8,2'-O-AnhG-$(TMS)_4$, (Xf)

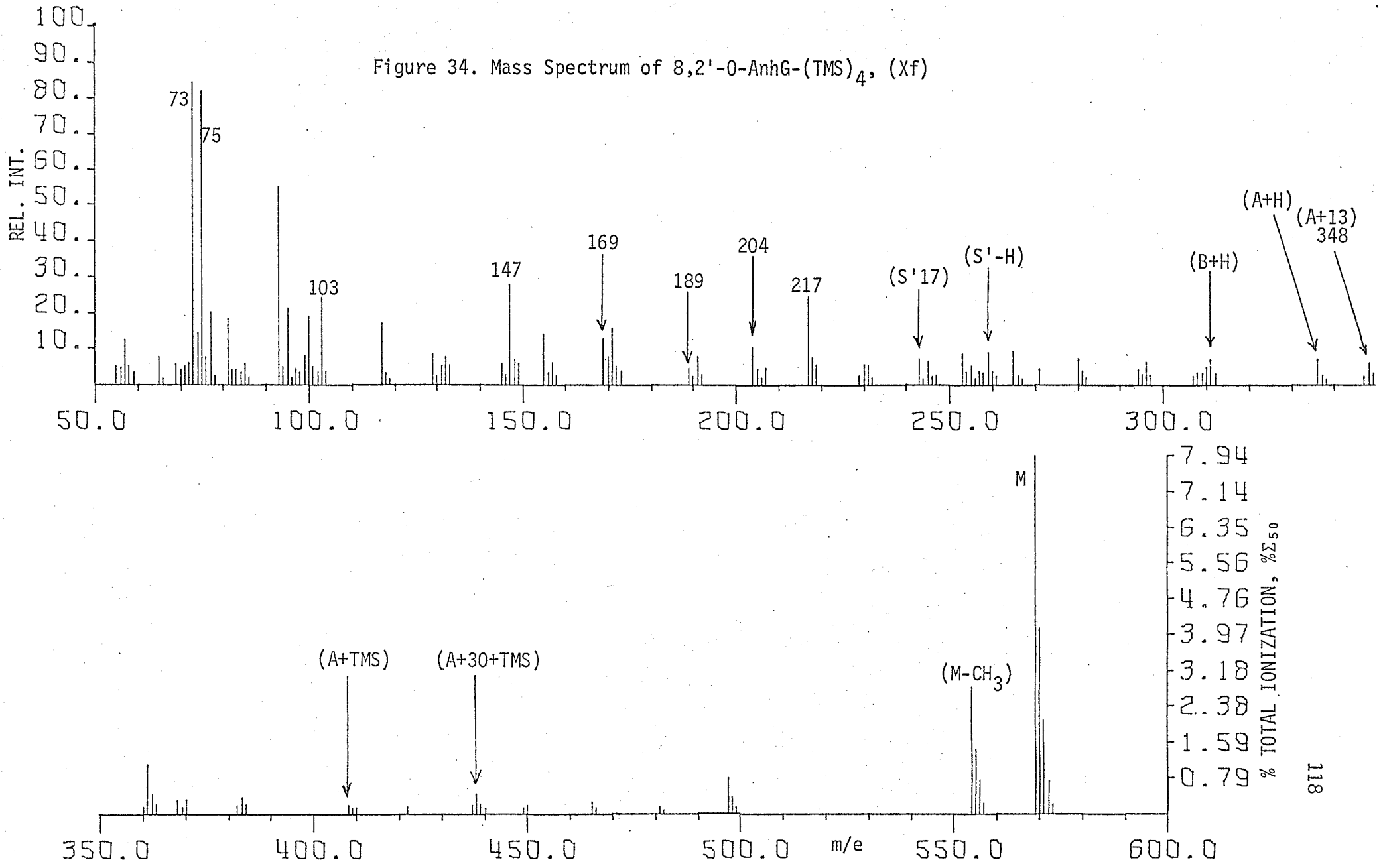
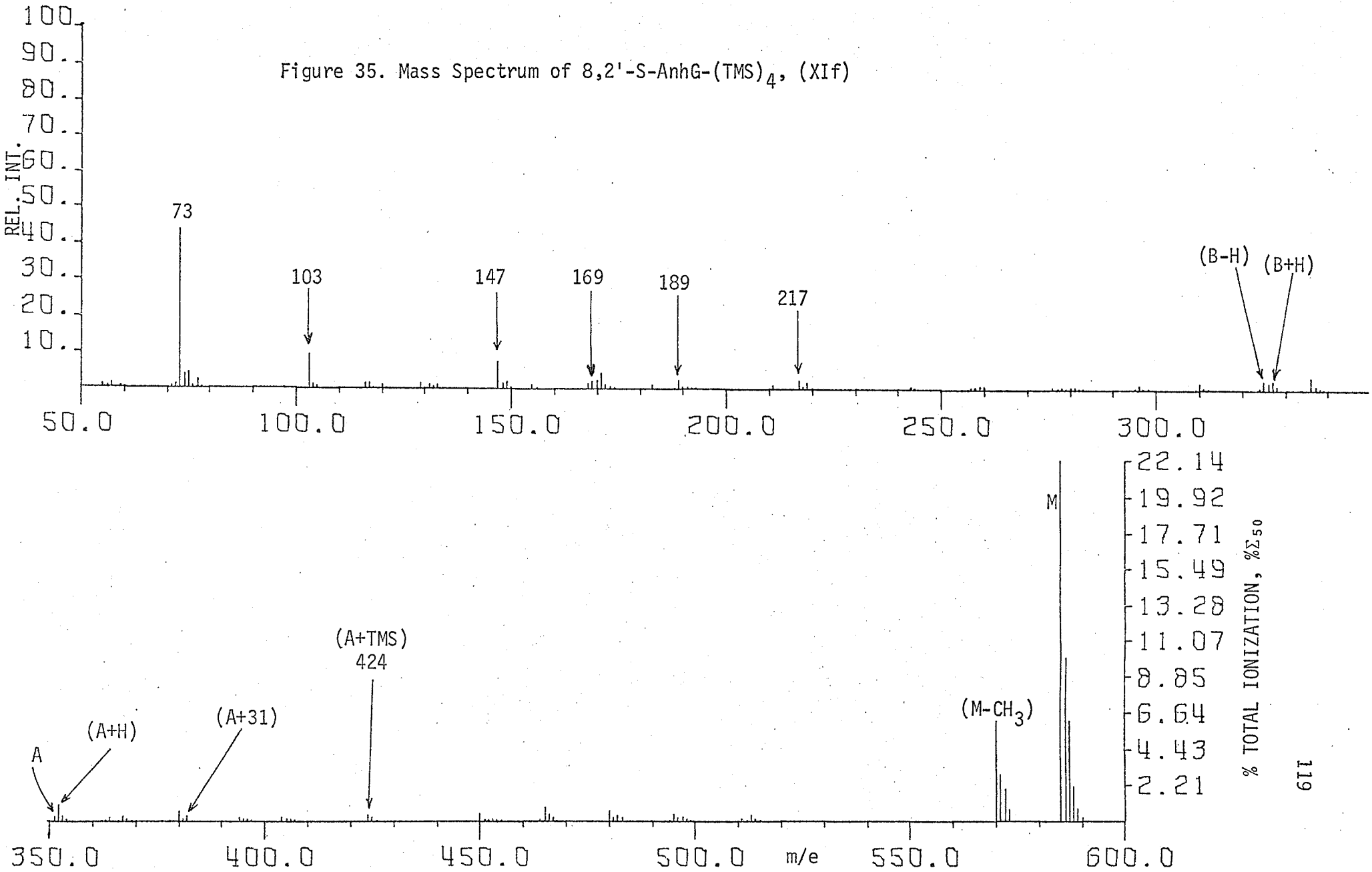


Figure 35. Mass Spectrum of 8,2'-S-AnhG-$(TMS)_4$, (XIf)



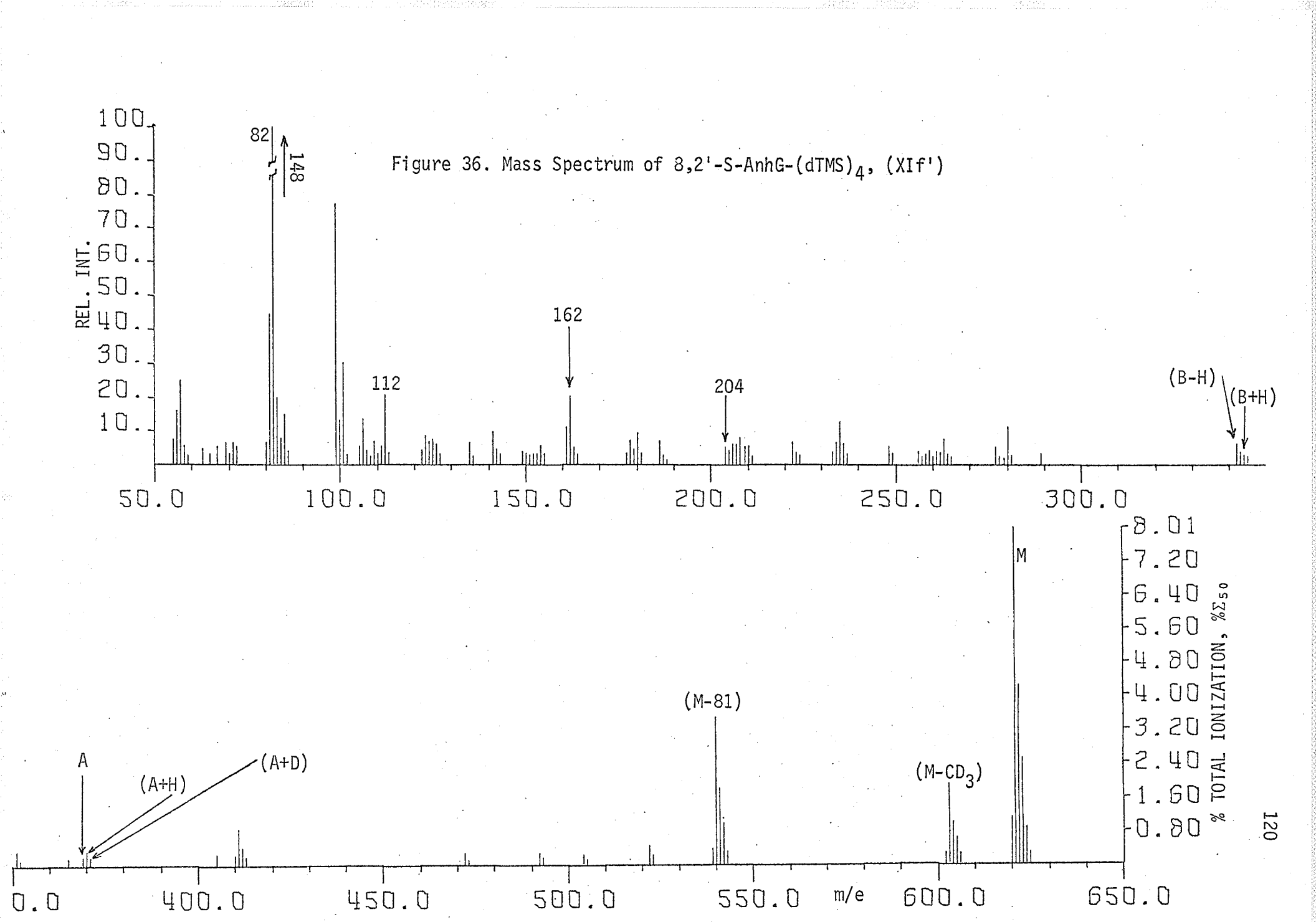


Figure 36. Mass Spectrum of 8,2'-S-AnhG-$(dTMS)_4$, (XIf')

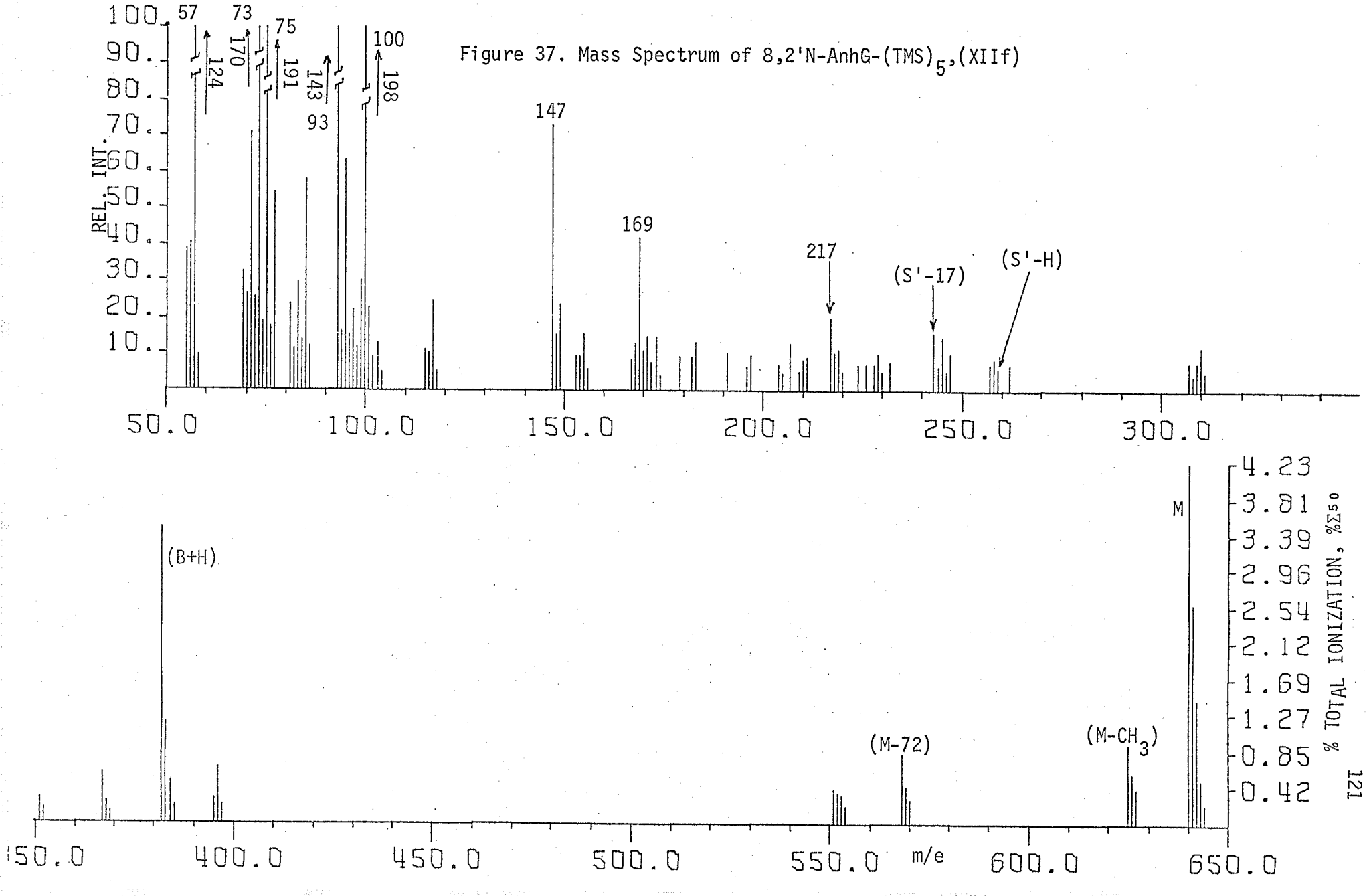


Figure 37. Mass Spectrum of 8,2'N-AnhG-$(TMS)_5$,(XIIf)

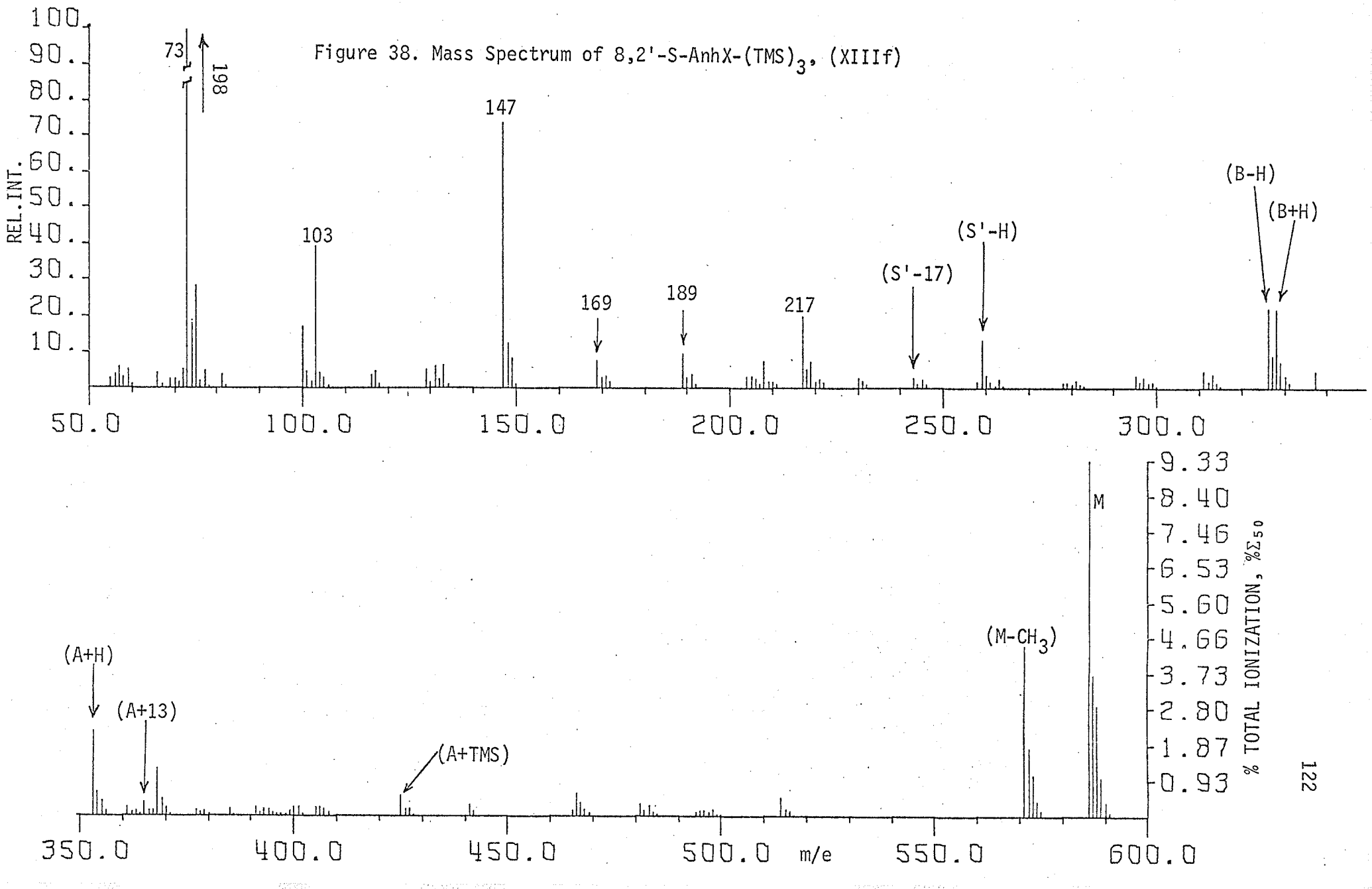
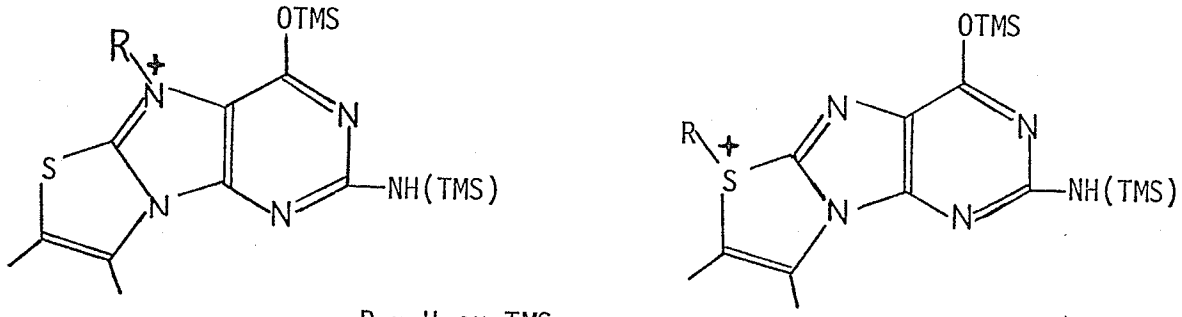


Figure 38. Mass Spectrum of 8,2'-S-AnhX-$(TMS)_3$, (XIIIf)

purine 8,2'-anhydronucleosides. Migration of hydrogen or the TMS group leads to structural analogues to those of the pyrimidine compounds, as illustrated for 8,2'-S-AnhG-$(TMS)_4$, XIf:



R = H or TMS

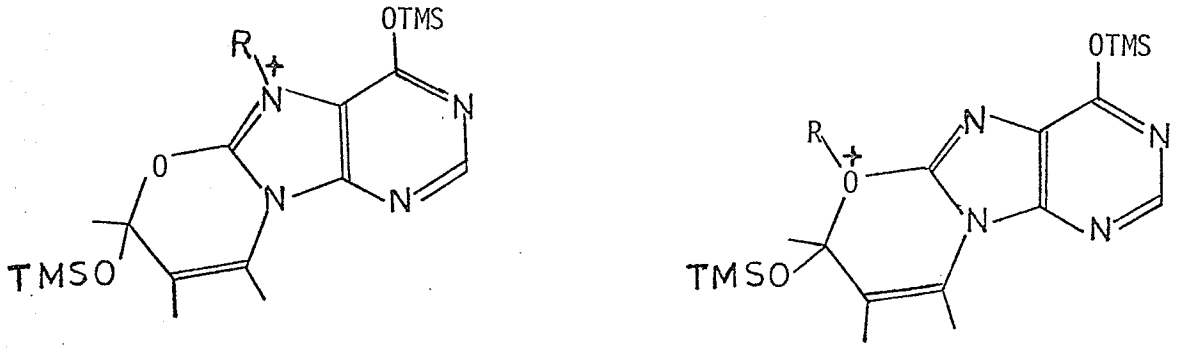
Comparison of the spectra of protium and deuterium-labelled TMS derivatives shows that hydrogen transfer can occur from one or either of the TMS groups, or from the ribose skeleton.

$\underline{A^+}$

This ion which was shown to be one of the most prominent ions in the mass spectrum of 8,2'-N-AnhA (VI), is present in all the spectra of the TMS derivatives of the purine 8,2'-anhydronucleosides studied, but its abundance differs substantially in different compounds. It is almost unobservable in the O-anhydro and in some of the S-anhydro compounds (below 2%, Rel. Int.) but it gives a peak of about the same intensity as $(A+H)^+$ in the N-anhydro compounds, 8,2'-N-AnhA-$(TMS)_4$, (VIf), and 8,2'-N-AnhI-$(TMS)_4$, (IXf). (The spectrum of 8,2'-N-AnhG-$(TMS)_5$ is dominated by M^+, $(M-15)^+$ and $(B+H)^+$ ions.)

$(A+31)^+$ and $(A+30+TMS)^+$

These ions are of generally low abundance in the mass spectra of the TMS derivatives of the purine 8,2'-anhydronucleosides except for those of 8,2'-O-AnhA (IV), 8,2'-O-AnhI (VII) and 8,2'-S-AnhI (VIII). Two requirements have been proposed earlier for the formation of these ions: (i) ring expansion to incorporate the C-3' atom into a six-membered ring and (ii) migration of H or TMS to the base moiety or heteroatom of the anhydro-linkage to give well-stabilized ions; for example, for VIIf the structures for these ions are:



R = H or TMS

The trends in the relative intensities of these ions in the mass spectra suggest that the reactions leading to these ions occur more readily when O rather than S or NH is present in the anhydro-linkage.

It should be noted that the $(A+31)^+$ and $(A+30+TMS)^+$ ions can be formed from the purine 8,3'-anhydronucleosides without the necessity for ring expansion. Comparison of the relative intensities of these ions and those of the $(A+H)^+$ and $(A+TMS)^+$ ions may form the basis of

a distinction between the 2' and 3' linked isomers. Formation of $(A+H)^+$ and $(A+TMS)^+$ from 3' linked compounds would require ring contraction and their formation should represent an unfavorable fragmentation process.

$(A+13)^+$

This ion is significant in all the spectra studied except for those of the thio and amino-anhydroguanine compounds where the spectra are dominated by M^+, $(M-15)^+$and $(B+H)^+$ ions. Its formation would be by the pathway proposed in the discussion of IV, V and VI. Mass shifts observed in the mass spectra of the dTMS derivatives support the proposed composition of this ion.

The ring expansion invoked for the formation of this ion appears to occur more readily for O-anhydro than for the NH- or S- anhydro compounds. This trend parallels that found for the formation of the $(A+31)^+$ and $(A+30+TMS)^+$ ions. This ion may also be expected to be of enhanced intensity in the spectra of 8,3'-anhydronucleosides.

$(B-H)^+$, B^+, $(B+H)^+$, $(B+2H)^+$

Comparison of the relative intensities of the ions in this group reveals that hydrogen transfer to the base moiety is not as extensive as for the pyrimidine analogues. In particular, $(B-H)^+$, is sometimes prominent in this group, where it is usually insignificant for the pyrimidine compounds.

$(S'\text{-}H)^+$

This ion which is characteristic of the sugar moiety, occurs at m/e = 259, and is of useful intensity in most spectra. Its sometimes extremely low intensity is probably caused by the preferred localization of charge on the purine base, especially of the guanine type. Its formation should not differ from the proposal suggested in connection with the pyrimidine analogues.

Other ions

The remaining prominent ions in the spectra, i.e. m/e = 243, 217, 189, 169, 147, 103, 75 and 73 are either ribose or TMS derived fragments.

Trimethylsilyl derivatives of purine 8,3'-anhydronucleosides

In the previous section the possibility of distinguishing purine 8,3'-anhydronucleosides from their 8,2'-isomers was discussed. The 8,5'-isomers can be easily identified[35] by the absence of the 5'-hydroxyl group and therefore the absence or low intensity of peak at m/e = 103 in the mass spectra of the TMS derivatives. A few of the purine 8,3'-anhydronucleosides were available and to minimize their thermal rearrangements if any, their TMS derivatives were studied. The mass spectra of 8,3'-O-AnhA-$(TMS)_3$, XIVf; 8,3'-S-AnhA-$(TMS)_3$, XVf; 8,3'-O-AnhI-$(TMS)_3$, XVIf; 8,3'-S-AnhI-$(TMS)_3$, XVIIf and 8,3'-S-AnhG-$(TMS)_4$, XVIIIf are shown in Figures 39, 40, 41, 42 and 43 respectively. Generally

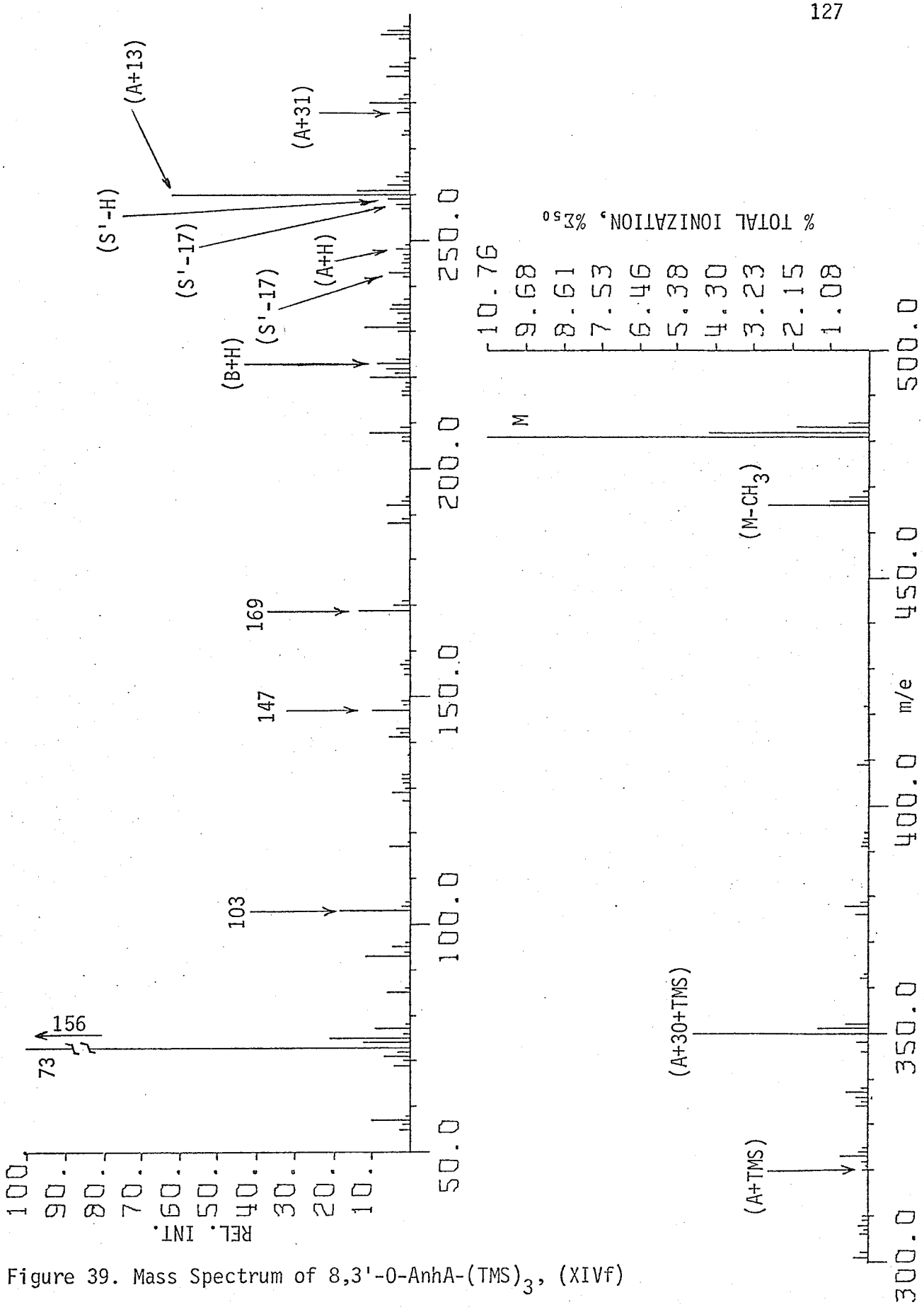


Figure 39. Mass Spectrum of 8,3'-O-AnhA-$(TMS)_3$, (XIVf)

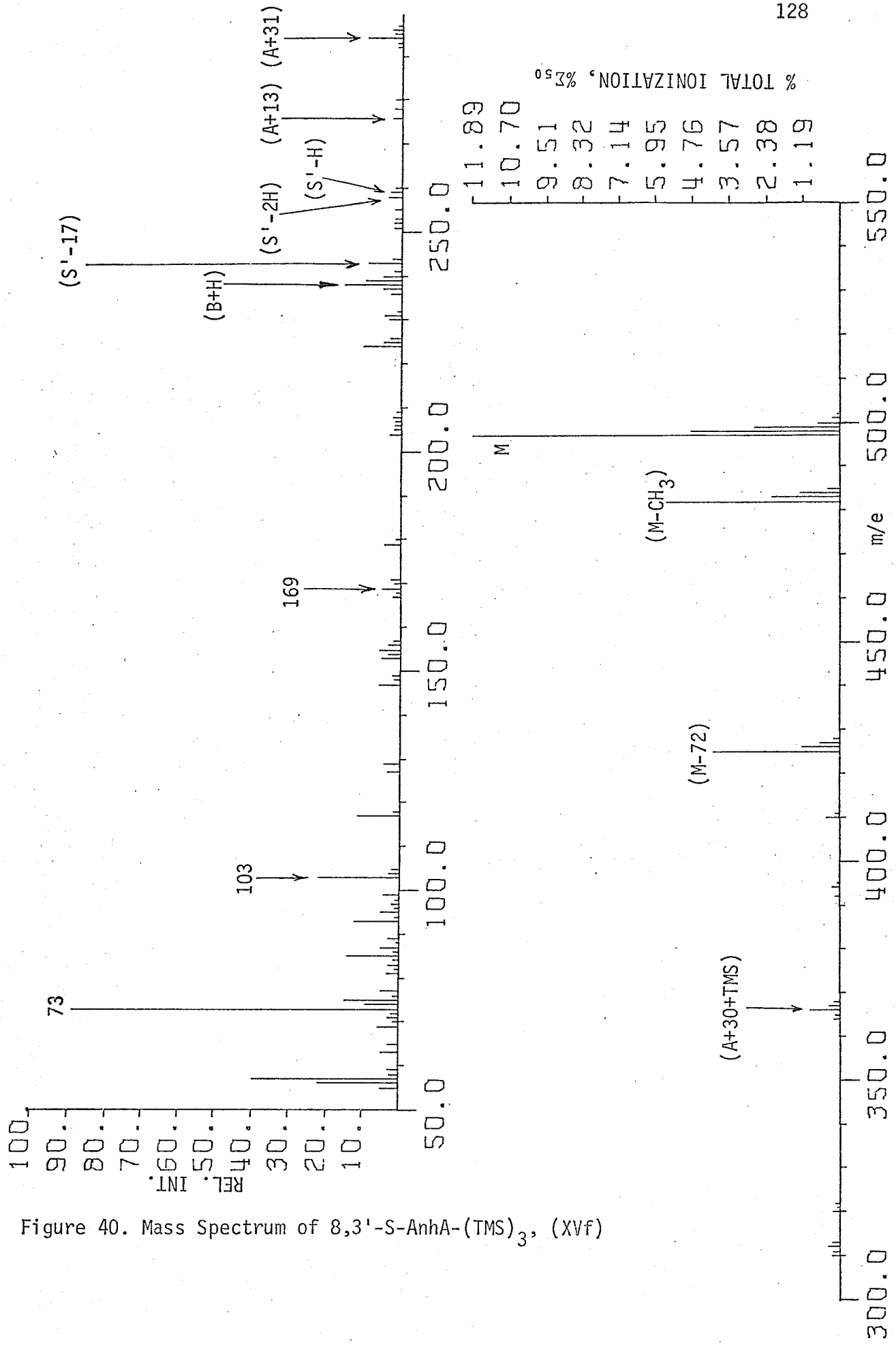


Figure 40. Mass Spectrum of 8,3'-S-AnhA-$(TMS)_3$, (XVf)

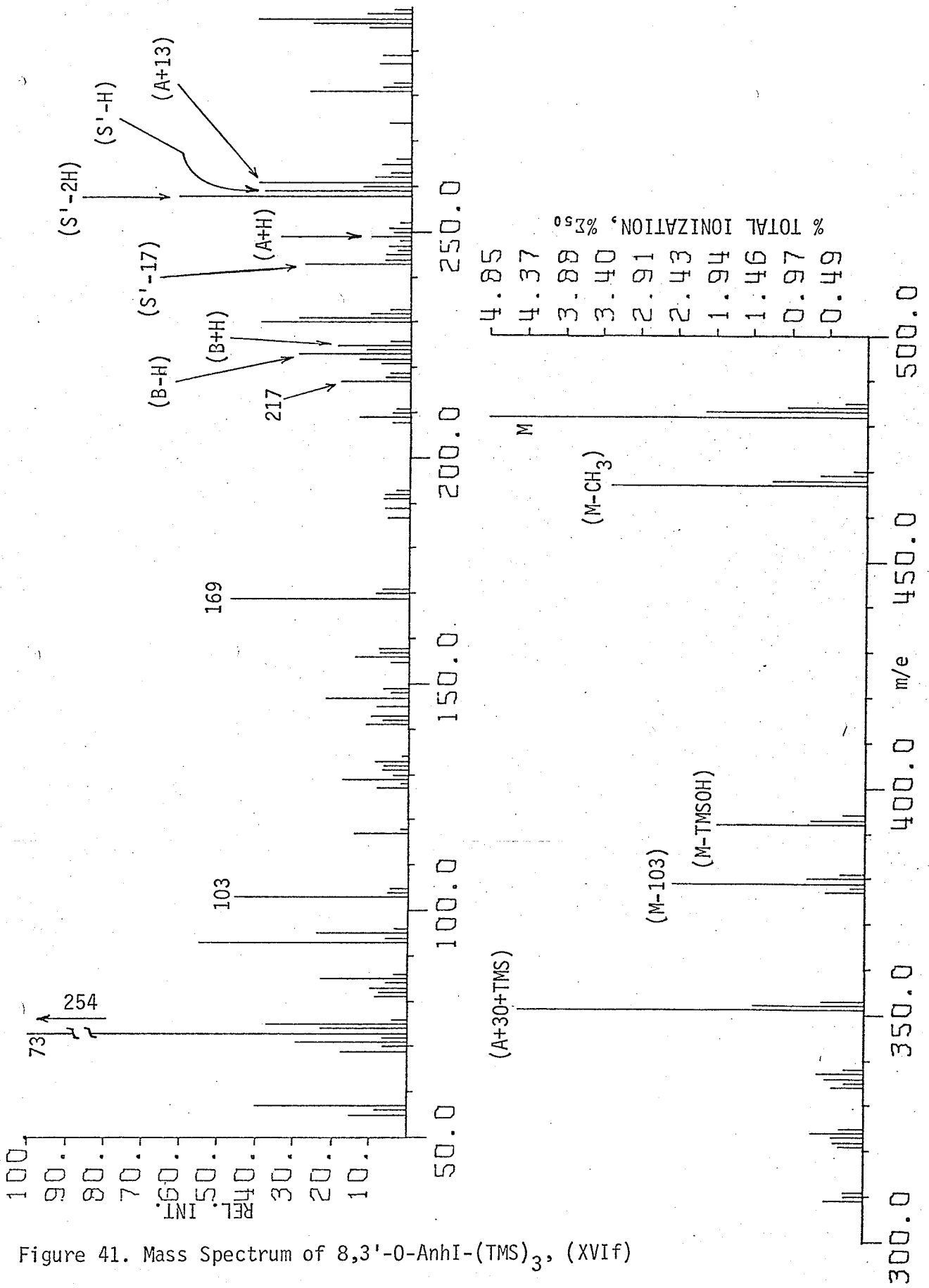


Figure 41. Mass Spectrum of 8,3'-O-AnhI-$(TMS)_3$, (XVIf)

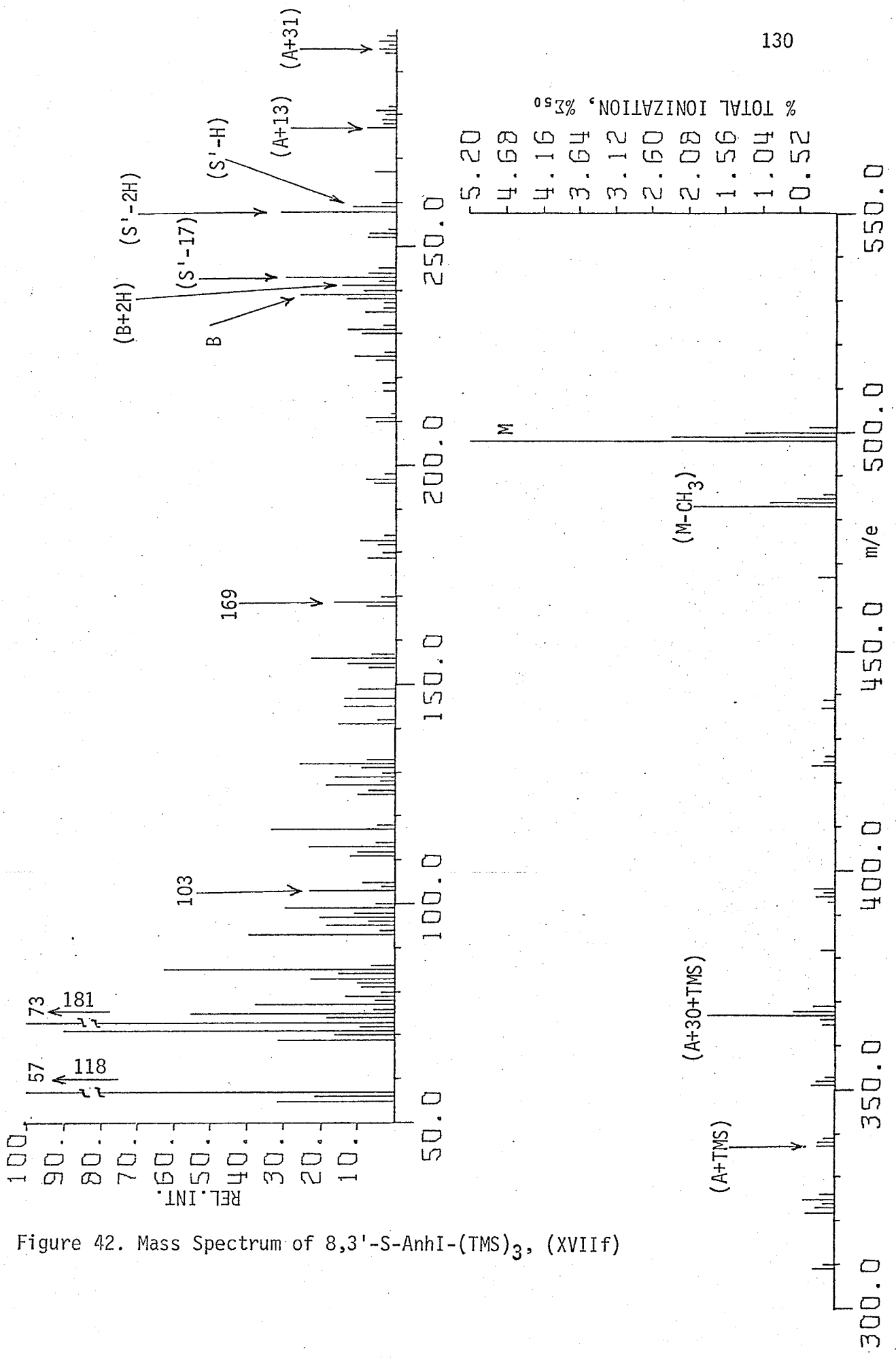
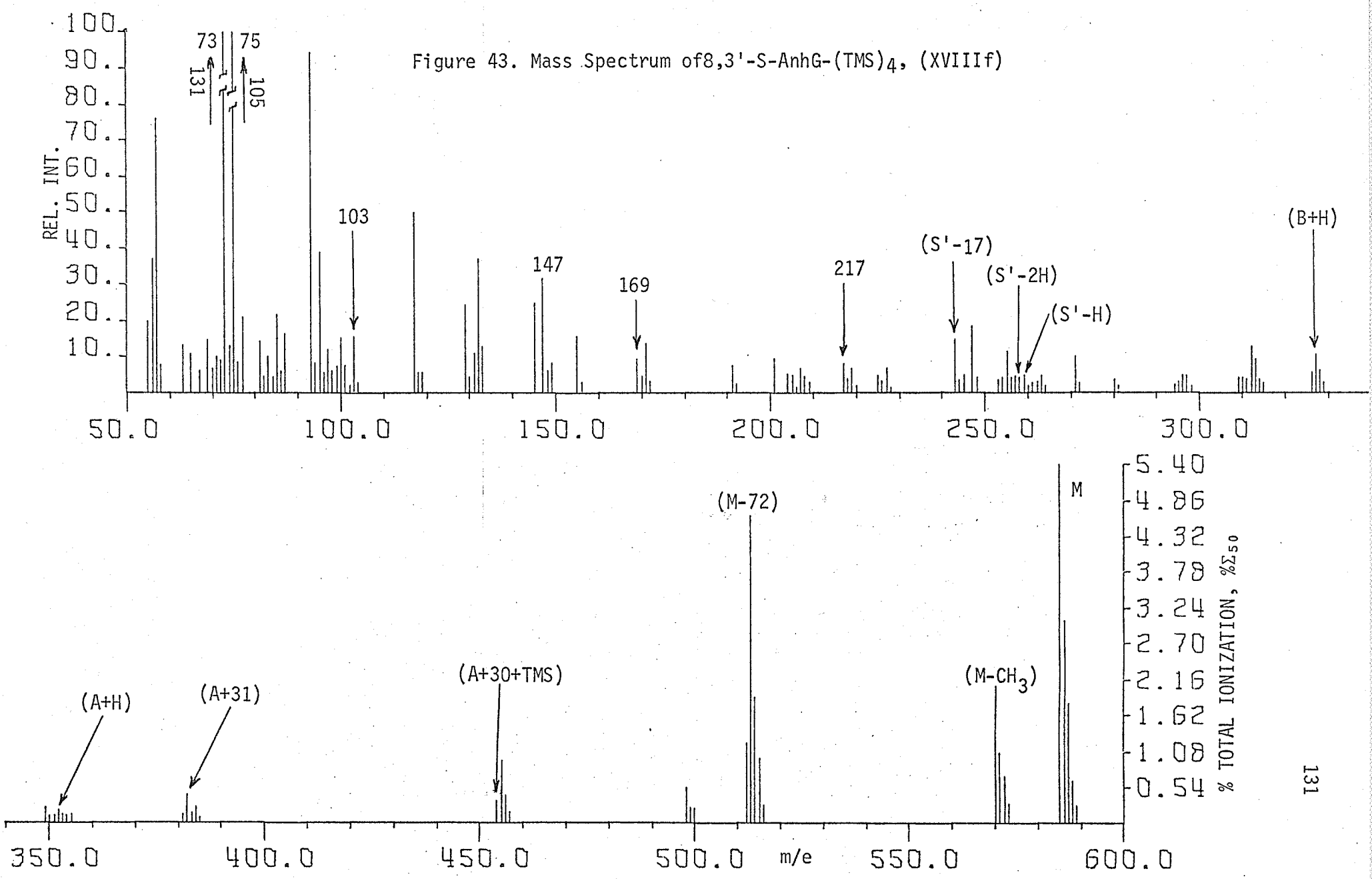


Figure 42. Mass Spectrum of 8,3'-S-AnhI-$(TMS)_3$, (XVIIf)

Figure 43. Mass Spectrum of8,3'-S-AnhG-$(TMS)_4$, (XVIIIf)



the proposals made as to how the 8,2'- and 8,3'- isomers can be distinguished are substan iated. The significant points of differences will be discussed individually. The other ions in the spectra of 8,3'- isomers can easily be explained with mechanisms analogous to their 8,2'- isomers. The $(M-72)^+$ ion present in XVf and XVIIIf is suspected to be caused by hydrolysis in the ion source.

$(A+H)^+$, $(A+TMS)^+$

The formation of these ions have been anticipated to be of lower abundance in the 8,3'- isomers since in these cases, unfavorable ring contractions are required. Indeed, they are either absent or of lower abundance in the 8,3'- isomers (See TABLE III).

$(A+31)^+$, $(A+30+TMS)^+$, $(A+13)^+$

These ions are generally of higher intenities, with some exceptions, in the 8,3'-isomers than in the 8,2'-isomers (See TABLE III). Their formation is rendered more favorable because of the presence of six-membered anhydro-linkage whereas in the 8,2'- isomers a ring expansion mechanism is required. This ring expansion process seems to be more easily achieved in the 8,2'-O-anhydro compounds; however, the intensity of this peak in these O-anhydronucleosides is still lower than that of their 8,3'-O- isomers.

$(S'-2H)^+$, $(S'-17)^+$

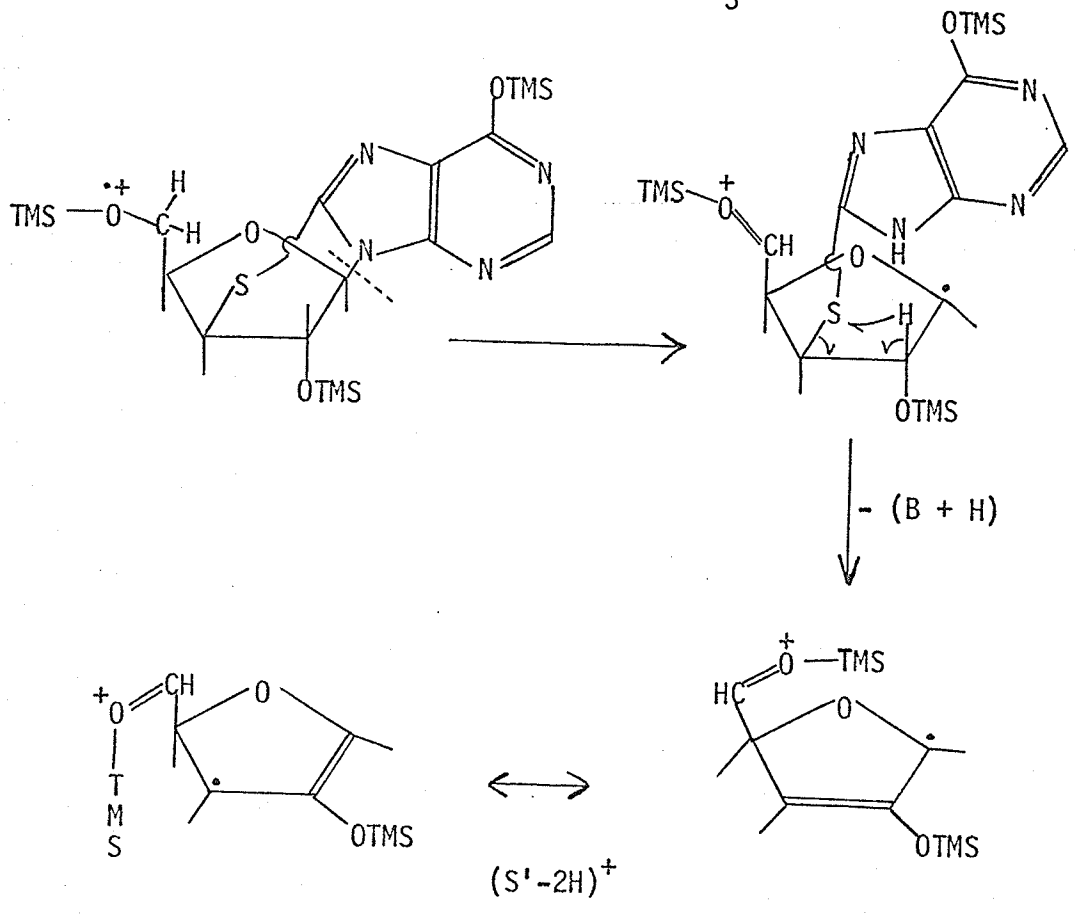
The ion $(S'-2H)^+$, with m/e value = 258 was absent or usually not

TABLE III

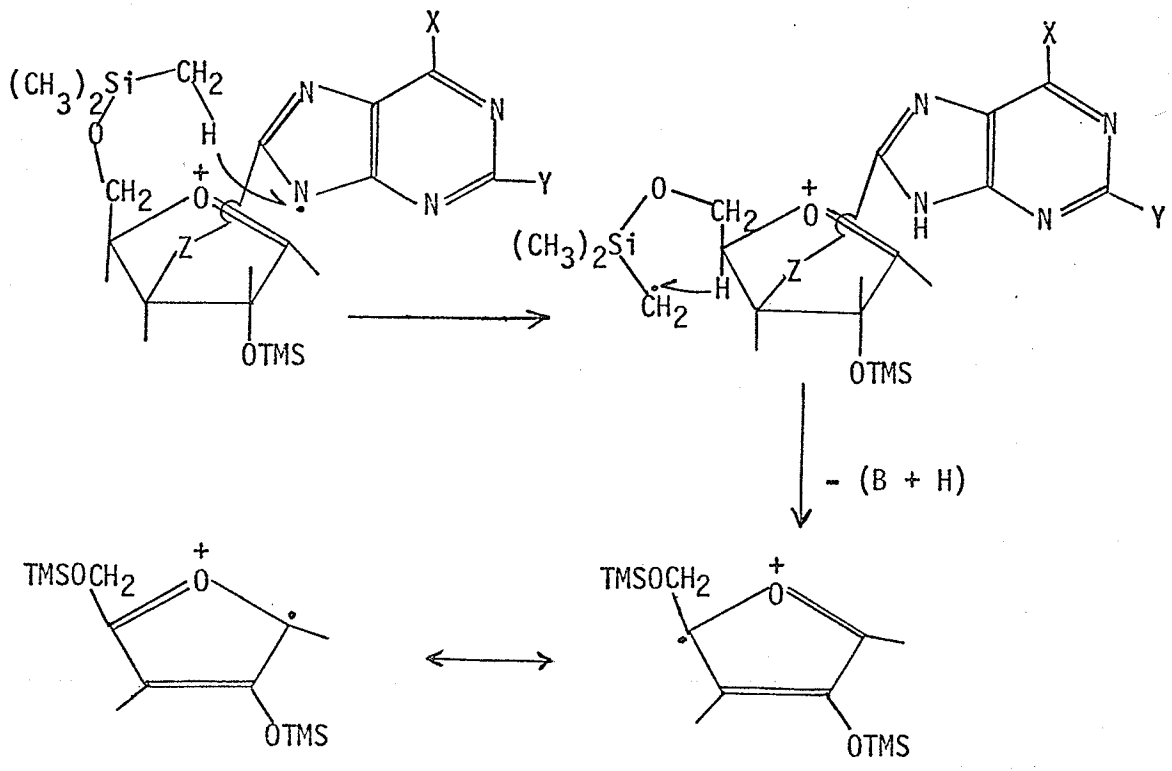
Comparison of relative intensities of important ions between the TMS derivatives of purine 8,2'-anhydro and purine 8,3'-anhydro nucleosides. The data presented are: (m/e); Rel. Int. for 8,2'-isomer: Rel. Int. for 8,3'-isomer.

	O-AnhA	S-AnhA	O-AnhI	S-AnhI	S-AnhG
$(A+H)^+$	(248) 7.9:3.5	(264) 15.4:0	(249) 19.7:11.1	(265) 42.7:0	(352) 4.6:3.6
$(A+TMS)^+$	(320) 3.1:1.3	(336) 2.8:0	(321) 10.2:0	(337) 18.3:4.6	(424) 1.7:0
$(A+13)^+$	(260) 29.0:61.5	(276) 6.5:2.5	(261) 15.7:39.8	(277) 11.5:7.9	(364) 0:0
$(A+31)^+$	(278) 2.2:3.3	(294) 0:9.4	(279) 11.0:0	(295) 0:4.6	(382) 1.6:8.0
$(A+30+TMS)^+$	(350) 18.5:45.2	(366) 3.7:8.1	(351) 25.9:91.8	(367) 15.5:34.6	(454) 0:6.3
$(S'-2H)^+$	(258) 4.9:3.2	(258) 1.7:3.6	(258) 13.3:60.8	(258) 0:31.2	(258) 0.4:4.4
$(S'-H)^+$	(259) 14.3:5.9	(259) 12.7:3.0	(259) 68.5:38.5	(259) 52.6:11.7	(259) 0.9:5.2
$(S'-17)^+$	(243) 7.3:5.5	(243) 3.0:9.0	(243) 14.2:28.0	(243) 0:29.6	(243) 0:14.8

prominent in the mass spectra of the TMS derivatives of the purine 8,2'-anhydronucleosides but it is present in all the spectra of the 8,3'-isomers. The peak at m/e = 258 is usually of higher intensity than the peak for $(S'-H)^+$ with m/e = 259, whereas the reverse is true for the 8,2'-isomers. $(S'-2H)^+$ has also been reported to be present in 2,3'-O-anhydrouridine-$(TMS)_2$[35], in agreement with results of this author, but no structure for this ion was proposed. In the absence of detailed labelling studies the formation of this ion is ambiguous and at least two logical routes to its formation can be suggested. For the first mechanism molecular models show that N-9 can come into close proximity to H-5' once the glycosidic bond has been cleaved. Thus the formation of $(S'-2H)^+$ can be envisaged by the initial localization of the positive charge on O-5' and fission of the glycosidic bond. For example, for 8,3'-S-AnhI-$(TMS)_3$, XVIIf:

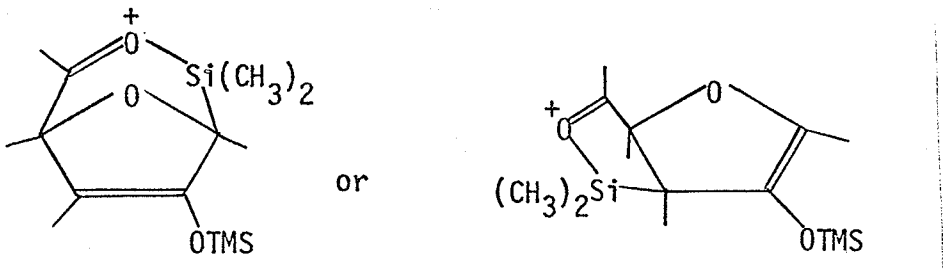


All of the TMS hydrogens are retained in the ion structure in this route. To account for the observation that one hydrogen atom from a TMS group is lost in the formation of the ion it can either be assumed that exchange of a TMS hydrogen with a hydrogen elsewhere in the molecule occurs[35] or an alternative mechanism can be proposed:



In either mechanism a stable neutral molecule, the purine base, is eliminated. Very similar mechanisms can be proposed for the formation of m/e = 258 for 2'-anhydronucleosides. Any of the above $(S'-2H)^+$ ion structures are in a favorable configuration to lose $\cdot CH_3$ for the formation of the $(S'-17)^+$ ion whose intensity is enhanced in the mass

spectra of the 8,3'-isomers; so apart from the structures proposed for $(S'-17)^+$ for the 8,2'-isomers, the following ions may contribute to the intensity of the peak at m/e = 243:



A metastable ion for the loss of $\cdot CH_3$ from $(S'-2H)^+$ was detected for 2,3'-AnhU-$(TMS)_2$[35].

As the results in TABLE III show, the general trends can be used to distinguish between the TMS derivatives of purine-8,3'-anhydro and purine-8,2'-anhydro nucleosides but caution must be exercised and all the ions listed in the table should be considered. This is especially so for the O-anhydro-adenosines where the 8,2'-isomer seems to readily undergo ring expansion.

CHAPTER FIVE: CONCLUSION

The mass spectra studied in the previous two chapters clearly indicate that the 2'-anhydronucleosides and their derivatives can be characterized by mass spectrometry. Furthermore, the study of the fragment ions points out that they carry structural information. The spectra themselves are different from those of natural nucleosides in that the anhydro-linkages remain intact in a lot of the diagnostically inportant ions namely M^+, $(M-31)^+$, A^+, $(A+H)^+$, $(A+13)^+$, $(A+31)^+$, $(A+TMS)^+$, $(A+30+TMS)^+$, C^+ and $(F+H)^+$. Other prominent ions include base or sugar moiety containing ions such as $(B-H)^+$, B^+, $(B+H)^+$, $(B+2H)^+$, $(S'-H)^+$, $(S'-2H)^+$, $(S'-17)^+$ and T^+. There are still other ions which are specific for particular substituents for example $(M-CH_3CO_2)^+$ for a 3'-acetyl group.

The information gathered in this study confirms the structures of known anhydronucleosides; checks their purity and will assist the synthetic chemist in identifying structures of novel anhydronucleosides which may be produced as a result of a variety of reactions of less well characterized nature. The ability of mass spectrometry to distinguish the position of substituents on the sugar moiety of anhydronucleosides as demonstrated by various synthetically related derivatives of 2'AnhU should be useful since the usual methods of UV, IR and paper chromatography (Rf values) are sometimes not sufficient to identify them. Further this study indicates that a

similar investigation extended to natural nucleoside derivatives may prove fruitful.

APPENDIX

PROGRAM FOR PLOTTING MASS SPECTRA

```
C     MASSPLOT
C
C     PROGRAM TO CALCULATE AND PLOT A NORMALIZED MASS SPECTRUM
C
C      INPUT PARAMETERS:
C     CARD #1. FORMAT: (2I1,I4,10A4)
C         PARAMETER 1: INTEGER DENOTING MASS SPECTROMETER USED AND
C               TREATMENT OF THE SPECTRUM
C           0 - HITACHI RMU-6D, ACTUAL SPECTRUM
C           1 - 1015 QUADRUPOLE MASS SPECTROMETER, ACTUAL SPECTRUM AND
C               SPECTRUM NORMALIZED TO CONSTANT SENSITIVITY
C           2 - 1015 QUADRUPOLE, SPECTRUM NORMALD TO CONSTANT SENSITIVIT
C           3 - 1015 QUADRUPOLE, ACTUAL SPECTRUM
C         PARAMETER 2: INTEGER DENOTING FORMAT OF DATA INPUT
C           0 - FORMAT A, SEE CARD #3
C           1 - FORMAT B, SEE CARD #3
C           2 - FORMAT C, SEE CARD #3
C           3 - FORMAT D, SEE CARD #3
C         PARAMETER 3: IDENTIFICATION NUMBER OF THE SPECTRUM. IF THE
C               NUMBER IS NOT ±0 EXECUTION WILL TERMINATE
C         PARAMETER 4: TITLE OR FURTHER IDENTIFICATION OF THE SPECTRUM.
C               TITLE IS NOT LONGER THAN 40 CHARACTERS
C   EG.  119999SUPER-8-CALIFRAGIL-2-LISTICEXPLIALIDOSHA
C     CARD #2. FORMAT: (8F10.4)
C         PARAMETER 1: MASS SCALE IN UNITS OF MASS NUMBER/CM.
C         PARAMETER 2: MASS NUMBER AT WHICH PLOT IS TO BE STARTED
C         PARAMETER 3: MASS NUMBER AT WHICH PLOT IS TO BE STOPPED
C         PARAMETER 4: HEIGHT, IN CM., DESIRED OF THE PLOT
C         PARAMETERS 5, 6 AND 7: VALUES OF MSTART, MSTOP AND MAGNIFY
C               RESPECTIVELY. A MAGNIFICATION OF INTENSITIES OCCURS
C               (DURING PLOTTING) OF ALL PEAKS BETWEEN MSTART AND MSTOP,
C               BY A FACTOR MAGNFY. IF MSTART = MSTOP = 0.0 THERE IS NO
C               MAGNIFICATION.
C         PARAMETER 8: NUMBER OF PLOTS OF THE SAME SPECTRUM REQUIRED
C EG.  10.        50.       250.      15.     0.       0.     0.      3
C
C     CARD #3 ONWARDS: DATA CARDS - MAY BE SUPPLIED IN FORMAT A, B OR C
C
C FORMAT A: (16F5.1)
C               MASS NO. OBTD FROM POSN OF INTENSITY VALUE ON DATA CARD
C         PARAMETERS 1 - 20: INTENSITIES IN ORDER OF MASS, AT TWENTY HAL
C               MASS INTERVALS, FROM E.G. 50.0 TO 59.5, EVEN WHEN THERE
C               IS NO PEAK AT THAT MASS. COMPLETION OF DATA IS DENOTED
C               WHEN INTENSITY = 9999.
C EG.     26.2                  17.4 50.0           333.5
```

```
C FORMAT B: (2F10.4) MASS NUMBER AND INTENSITY OF THE PEAKS IN THIS
C              SPECTRUM.DENOTE THE AND OF THIS SERIES BY A BLANK CARD.
C EG.     16.0       10.0
C         20.1225    12.1531
C
C FORMAT C: (I1,F7.2,9F8.2)
C              MASS NO. OBTD FROM POSN OF INTENSITY VALUE ON DATA CARD
C         PARAMETER 1: INTEGER DENOTING INCREMENTS OF MASS SCALE
C           0 - INCREMENT = 1.0 MASS UNIT
C           1 - INCREMENT = 0.5 MASS UNIT
C         PARAMETERS 2 - 11: INTENSITIES, IN ORDER OF MASS, OF TEN
C              MASSES FROM E.G. 51.0 TO 60.0, OR FROM 50.5 TO 55.0,
C              FOLLOWED BY 55.5 TO 60.0, EVEN IF THERE IS NO PEAK AT
C              THAT MASS. COMPLETION OF DATA IS  DENOTED WHEN INTENSITY
C               = 9999.
C EG.  1  152.              248.              45.
C
C FORMAT D: (8F10.2)
C              MASS NO. OBTD FROM POSN OF INTENSITY VALUE ON DATA CARD
C         PARAMETERS 1 - 10: INTENSITIES IN ORDER OF MASS, AT TEN
C              MASS INTERVALS, FROM E.G. 50.0 TO 59.0, EVEN WHEN THERE
C              IS NO PEAK AT THAT MASS. COMPLETION OF DATA IS DENOTED
C              WHEN INTENSITY = 9999.
C
C    CARD #N: A BLANK  CARD WILL TERMINATE THE PROGRAM; HOWEVER IF
C               A SERIES OF CARDS FROM #1 ABOVE DOWN TO #3 IS INSERTED,
C               CONTROL WILL BRANCH TO THE BEGINNING OF THE PROGRAM.
C
      INTEGER IBUF(1000),TITLE(10),ID,I,AST(100),ITEMP1
      REAL MASSNO(230),INTEN(230),YHGHT,PCTG,BIGPK,SUMINT,TEMP,TEMP1,
     &XSTART,XSTOP,MSTART,MSTOP,MAGNFY,XEROX,PKHT(10)
      DO 10 J=1,100
   10 AST(J)=11
      CALL PLOTS(IBUF,1000,9)
   20 READ (5,480,END=460) K,L,ID,TITLE
      IF (ID.LE.0) GO TO 470
   30 READ (5,490,END=460) XSCALE,XSTART,XSTOP,YHGHT,MSTART,MSTOP,MAGNFY
     &,XEROX
      IF (XSCALE.LE.0.0) XSCALE=5.0
      IF (YHGHT.LE.0.0) YHGHT=10.00
      IF (MSTOP.LE.MSTART.OR.MAGNFY.LE.0.0) MSTOP=-99.0
      I=1
      IF (L.EQ.3) GO TO 60
      IF (L.EQ.2) GO TO 90
      IF (L.EQ.0) GO TO 85
   40 READ (5,490,END=460) MASSNO(I),INTEN(I)
      IF (INTEN(I).LT.1.0E-10) GO TO 50
      I=I+1
      GO TO 40
   50 NOPKS=I-1
      GO TO 150
```

```
 60 TMASS=XSTART-1.0
 70 READ (5,500,END=460) (PKHT(J),J=1,10)
    DO 80 J=1,10
    TMASS=TMASS+1.0
    IF (PKHT(J).LE.1.0E-10) GO TO 80
    INTEN(I)=PKHT(J)
    MASSNO(I)=TMASS
    I=I+1
 80 CONTINUE
    IF (INTEN(I-1).GT.9998.) GO TO 140
    GO TO 70
 85 TMASS=XSTART-0.5
 86 READ (5,501,END=460) (PKHT(J),J=1,20)
    DO 87 J=1,20
    TMASS=TMASS+0.5
    IF (PKHT(J).LE.1.0E-10) GO TO 87
    INTEN(I)=PKHT(J)
    MASSNO(I)=TMASS
    I=I+1
 87 CONTINUE
    IF (INTEN(I-1).GT.9998.) GO TO 140
    GO TO 86
 90 TMASS=XSTART
100 READ (5,510,END=460) M,(PKHT(J),J=1,10)
    IF (M.EQ.0) GO TO 110
    XINC=0.5
    GO TO 120
110 XINC=1.0
120 DO 130 J=1,10
    TMASS=TMASS+XINC
    IF (PKHT(J).LE.1.0E-10) GO TO 130
    INTEN(I)=PKHT(J)
    MASSNO(I)=TMASS
    I=I+1
130 CONTINUE
    IF (INTEN(I-1).GT.9998.) GO TO 140
    GO TO 100
140 NOPKS=I-2
150 BIGPK=-1.0E49
    SUMINT=0.0
    IF (K.NE.2) GO TO 180
160 DO 170 I=1,NOPKS
170 INTEN(I)=INTEN(I)*MASSNO(I)
180 DO 190 I=1,NOPKS
    TEMP=INTEN(I)
    SUMINT=SUMINT+TEMP
    IF (TEMP.GT.BIGPK) BIGPK=TEMP
190 CONTINUE
200 SCALE=100.0/BIGPK
    SUMINT=SUMINT*SCALE
    IF (XSTOP-XSTART) 210,460,220
```

```
210 TEMP=XSTART
    XSTART=XSTOP
    XSTOP=TEMP
220 XSCAL2=1.0/XSCALE/2.54
    YSCAL2=YHGHT/BIGPK/2.54
    COPY=XEROX-0.1
230 COPY=COPY-1.0
    WRITE (6,520) ID,TITLE
    IF (K.EQ.0) GO TO 240
    IF (K.EQ.1) GO TO 250
    IF (K.EQ.2) GO TO 260
    IF (K.EQ.3) GO TO 250
240 WRITE (6,530)
    GO TO 270
250 WRITE (6,540)
    GO TO 270
260 WRITE (6,550)
270 WRITE (6,560) XSCALE,XSTART,XSTOP,YHGHT,MSTART,MSTOP,MAGNFY
    CALL PLOT(6.0,-5.0,-3)
    CALL PLOT(0.0,1.0,-3)
    X=1.5
    CALL SYMBOL(0.4,YHGHT/2.54+0.85,0.14,TITLE,0.0,40)
    IF (K.EQ.0) GO TO 280
    IF (K.EQ.1) GO TO 290
    IF (K.EQ.2) GO TO 300
    IF (K.EQ.3) GO TO 290
280 CALL SYMBOL(0.4,YHGHT/2.54+0.60,0.14,28HSPECTRUM RECORDED ON HITAC
   &HI,0.0,28)
    CALL SYMBOL(0.4,YHGHT/2.54+0.35,0.14,24HRMU-6D MASS SPECTROMETER,
   &0.0,24)
    GO TO 310
290 CALL SYMBOL(0.4,YHGHT/2.54+0.60,0.14,25HSPECTRUM RECORDED ON 1015,
   &0.0,25)
    CALL SYMBOL(0.4,YHGHT/2.54+0.35,0.14,28HQUADRUPOLE MASS SPECTROMET
   &ER,0.0,28)
    GO TO 310
300 CALL SYMBOL(0.4,YHGHT/2.54+0.60,0.14,54HSPECTRUM RECORDED ON 1015
   &QUADRUPOLE MASS SPECTROMETER,0.0,54)
    CALL SYMBOL(0.4,YHGHT/2.54+0.35,0.14,37HAND NORMALIZED TO CONSTANT
   &SENSITIVITY,0.0,37)
310 CALL SYMBOL(X,-0.55,0.14,11HMASS NUMBER,0.0,11)
    CALL PLOT(0.0,0.0,3)
    X=XSTART-10.
    I=5
320 X=X+10.
    TEMP=(X-XSTART)*XSCAL2
    CALL PLOT(TEMP,0.0,2)
    CALL PLOT(TEMP,-0.05,2)
    I=I+1
    IF (I.LT.5) GO TO 340
    CALL PLOT(TEMP,-0.15,2)
```

```
330 CALL NUMBER(TEMP-0.28,-0.35,0.14,X,0.0,1)
    I=0
340 CALL PLOT(TEMP,0.0,3)
    IF (X.LT.XSTOP) GO TO 320
350 TEMP=(XSTOP-XSTART)*XSCAL2+1.0
    CALL SYMBOL(TEMP,1.0,0.14,21H% OF TOTAL IONIZATION,90.0,21)
    TEMP=TEMP-1.0
    CALL PLOT(TEMP,0.0,3)
    TEMP1=YHGHT/25.4
    Y=0.0
    X=0.0
    PCTG=1000.0/SUMINT
360 Y=Y+TEMP1
    CALL PLOT(TEMP,Y,2)
    CALL PLOT(TEMP+0.05,Y,2)
    X=X+PCTG
    CALL NUMBER(TEMP+0.11,Y-0.07,0.14,X,0.0,2)
370 CALL PLOT(TEMP,Y,3)
    IF (Y.LT.(YHGHT/2.54-0.1)) GO TO 360
380 CALL SYMBOL(-0.65,1.5,0.14,20HRELATIVE INTENSITY,%,90.0,20)
    CALL PLOT(0.0,0.0,3)
    Y=TEMP1
    X=0.0
390 X=X+10.00
    CALL PLOT(0.0,Y,2)
    CALL PLOT(-0.05,Y,2)
    CALL NUMBER(-0.45,Y,0.14,X,0.0,0)
    CALL PLOT(0.0,Y,3)
    Y=Y+TEMP1
    IF (X.LT.99.0) GO TO 390
400 CALL PLOT(0.0,0.0,3)
    DO 410 I=1,NOPKS
    TEMP=MASSNO(I)
    TEMP1=INTEN(I)*SCALE
    ITEMP1=TEMP1
    PCTG=TEMP1/SUMINT*100.00
    WRITE (6,570) TEMP,TEMP1,PCTG,(AST(J),J=1,ITEMP1)
    TEMP1=INTEN(I)
    IF (TEMP.GT.MSTART.AND.TEMP.LT.MSTOP) TEMP1=TEMP1*MAGNFY
    CALL PLOT((TEMP-XSTART)*XSCAL2,0.0,3)
    CALL PLOT((TEMP-XSTART)*XSCAL2,TEMP1*YSCAL2,2)
    CALL PLOT((TEMP-XSTART)*XSCAL2,0.0,3)
410 CONTINUE
    IF (MSTOP.LT.0.0) GO TO 450
420 TEMP=(MSTART-XSTART)*XSCAL2
    YHGHT=YHGHT/2.54
    CALL PLOT(TEMP,0.0,3)
    CALL PLOT(TEMP+0.25,YHGHT/2.0,2)
    CALL PLOT(TEMP+0.50,YHGHT/2.0,2)
    CALL SYMBOL(TEMP+0.55,YHGHT/2.0,0.14,1H*,0.0,1)
    CALL NUMBER(TEMP+0.70,YHGHT/2.0,0.14,MAGNFY,0.0,1)
```

```
      IF (ABS(MSTOP-XSTOP).LT.1.0E-05) GO TO 440
  430 TEMP=(MSTOP-XSTART)*XSCAL2
      CALL PLOT(TEMP-0.50,YHGHT/2.0,3)
      CALL PLOT(TEMP-0.25,YHGHT/2.0,2)
      CALL PLOT(TEMP,0.0,2)
  440 YHGHT=YHGHT*2.54
  450 CALL PLOT((XSTOP-XSTART)*XSCAL2,0.0,-3)
      IF (COPY.GT.0.0) GO TO 230
      IF (K.NE.1) GO TO 20
      K=K+1
      GO TO 150
  460 WRITE (6,580)
  470 WRITE (6,590)
      CALL PLOT(0.0,0.0,999)
      CALL EXIT
  480 FORMAT (2I1,I4,10A4)
  490 FORMAT (8F10.4)
  500 FORMAT (8F10.2)
  501 FORMAT (16F5.1)
  510 FORMAT (I1,F7.2,9F8.2)
  520 FORMAT (1H1,//1H  ,15X,I4,2X,10A4)
  530 FORMAT (1H  ,15X,'SPECTRUM RECORDED ON HITACHI RMU-6D MASS SPECTRC
     &METER')
  540 FORMAT (1H  ,15X,'SPECTRUM RECORDED ON 1015 QUADRUPOLE MASS SPECTF
     &OMETER')
  550 FORMAT (1H  ,15X,'SPECTRUM RECORDED ON 1015 QUADRUPOLE MASS SPECTF
     &OMETER AND NORMALIZED TO CONSTANT SENSITIVITY')
  560 FORMAT (1H  ,//1H  ,15X,'INPUT PARAMETERS: ',/1H  ,15X,'X-SCALE ='
     &,F7.1,' MASS UNITS/CM.',/1H  ,15X,'X-START =',F7.1,' MASS UNITS'/1
     &H  ,15X,'X-STOP  =',F7.1,' MASS UNITS',/1H  ,15X,'Y-HEIGHT=',F7.1,
     &' CM.',/1H  ,15X,'MAGNIFICATION FROM',F10.4,' TO',F7.1,'MASS UNIT.
     &',/1H  ,15X,'MAGNIFICATION RATIO=',F10.4,//1H  ,' MASS    INTENSIT
     &Y    %',/1H  ,'NUMBER  (NMLIZED)   IONTN')
  570 FORMAT (1H  ,F7.2,F10.3,F9.3,4X,100I1)
  580 FORMAT (/////1H  ,45X,'***DATA ERROR***')
  590 FORMAT (///1H  ,45X,'***END OF THIS JOB***')
      END
```

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