CHROMATOGRAPHY AND MASS SPECTROMETRY OF STERICALLY CROWDED TRIALKYLSILYL DERIVATIVES OF NUCLEOSIDES AND STEROIDS

by

Michael A. Quilliam

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements for the
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Department of Chemistry
The University of Manitoba
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to Noreen

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ABSTRACT

The chromatography and mass spectrometry of a new series of silyl derivatives of two classes of biologically important compounds have been studied: "sterically crowded trialkylsilyl" (SCTASi) ethers of nucleosides and steroids. Such derivatives are of interest for two reasons. Firstly, these new silyl derivatives are valuable in synthetic work for the protection of hydroxyl functions. The initial part of this study was performed in conjunction with the application of SCTASi blocking groups to nucleic acid synthesis. The separation and characterization of isomeric substituted silyl derivatives of nucleosides were of particular interest in this respect. Therefore, the techniques of "gas phase analytical chemistry", namely chemical derivatization, liquid chromatography (LC), gas chromatography (GC), and mass spectrometry (MS), were examined as solutions to the problem. Secondly, the SCTASi derivatives are important because they have considerable potential as analytical derivatives for the gas phase analytical chemistry of compounds of biological interest. Therefore, the second part of the study investigated the chemistry of sterically crowded trialkylsilylation and the suitability of SCTASi derivatives of nucleosides and steroids for thin layer chromatography (TLC), high performance liquid chromatography (HPLC), GC, and electron-impact MS.

Three SCTASi groups were studied in detail, namely: *tert*-butyldimethylsilyl (TBDMSi), *cyclo*-tetramethylene-*tert*-butylsilyl (TMTBSi), and *cyclo*-tetramethylene-*iso*-propylsilyl (TMIPSi). Other types of protecting groups (trimethylsilyl (TMSi), acetyl (Ac), and trifluoroacetyl (TFA)) were also examined, not only for purposes of comparison but for the preparation of "mixed derivatives" which were valuable for the analysis of partial O-SCTASi derivatives and for the elucidation of MS

fragmentation mechanisms.

SCTASi derivatives of compounds with primary and sterically unhindered secondary hydroxyls are easily prepared in quantitative yield under mild reaction conditions. Their hydrolytic stability allows easy handling, the isolation of pure standards, and quantitative analysis by LC. The low polarity of SCTASi ethers eliminates tailing and non-quantitative elution problems encountered in the LC of polar compounds and allows the use of low polarity, volatile mobile phases. Isomeric partially silylated derivatives of nucleosides could be separated by TLC and HPLC methods.

Most of the derivatives of nucleosides and steroids displayed sufficient volatility and thermal stability for quantitative GC analysis. Isomeric substituted nucleoside derivatives could be separated and methods were developed for monitoring the formation and hydrolysis of SCTASi derivatives of nucleosides. For steroids, it was found that SCTASi ethers can give improved separations of isomers and mixtures by GC.

The electron-impact mass spectra of SCTASi derivatives of nucleosides and steroids were examined in detail. These spectra are quite different from those of the widely-used TMSi derivatives because of the much greater tendency of the SCTASi derivatives to yield siliconium ions by elimination of a bulky alkyl radical from a silyl group in the molecular ion. Subsequent fragmentations and "siliconium ion rearrangements" provide detailed information on various structural features such as the sugar and base moieties and isomeric substitution in nucleosides, and stereochemistry in steroids.

The methods that were developed were tested with several applications to synthetic problems and the analysis of a mixture of steroid metabolites.

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ABBREVIATIONS

Ac	acetyl
AcAnh	acetic anhydride
AcIm	acetyl imidazole
AcOH	acetic acid
amu	atomic mass units
B	base unit of nucleoside
B'	B + a protecting group on N-6
CC	column chromatography
dAc	acetyl-d3
DMF	N,N-dimethylformamide
dTMSi	trimethylsilyl-d_9
Et	ethyl
GC	gas chromatography
HPLC	high performance liquid chromatography
Im	imidazole
Im·HCl	imidazole hydrochloride
i-Pr	*iso*-propyl
M^+	molecular ion
m*	mass of metastable peak
m_d	mass of daughter ion
m_p	mass of parent ion
Me	methyl
MS	mass spectrometry
MW	molecular weight
PYR	pyridine
RMR	relative molar response
S	sugar unit of nucleoside
SCTASi	sterically crowded trialkylsilyl
SIR	selected ion recording
TBDMSi	*tert*-butyldimethylsilyl
*t*Bu	*tert*-butyl
TEA	triethylamine
TFA	trifluoroacetyl
TFAA	trifluoracetic anhydride

TFAIm	trifluoroacetyl imidazole
THF	tetrahydrofuran
TLC	thin layer chromatography
TMIPSi	*cyclo*-tetramethylene-*iso*-propylsilyl
TMSi	trimethylsilyl
TMSiIm	N-trimethylsilyl imidazole
TMTBSi	*cyclo*-tetramethylene-*tert*-butylsilyl

SYMBOLS

α	separation factor
δI	retention increment due to change of substituent
ΔI	difference in retention indices on two different columns
H^*	deuterium labelled hydrogen
$\xrightarrow{*}$	fragmentation pathway supported by metastable peak
	single electron movement
	double electron movement
	*iso*-propyl
	*tert*-butyl

I. INTRODUCTION

This thesis will present the results of two separate but related studies: the application of the techniques of "gas phase analytical chemistry" to the development and analysis of isomeric substituted nucleoside derivatives of importance in nucleic acid synthesis; and the development of new derivatives for the chromatography and mass spectrometry of biologically important compounds. These areas are related through a new series of derivatives: "sterically crowded trialkylsilyl" (SCTASi) ethers.

The research involved the following specific topics: (a) the use of GC/MS to help synthesize a series of new silylating reagents; (b) the development of chromatographic and mass spectral techniques for the separation and characterization of isomeric substituted nucleoside derivatives; (c) the application of these techniques to the study of the reactions of the new silylating reagents with different functional groups, and the stability of the SCTASi derivatives under various conditions; (d) the investigation of SCTASi derivatization for the analysis of various biologically important compounds by liquid chromatography, gas chromatography, and mass spectrometry; and (e) the elucidation of the unusual electron-impact induced fragmentation behavior of SCTASi derivatives.

The results from each of these areas were complementary and should be of value in future synthetic and analytical studies.

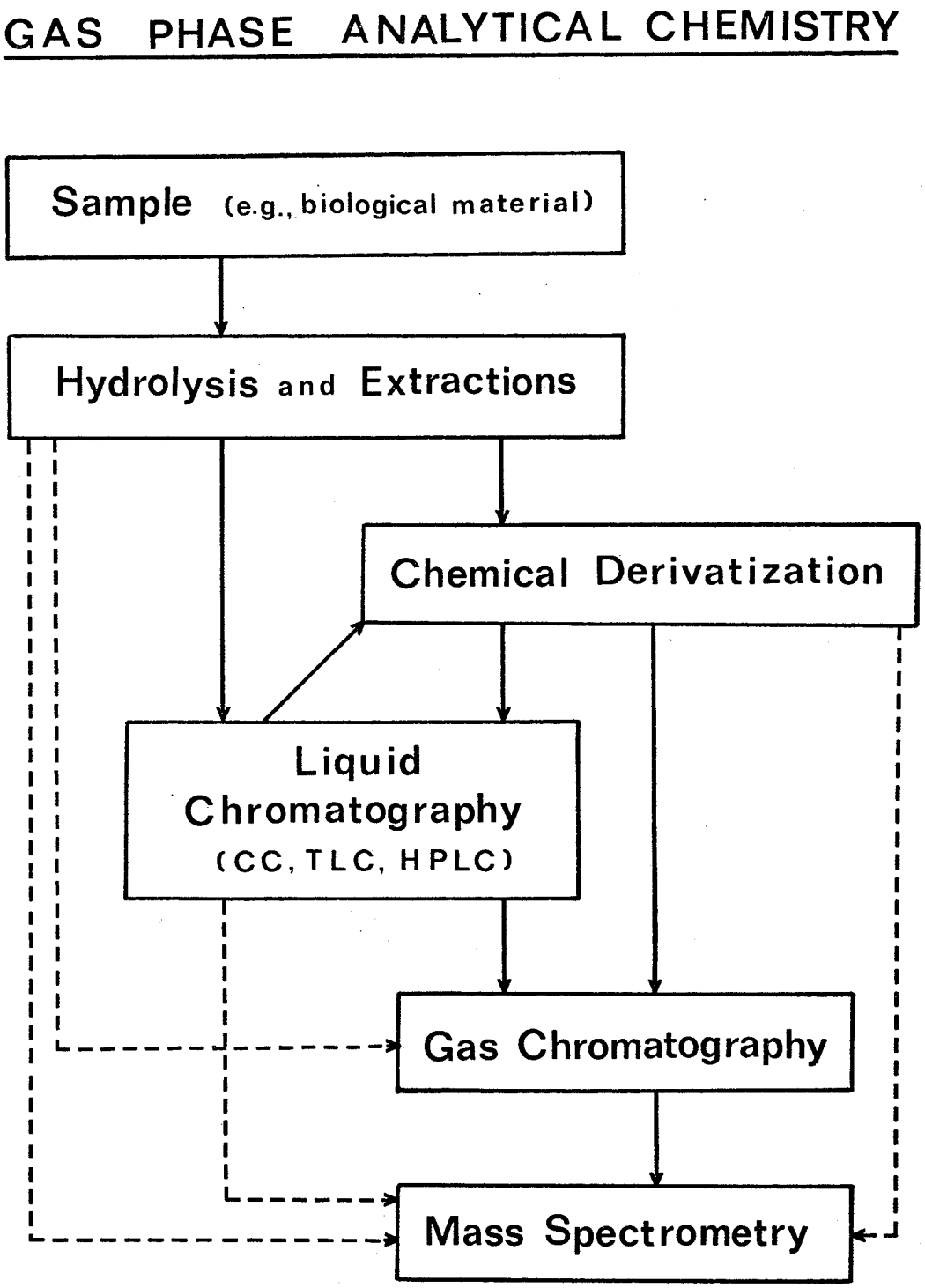
A. Gas Phase Analytical Chemistry

Few methods in organic analytical chemistry have had as wide and diverse application as gas chromatography (GC) and mass spectrometry (MS) (1-5). The field of biological chemistry is one area in which they have made a particularly strong impact. Numerous reviews have discussed the extensive applications of GC and MS in biochemistry, pharmacology, and medicine (5-9), and to various classes of biologically-important compounds such as steroids (10-13), carbohydrates (14), nucleic acid components (15), amino acids and peptides (16-18), and other natural products, in general (7,19,20).

Furthermore, GC and MS are the key elements of the rapidly expanding field of "gas phase analytical chemistry", a term first coined by Horning, Brooks and VandenHeuvel in 1968 (10). This field is a blend of organic, physical, and analytical chemistry that has evolved in response to the challenge of analyzing biological samples that contain very complex mixtures of organic compounds, often at submicrogram levels. It comprises the complete analytical procedure, from sample isolation and preparation to the final interpretation of data. The major techniques employed are chemical derivatization, thin layer, column and gas chromatography, and mass spectrometry. Figure 1 illustrates their relationship in various possible analytical schemes.

Most important in this scheme are GC and MS, where all operations are performed in the gas phase. Gas chromatography is one of the most effective methods for analyzing organic mixtures. In addition to fast, high-resolution separations, it can provide both qualitative and quantitative information, is suitable for trace analysis, and is an ideal method for introducing samples to a mass spectrometer. Mass spectrometry

Figure 1.


GAS PHASE ANALYTICAL CHEMISTRY
Sample (e.g., biological material)
Hydrolysis and Extractions
Chemical Derivatization
Liquid Chromatography (CC, TLC, HPLC)
Gas Chromatography
Mass Spectrometry

is the final and most powerful step in gas phase analytical schemes. With submicrogram quantities, it is possible to determine molecular weights (and the elemental compositions, with precise mass measurements), provide identification by "fingerprint" techniques, elucidate the structures of new substances, and even perform quantitative analyses.

The other steps in gas phase analytical chemistry are designed primarily to optimize analysis by GC/MS. For example, in the analysis of very complex mixtures, more than one chromatographic procedure is valuable--especially the combination of a high-capacity, low-resolution technique such as column chromatography, followed by a low-capacity, high-resolution method such as GC. Separation of mixtures into specific classes of compounds by liquid chromatography prior to gas phase analysis not only simplifies GC/MS, but also yields functional group information. High performance liquid chromatography (HPLC) (21) is a rapidly developing method that will fit well into the gas phase analytical chemistry scheme, especially when combined HPLC/MS systems are well established. There are other ancillary techniques also available. Possibly the most important of these is the application of modern computer technology to the processing of the vast amounts of data produced by a typical analysis.

Chemical derivatization is an essential step in the application of gas phase analysis to most compounds of biological interest. Such compounds are usually nonvolatile and thermally labile due to intermolecular hydrogen-bonding and reactive functional groups. Derivatization eliminates hydrogen-bonding and protects active sites. The types of derivatives used have been termed "nonclassical" (10), since the prime features desired are volatility, thermal stability, and freedom from

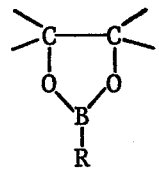
adsorption. The classical requirements of color, ease of crystallization, and sharp melting point are not important in gas phase analysis. Several reviews have dealt with derivatization for GC (22) and GC/MS (12,13,23,24). The most commonly used derivatives are listed in Table I.

Although the most important function of derivatization is to enhance volatility and thermal stability, a considerable effort has been devoted to the development of new derivatives that impart other useful properties and, in general, improve the compatibility of various chromatographic and mass spectral techniques. Such desirable properties include (a) simple, fast, quantitative preparation with controllable functional group selectivity, (b) hydrolytic stability, (c) better separations in GC, (d) structural information from GC retention behavior, (e) greater structural and stereochemical information by MS, and (f) improved detectability with GC detectors or with GC/MS selected ion recording (SIR) techniques.

Silylation (23), the substitution of a trialkylsilyl group for a labile hydrogen, has proven to be one of the most effective methods for derivatizing a wide variety of compounds. Trimethylsilyl (TMSi) derivatives of compounds with hydroxyl, amine, and carboxyl functions are readily prepared and have many of the valuable features listed above. Their formation can permit the volatilization of high molecular weight polyhydroxy compounds, stabilize thermally labile functions, and improve both chromatographic and mass spectral behavior. A striking example of trimethylsilylation leading to a better separation in GC is given in Figure 2. The underivatized steroid epimers, 3α-hydroxy-5α-androstan-17-one (androsterone) and 3β-hydroxy-5α-androstan-17-one (epiandrosterone), cannot be separated on most GC columns but are easily resolved as TMSi

TABLE I.

Derivatives most commonly used in gas phase analytical chemistry.

Reacting Functional Group	Reaction Name	Derivative Abbreviation	Derivative Group Structure
Carboxyls	Methylation	Me	$--CH_3$
Hydroxyls Amines	Acetylation	Ac	$--\overset{\overset{\displaystyle O}{\|}}{C}-CH_3$
	Trifluoroacetylation	TFA	$--\overset{\overset{\displaystyle O}{\|}}{C}-CF_3$
Hydroxyls Carboxyls Amines	Trimethylsilylation	TMSi	$--\underset{\underset{\displaystyle CH_3}{\|}}{\overset{\overset{\displaystyle CH_3}{\|}}{Si}}-CH_3$
Ketones	Methoxylation	MO	$>C=N-O-CH_3$
cis-Diols	Boronation	--	 (R = Me, *n*-Bu, Ph)

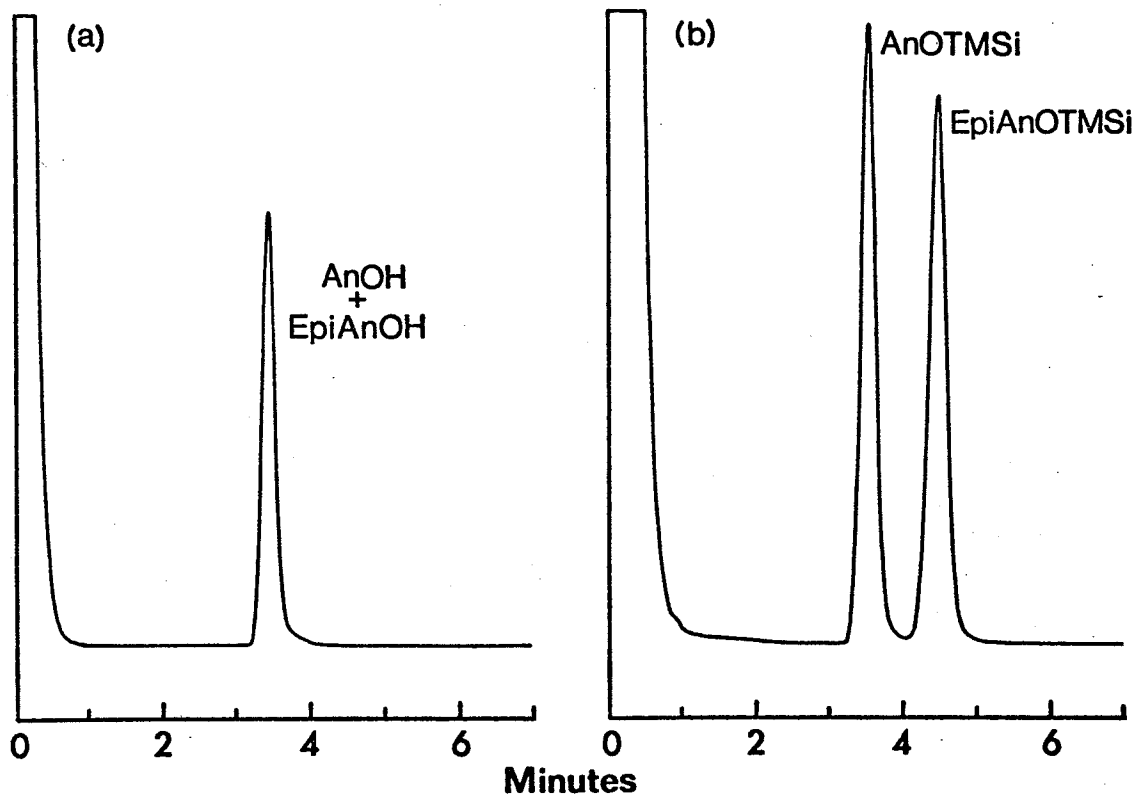


Figure 2. Trimethylsilylation for improved separation of epimeric steroids by GC with nonselective stationary phases: (a) the epimers, 3α-hydroxy-5α-androstan-17-one (AnOH) and 3β-hydroxy-5α-androstan-17-one (EpiAnOH) are not separated with a 1m X 2mm-ID, 10% OV-1 column at 230°; (b) the TMSi derivatives, on the other hand, are well separated under the same conditions.

derivatives on a short, nonselective column. Important aspects of improved mass spectral behavior include structurally informative fragmentations and stereochemically-dependent rearrangements. Trimethylsilylation can also be useful for certain separations in thin layer and column chromatography, where the hydrocarbon-like polarity of the silyl ether allows greater mobility (25-30). One of the disadvantages of TMSi derivatives is the ease of hydrolysis of certain classes (TMSi ethers of primary alcohols and phenols, TMSi esters, and TMSi amides). For this reason, most derivatives must be protected from atmospheric moisture.

Several alternative silyl derivatives have been investigated for gas phase analysis, including: dimethylsilyl (31-33) for lower molecular weight and retention time; halomethyldimethylsilyl (31,34-44) for electron-capture detection, SIR and MS; fluorocarbonsilyl (45-48) for electron capture detection and MS of steroids; tri-n-alkylsilyl (alkyl = Et, n-Pr, n-Bu, n-Hex) (49,50) for improved separation of complex mixtures; and dimethylalkylsilyl(alkyl = Et, n-Pr) (51) for GC/MS of steroids. Perdeuteriotrimethylsilyl (TMSi-d_9) groups have proven valuable in the interpretation of mass spectral fragmentations of TMSi derivatives (52,53). Still, despite this variety of available derivatives, other complementary or more powerful derivatives are worth investigating.

B. Nucleic Acid Synthesis

Three key discoveries stimulated a tremendous interest in the chemistry and biochemistry of nucleic acids (54,55): the demonstration by Avery in 1944 (56) of the genetic role of nucleic acids, the elucidation of the nature of the internucleotide linkage in 1952 by Todd

and coworkers (57), and the determination of the double helical structure of DNA by Watson and Crick in 1953 (58). A great effort has been devoted to the development of chemical methods for the synthesis of oligonucleotides of predetermined sequence. This area is still in a developing stage, as evidenced by the amount of literature on this subject.

One main concern in oligonucleotide synthesis is the availability of protective groups (59,60) which can block specific hydroxyls of different nucleoside units to allow formation of the appropriate internucleotide 3',5'-phosphate linkage. Scheme 1 illustrates a typical synthesis. The following features are desirable for blocking groups:

(a) A reagent system with high selectivity in reaction with different types of hydroxyls (3' and 5' in deoxynucleosides; 2', 3' and 5' in ribonucleosides).

(b) Easy introduction under relatively mild conditions.

(c) Stability under a variety of synthetic conditions such as phosphorylation, acylation, and acidic or basic medium.

(d) Facile removal under mild conditions that do not affect other blocking goups or other parts of the molecule.

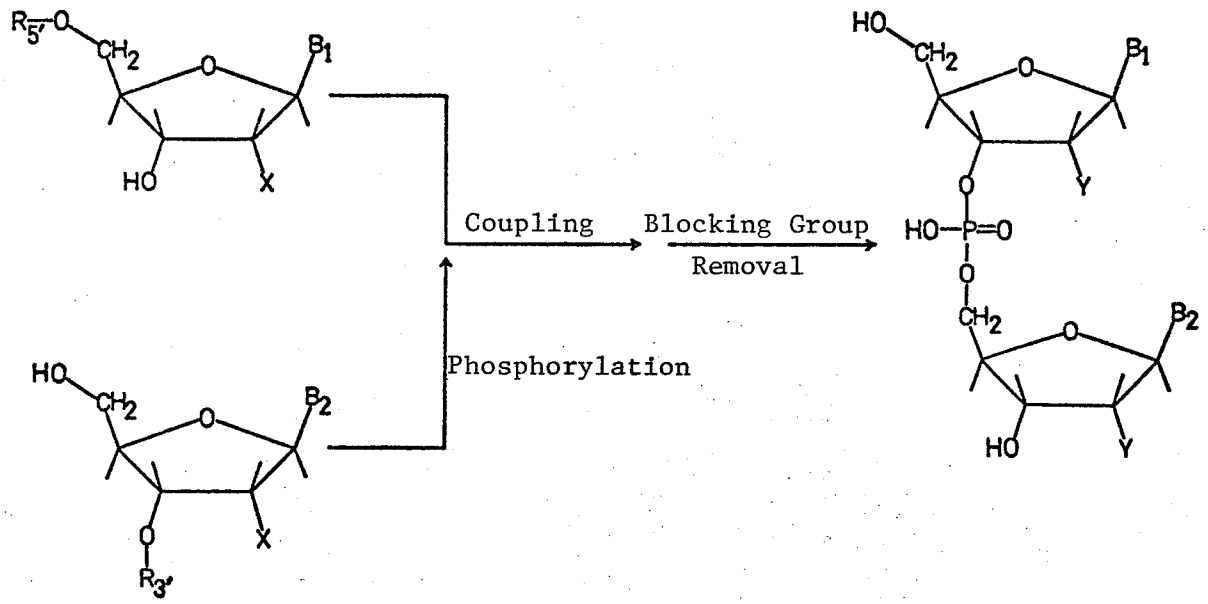
(e) Lipophilicity to facilitate reactions in aprotic solvents and purifications by techniques such as thin layer chromatography.

The trimethylsilyl group has a number of these features (easy introduction and removal, and lipophilicity), and has found many synthetic applications (61). However, it does not offer sufficient stability or selectivity for work in many areas such as nucleic acid synthesis.

Recently, another trialkylsilyl group, namely tert-butyldimethylsilyl (TBDMSi), has found wide application in synthesis. It was first

Scheme 1

Synthesis of a dinucleoside monophosphate with the natural 3'-5' phosphate linkage from partially protected nucleoside units.



R = blocking group (e.g., acetyl, trityl, etc.)

B = base (uracil, thymine, adenine, cytosine, guanine; see Scheme 3)

X,Y = H for 2'-deoxynucleosides

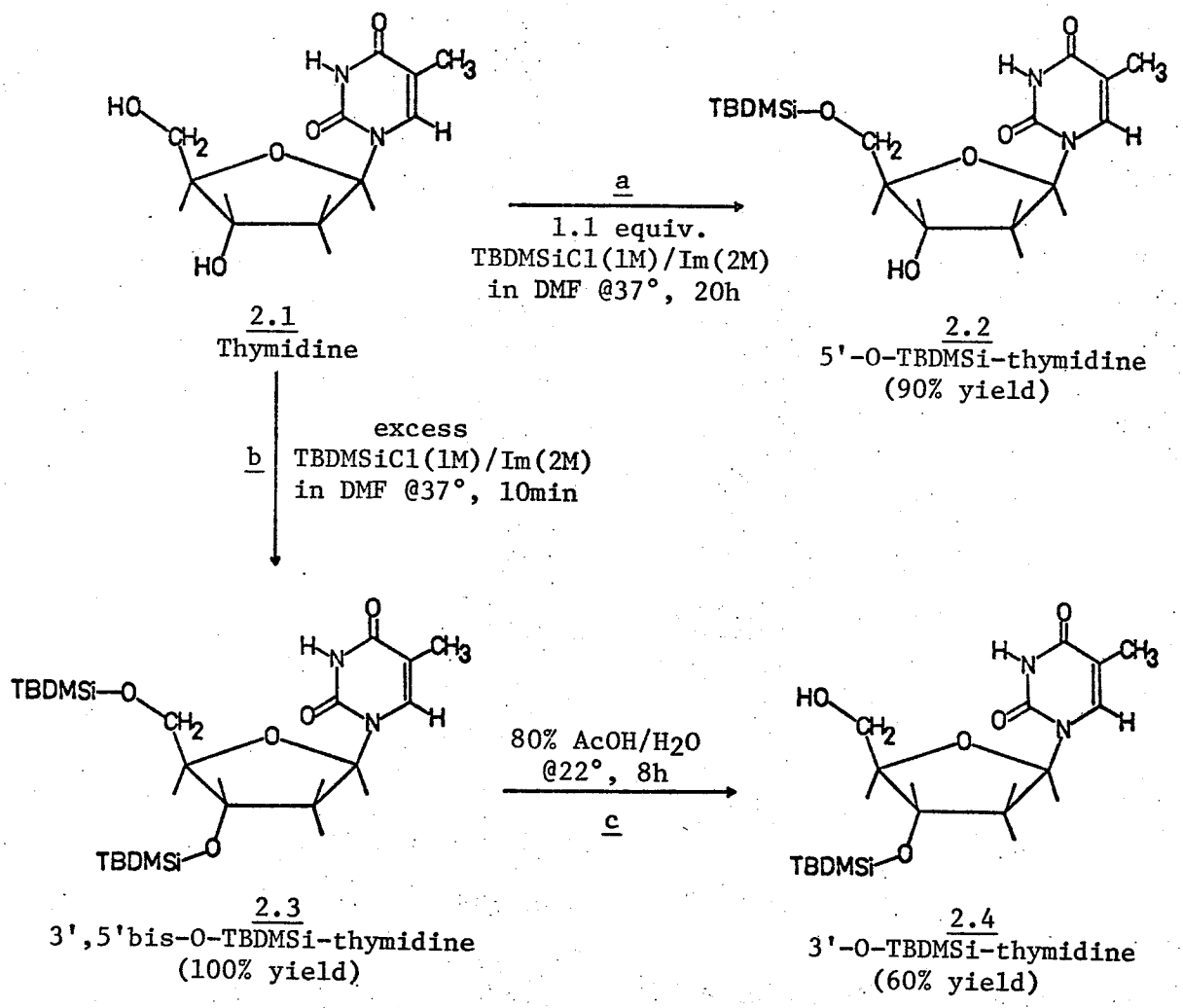
X = $OR_{2'}$, Y = OH for ribonucleosides

used in the isolation of ketone enolates in 1968 (62), but did not receive attention until Corey's classic application to hydroxyl protection in prostaglandin synthesis in 1972 (63). The TBDMSi group has a number of valuable features: a variety of alcohols can be silylated under mild conditions with a reagent mixture of chlorosilane (TBDMSiCl, 1M) and imidazole (2M) in N,N-dimethylformamide (DMF); the derivatives are stable towards mild aqueous or alcoholic base, and under conditions of acylation (e.g., acetic anhydride/pyridine), hydrogenolysis, and mild chemical oxidation and reduction; the TBDMSi group can be easily and quantitatively removed with tetra-n-butylammonium fluoride in tetrahydrofuran or under acidic conditions; and the derivatives are frequently crystalline solids with sharp melting points and possess good chromatographic behavior.

Following Corey's paper, Ogilvie (64,65) demonstrated that the TBDMSi group was suitable for oligonucleotide synthesis. Not only was it stable to phosphorylation conditions, but it was possible to selectively protect different types of hydroxyls in polyhydroxy compounds. The latter observation is due to the fact that the steric environment of a hydroxyl greatly influences its rate of reaction with the sterically crowded TBDMSi reagent, and under competitive (partial) silylation conditions, high yields of specifically protected derivatives are possible. Thus, silylation of thymidine with 1.1 equivalents of TBDMSi reagent gives primarily the 5'-TBDMSi ether (Step a, Scheme 2). Furthermore, it was observed by this author, Ogilvie and coworkers (66-69) that selective desilylation of fully protected derivatives under acidic conditions yields the more stable secondary alcohol derivatives. Thus, the 3',5'-bis-TBDMSi ether of thymidine when treated in 80% aqueous acetic acid, gives the 3'-TBDMSi-derivative in good yield (Step c, Scheme 2).

Scheme 2

Synthesis of partial O-TBDMSi derivatives of thymidine by selective silylation and desilylation.



The TBDMSi group has now been applied in the synthesis of a variety of compounds: prostaglandins (63,70-72), carbohydrates (73,74), steroids (75-78), nucleosides (64-69,79), and other natural products (80-82).

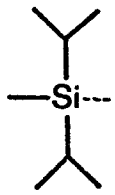
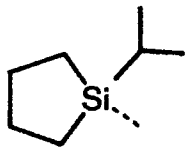
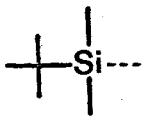
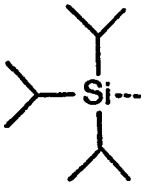
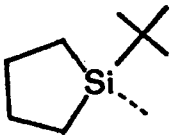
C. Sterically Crowded Trialkylsilyl Derivatives

The success of TBDMSi derivatives in nucleic acid synthesis led to a search by Ogilvie and coworkers for other suitable silyl blocking groups. Of particular interest was the development of a series of complementary silyl groups of varying hydrolytic stability that would allow versatile synthetic schemes. Four new silyl groups, in addition to TBDMSi, have been investigated (66-69). The entire series is given in Table II, in order of increased steric crowding and hence decreased susceptibility to nucleophilic attack. These groups have been called "sterically crowded trialkylsilyl" (SCTASi) groups. The synthesis of the chlorosilane reagents and their reactions with deoxynucleosides form the main subject of another doctoral thesis (83).

Since these silyl groups promised to be of major significance in the nucleoside and nucleotide field, it was important to develop good techniques for the analysis of synthetic derivatives. In particular, it was desirable to have methods for: (a) determining the purity of isomeric substituted derivatives; (b) characterizing the derivatives in terms of structure; and (c) monitoring the progress of silylation and desilylation reactions for the purpose of optimizing reaction conditions and yields, as well as to study mechanistic aspects.

The conventional techniques of liquid chromatography and NMR, IR, and UV spectroscopies have played important roles in the study of nucleosidic materials (84), but do have some limitations. Although gas phase analysis has a number of advantages (i.e., rapid, quantitative

TABLE II. Sterically crowded trialkylsilyl protecting groups.

Structure	Name of Group	Abbreviation
	methyl-di-*iso*-propylsilyl	MDIPSi
	*cyclo*-tetramethylene-*iso*-propylsilyl	TMIPSi
	*tert*-butyldimethylsilyl	TBDMSi
	tri-*iso*-propylsilyl	TIPSi
	*cyclo*-tetramethylene-*tert*-butylsilyl	TMTBSi

*Increasing steric bulk of silyl group and increasing hydrolytic stability of derivative.

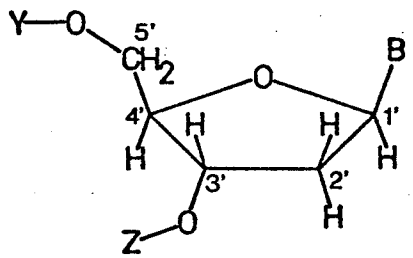
determinations on small samples, along with precise structural information and identification) it has not found widespread application in nucleic acid synthesis. This is in part due to the fact that many synthetic derivatives are not suitable for direct analysis by GC and MS.

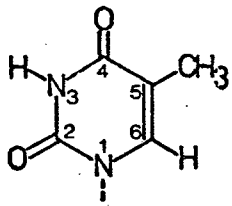
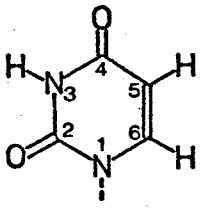
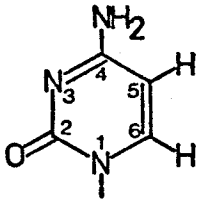
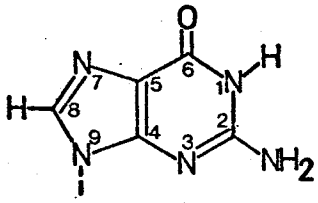
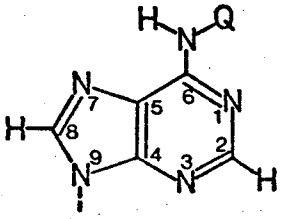
Fortunately, the new SCTASi groups impart properties to the nucleosides which make them quite amenable to gas phase analysis. Therefore, Chapter III of this thesis examines the separation, analysis, and characterization of SCTASi derivatives of nucleosides by the techniques of gas phase analytical chemistry, and Chapter IV presents the detailed interpretation of mass spectra.

Scheme 2 has already illustrated the types of silyl derivatives of the 2'-deoxynucleoside series that are of concern. The partially protected derivatives (such as 5'-O-TBDMSi-thymidine, 2.2, and 3'-O-TBDMSi-thymidine, 2.4) are the essential units for oligonucleotide synthesis. Fully protected derivatives (such as 3',5' bis-O-TBDMSi-thymidine, 2.3), although produced as side-products in partial silylation reactions, are also valuable as synthetic intermediates (as in the preparation of 2.4). In addition, they are useful as "analytical" derivatives, an aspect which will be discussed in Chapter V.

The entire series of deoxynucleoside derivatives examined by chromatography and MS are outlined in Scheme 3; the complete names and numbers of individual derivatives appear in Tables III and VIII (Chapter III). Derivatives are numbered according to the parent compounds (I = thymidine, II = uridine, etc.) with subscripts representing the 5'- and 3'-O-substituents, Y and Z respectively. Y and Z are designated by letters for various substituent groups (a = H, b = TBDMSi, c = TMTBSi, d = TMIPSi, etc.). Thus, Ibc represents the compound 5'-O-TBDMSi-3'-O-

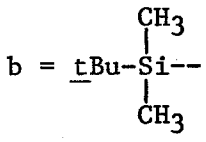
Scheme 3. Structural designation of 2'-deoxynucleoside derivatives.

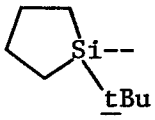


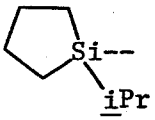
Base (B)	Compound Number	Parent Compound (Y,Z = H)
	I_{yz}	Thymidine
	II_{yz}	Deoxyuridine
	III_{yz}	Deoxycytidine
	IV_{yz}	Deoxyguanosine
	V_{yzq}	Deoxyadenosine

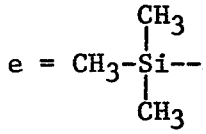
Substituents (Y,Z,Q)

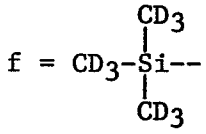
a = H

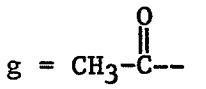
b =  (TBDMSi)

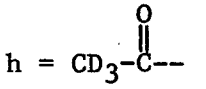
c =  (TMTBSi)

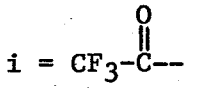
d =  (TMIPSi)

e =  (TMSi)

f =  (dTMSi)

g =  (Ac)

h =  (dAc)

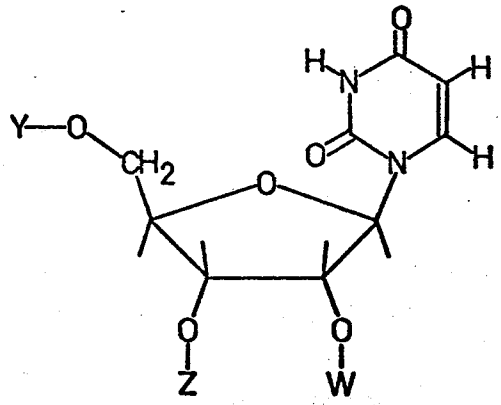
i =  (TFA)

TMTBSi-thymidine. In the case of deoxyadenosine, a few derivatives were further substituted at the N6 position of the base and are numbered Vyzq (where Q is the N6 substituent).

Only three SCTASi groups are discussed in this thesis: TBDMSi, TMTBSi, and TMIPSi. Their behavior represents that of the entire series of SCTASi groups, and these three are essential for the interpretation of mass spectra. Substituents (e) to (i) were employed in this study for analytical derivatization to improve the chromatographic and mass spectral properties of partial SCTASi derivatives.

Most of the SCTASi derivatives were prepared and characterized (by conventional techniques: NMR, IR, UV, melting point, and elemental analysis) by K.K.Ogilvie and coworkers (64-69) and E.A.Thompson (83).

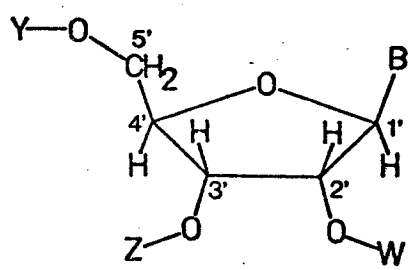
The ribonucleoside series of derivatives have not been examined in as much detail as the deoxy series. This is partly due to the fact that the synthetic work is still incomplete, and partly due to the number of partial derivatives available. Scheme 4 illustrates the compounds investigated. Most of the work has been performed on TBDMSi derivatives of uridine (VI), which have been synthesized and characterized by K.L.Sadana (85). As shown in Scheme 5 there are seven such derivatives possible: three mono-silyl and three bis-silyl partial derivatives, and one fully protected compound.

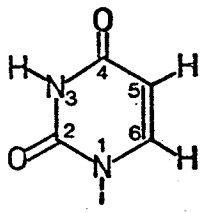
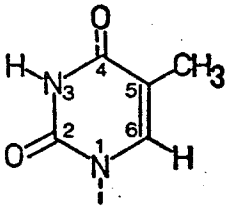
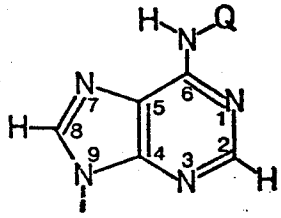


Scheme 5. TBDMSi-uridine derivatives.

#	Y(5')	Z(3')	W(2')
VIbaa	TBDMSi	H	H
VIaba	H	TBDMSi	H
VIaab	H	H	TBDMSi
VIbba	TBDMSi	TBDMSi	H
VIbab	TBDMSi	H	TBDMSi
VIabb	H	TBDMSi	TBDMSi
VIbbb	TBDMSi	TBDMSi	TBDMSi

Scheme 4. Structural designation of ribonucleoside derivatives.



Base (B)	Compound Number	Parent Compound (Y,Z,W = H)	Substituents (Y,Z,W,Q)
	VI_{yzw}	Uridine	a = H b = TBDMSi c = TMTBSi d = TMIPSi e = TMSi g = Ac i = TFA
	VII_{yzw}	5-Methyluridine	
	VIII_{yzwq}	Adenosine	

D. New Analytical Derivatives

With the application of gas phase analytical chemistry to synthetic nucleoside derivatives, it became apparent that SCTASi ether derivatives had certain useful properties which suggested their use as analytical derivatives. They possess some of the valuable features of nonclassical, as well as classical, derivatives: hydrolytic and thermal stability, crystallinity and sharp melting points, volatility, and good TLC, GC, and MS behavior. Therefore, some of the results in Chapters III and IV that illustrate the value of these derivatives for chromatography and MS of nucleosides, are discussed further in Chapter V from an analytical viewpoint. Also presented in that Chapter are the results of an investigation into the application of SCTASi derivatives to the gas phase analysis of another class of biologically important compounds: steroids.

The results of this work have been reported in a number of conference proceedings (68, 86-88) and publications (66,67,69,89-93). Other reports are in preparation. Recently, several papers have demonstrated the use of TBDMSi derivatives for GC, MS, and SIR of prostaglandins (94), steroids (95-97), carbohydrates (73,98,99), and fatty acids (100). The results, where comparable, are in good agreement with those reported in this thesis and serve to indicate the intense interest in this area of research.

II. EXPERIMENTAL

A. Liquid Chromatography

Thin layer chromatography R_f values were measured on strips of Eastman Chromagram sheets (No. 6060, silica gel with fluorescent indicator) with a solvent migration distance of 10 cm. Large scale separations were performed by thick layer chromatography using 5 X 20 cm glass plates coated with a 250 μ layer of silica gel GF ("Prekotes" from Applied Science Labs Inc., State College, Pennsylvania). These plates were activated for 30 min at 110° prior to use. Reaction mixtures could be applied directly, unless this involved large amounts, in which case DMF or pyridine was first removed *in vacuo* and the residue was dissolved in a small amount of diethyl ether or dichloromethane for application. A nitrogen stream was used for drying applied spots or bands. Developing solvent systems employed were as follows: A = diethyl ether; B = ethyl acetate; C = chloroform/ethanol (8:2, v/v); D = diethyl ether/hexane (2:1, v/v); and E = cyclohexane/ethyl acetate (9:1, v/v). Visualization was achieved with a UV lamp for nucleosides or with iodine vapor for steroids. Compounds were eluted from Prekote plates with diethyl ether. This elution solution could be analyzed directly by GC/MS, or a few microliters could be evaporated in a solid probe sample tube for direct MS analysis.

Sephadex LH-20 column chromatography of silylated steroids was performed by a method similar to that of Kelly and Taylor (97). Up to 200 μl of silylation reaction mixtures was applied to the top of a 0.5 X 6 cm column of Sephadex LH-20 (Pharmacia AB, Uppsala, Sweden) swollen in heptane/ethyl acetate (3:1, v/v) and then eluted with 10 ml

of the same solvent. Fractions (1 ml) were collected and evaporated to dryness. Silylated steroids eluted within the first 2 ml as determined by TLC or GC analysis.

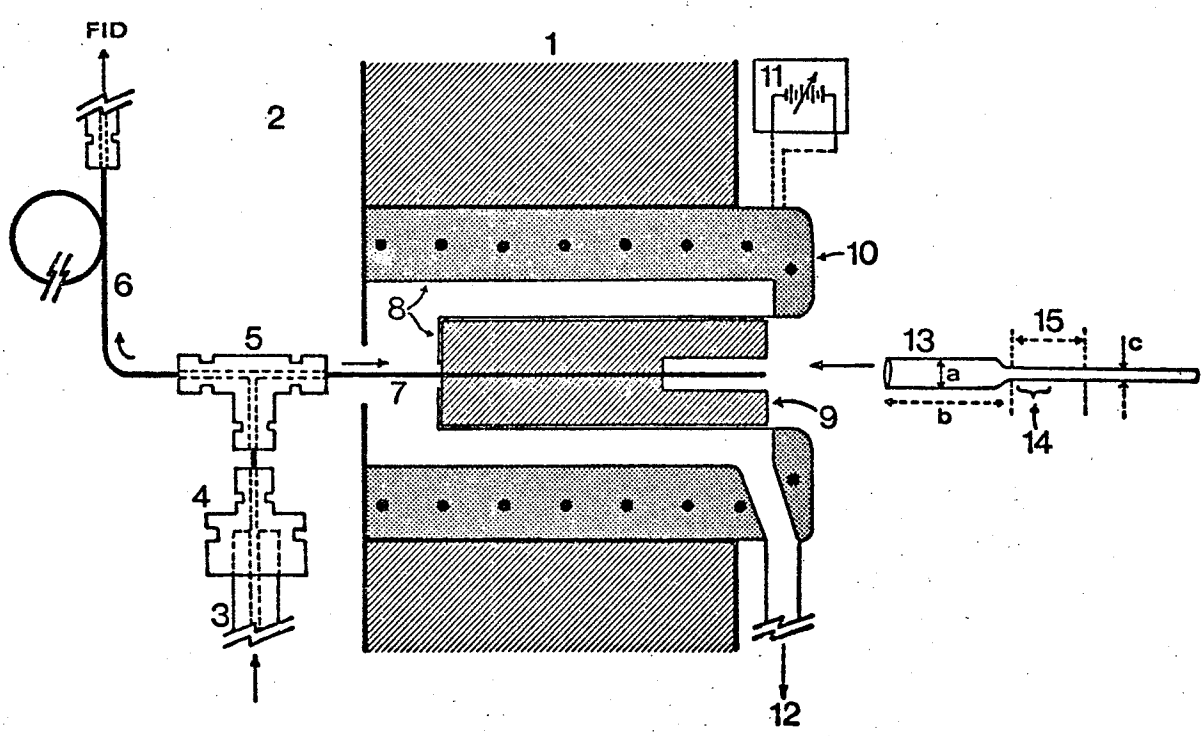
High performance liquid chromatography was performed on a Spectra-Physics Model 3500 instrument with a 10 μl volume valve injector and a UV absorption monitor (λ = 280 nm). The column was 250 mm X 3 mm-ID packed with 5μ Spherisorb Silica. Ethyl acetate was used as mobile phase with flow rates from 0.4 to 3.2 ml/min.

B. Gas Chromatography

Gas chromatography was performed on two different instruments. The first was a Hewlett-Packard model 5711A gas chromatograph equipped for dual flame ionization detection (FID). The off-column injectors and the detectors had glass inserts and were at 250° and 300°, respectively. Columns A and B (glass, 1m X 2mm-ID X 6mm-OD) were packed with 10% OV-1 and 10% OV-17, respectively, on 80/100 mesh Gas Chrom Q (Applied Science Labs Inc.). Column C was 3.75m X 2.4mm-ID (glass) packed with 10% OV-1 on 80/100 mesh Gas Chrom Q. All column tubing was treated with 5% dimethyldichlorosilane/toluene, washed with methanol, and dried prior to packing. Columns were routinely treated *in situ* with injections of Silyl-8 (Pierce Chemical Co.) to block any active sites. Nitrogen carrier gas flow rates were 30 ml/min and the column oven was operated isothermally. In order to collect effluent for further studies (TLC, MS, derivatization and reinjection, etc.), a column could be connected to an effluent splitter between the detector and a collector in the ratio 1:4. The design of this splitter/collector system is given in Figure 3. For mass spectrometry of the effluents, the glass capillary trap could be fitted into the solid probe of the MS.

Figure 3. Cross-sectional view of the splitter/collector system developed for the Hewlett-Packard GC. The column effluent is split in a 1:4 ratio between the flame ionization detector (FID) and collector by a stainless steel (ss) splitter. The collector unit is designed to maintain a constant temperature up to the point of collection by a combination of insulation, heating wire, and air flow from the column oven. High resolution is preserved by a low dead volume and efficient glass traps.

1 = column oven wall; 2 = column oven; 3 = column (1/4"-OD X 2mm-ID); 4 = Swagelok reducer union (1/4"-1/16",ss); 5 = Swagelok union tee (1/16",ss); 6,7 = capillary tubing (1/16"-OD X 0.01"-ID,ss) of lengths 20cm and 5cm, respectively; 8 = glass collector housing; 9 = drilled Teflon cylinder; 10 = asbestos insulation; 11 = Variac voltage regulator and Nichrome heating wire; 12 = air flow maintained by house vacuum; 13 = glass trap with dimensions of a = 3mm, b = 10mm, c = 2mm; 14 = point of collection (can be cooled by a stream of liquid nitrogen vapor); 15 = 20mm section for insertion into solid probe of Finnigan MS.



It should be noted that, although the analysis of complex mixtures is best accomplished with longer columns with lower percentage liquid phase loadings (1 to 3%), temperature programming, and combined GC/MS, this work utilized short 10% liquid phase columns in order to achieve fast analyses of simple mixtures, the measurement of isothermal retention indices, and semi-preparative isolation of effluents.

The second GC was a Varian model 1700 equipped with FID, as well as an interface to a Finnigan 1015 mass spectrometer. The interface (described in Figure 4) allowed simultaneous GC/FID/MS. An off-column glass-lined injector was maintained at 225° and the FID and separator were at 250°. Column D (10% OV-1, identical to column A) was operated with helium carrier gas at 40 ml/min and with temperature programming.

The Hewlett-Packard GC was used for quantitative studies and peak areas were measured by either triangulation (for results in Chapter III) or disc integration (for results in Chapter V).

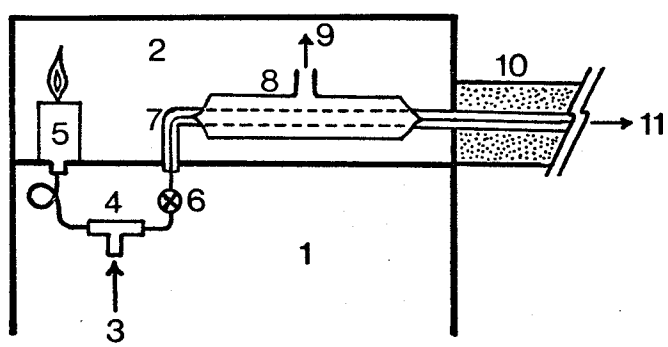
Kovat's retention indices (I) (101) were calculated with a program developed for the Hewlett-Packard 9100A calculator (see Appendix). This performs a linear interpolation of the plot of retention time (measured for a mixture of n-alkanes and the compound of interest, at a particular temperature) versus carbon number x 100. The I values reported have a precision of ±5 units. Separation factors (retention time ratios; always ≥ 1) of isomeric derivatives were measured at temperatures which gave reasonable retention times.

C. Mass Spectrometry

Three different types of instruments were employed. A Finnigan 1015 RF-quadrupole mass spectrometer was used for most compounds since it provided fast analyses on very small samples. Solids, trapped effluents

Figure 4. GC/FID/MS-interface developed for coupling the Varian 1700 GC to a Finnigan 1015 MS with simultaneous flame ionization detection. When the micro-valve is open, the column effluent is split in a 1:4 ratio between FID and MS, respectively; when it is closed, the MS is isolated to prevent introduction of solvent or other components, or for independent operation of the GC/FID and MS.

1 = column oven; 2 = detector oven; 3 = column; 4 = splitter (same construction as in Figure 3); 5 = FID; 6 = high-temperature micro-valve (Precision Sampling Corp., Baton Rouge, Louisiana); 7 = glass capillary tubing (pressure drop restrictor); 8 = Watson-Biemann glass frit carrier gas separator; 9 = rough vacuum for He removal; 10 = heated manifold to MS; 11 = MS ion source.



from the GC, or evaporated TLC eluates were introduced with the solid probe inlet system. Only mild heating was required for most samples. The analysis of mixtures of silylating reagents was accomplished by combined GC/MS using the interface described in Figure 4. The electron energy was 70eV and the ionization chamber was maintained at 200°. Special care was taken that spectra were scanned only when a constant rate of evaporation had been achieved (or at peak maxima in GC/MS) in order to avoid distortion due to concentration changes. The instrument was carefully tuned for "unit resolution" ($\Delta M = 1$, or where the resolution is equal to the mass of the ion transmitted) and optimum sensitivity at high mass since the spectra are very sensitive to quadrupole operating conditions (102). The serious effect of tuning on the appearance of a spectrum is illustrated in Figure 5. The reproducible tuning of the quadrupole system was assisted by using 2',3',5'tris-O-TBDMSi-uridine (VIbbb) as a standard. Figure 6 illustrates that the conditions selected gave a spectrum on the quadrupole instrument, after correction for mass discrimination (vide infra), that was quite similar to the one produced on a Hitachi RMU-6D magnetic MS. In addition, this tuning is close to that proposed by Eichelberger et al.(102) who used decafluorotriphenylphosphine as a standard (Figure 7).

Theoretically and experimentally, the transmission of a quadrupole mass filter is inversely proportional to the resolution provided that resolution is greater than about 70 to 80 (103-105). In order to compare the abundance of fragment ions of different mass from different compounds, and to give a spectrum similar to that on a magnetic instrument, correction for this mass discrimination was achieved by multiplying the intensity of each ion by its mass before the spectrum was normalized (Figure 6).

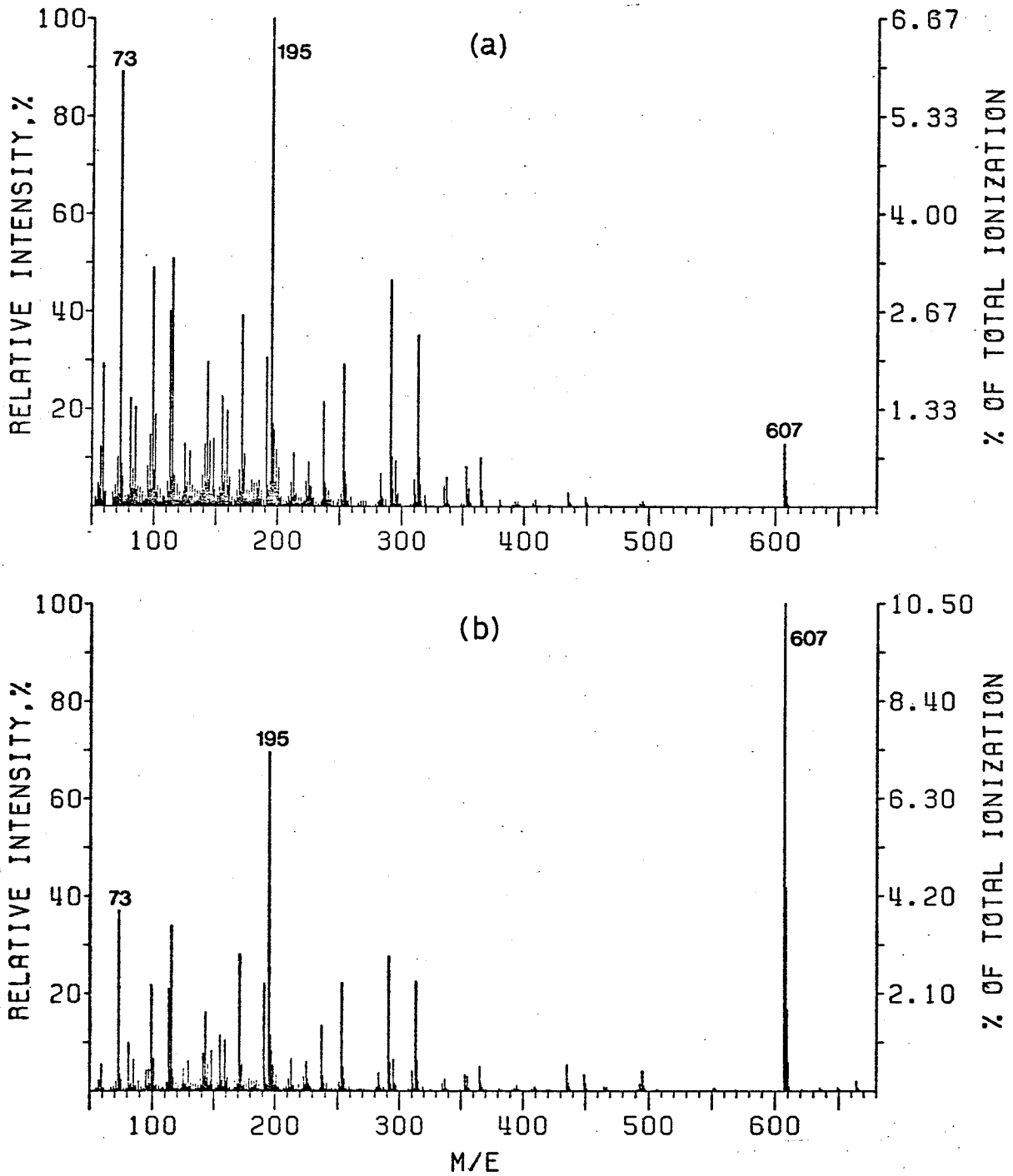


Figure 5. Mass spectra of 2',3',5'tris-O-TMTBSi-uridine (VIccc) recorded on the Finnigan quadrupole MS at 70 eV with different tuning conditions: (a) poor tuning (greater than "unit resolution"); (b) "standard tuning" (see text). Both spectra have been corrected for mass discrimination.

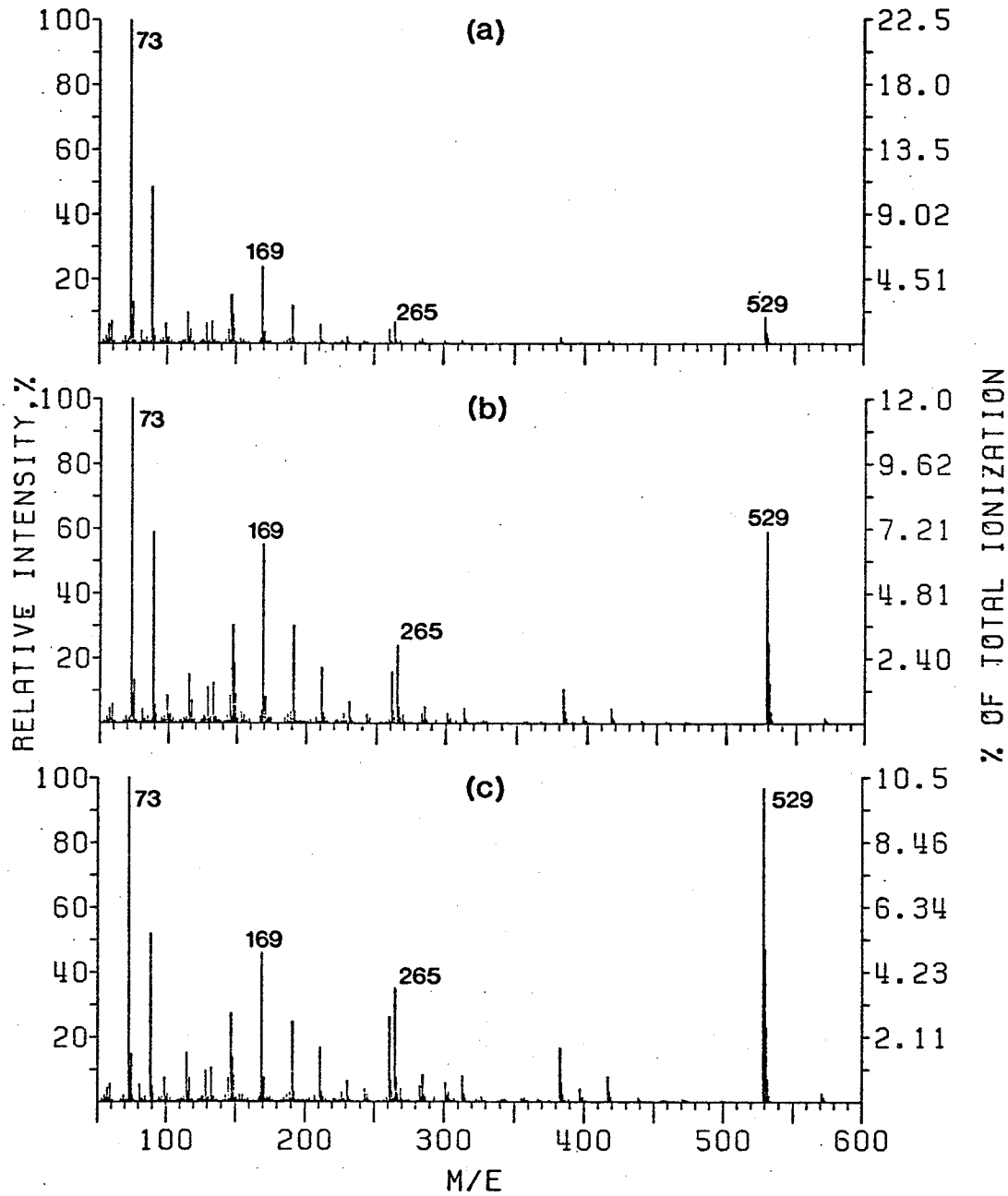


Figure 6. Mass spectra of 2',3',5'tris-O-TBDMSi-uridine (VIbbb) recorded on the Finnigan quadrupole instrument at 70 eV (a) and on the Hitachi magnetic sector instrument at 50 eV (c). Spectrum (b) is the same as (a) except that correction has been made for the mass discrimination of the quadrupole mass filter by multiplying the intensity of each ion by its mass.

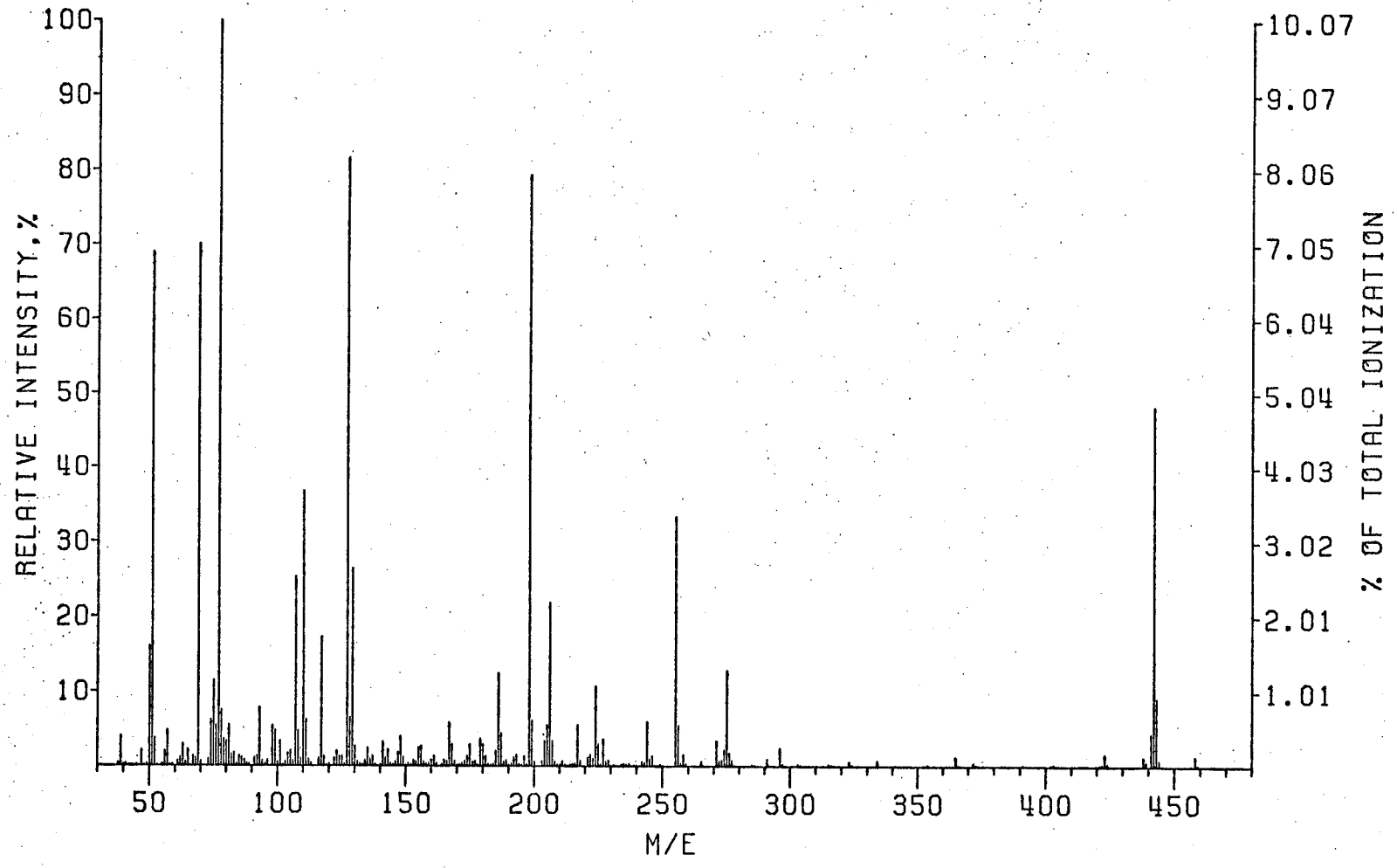


Figure 7. Mass spectrum of decafluorotriphenylphosphine recorded on the Finnigan quadrupole MS at 70 eV under "standard" tuning conditions and no correction for mass discrimination.

The second instrument was a Hitachi-Perkin Elmer RMU-6D single-focusing 8" radius magnetic sector MS. All samples were introduced with the direct insertion probe. A total ion monitor was used to determine when the rate of sample evaporation was constant. Spectra were recorded at 50eV or 20eV with a nominal accelerating potential of 950V and with the ionization chamber at 200°. Although this instrument required more sample for a good spectrum, it provided metastable peaks and higher resolution than the quadrupole.

The mass spectra produced by the above instruments were measured manually from the oscillographic chart record, keypunched, and plotted with the computer program given in the Appendix. Metastable peaks were matched according to the equation, $m^* = m_d^2/m_p$ (where m_p = parent ion mass, m_d = daughter ion mass), by a computer program similar to that reported by Lin (106).

The third instrument was the CEC-110B double-focusing MS of the Upjohn Company (Kalamazoo, Michigan) (107). Its high-resolution output was in the form of a photographic plate and an offline plate reader and computer data acquisition system provided exact mass values for all significant peaks above m/e 70 in the mass spectra. Only compounds Iba, Iab, Ibb, and VIbbb were run on this system.

D. Reagents

Reagent grade pyridine (PYR) was distilled from p-toluenesulfonyl chloride, redistilled from calcium hydride, and stored over Linde molecular sieve. Reagent grade N,N-dimethylformamide (DMF) was refluxed over calcium hydride, followed by distillation and storage over Linde molecular sieve. Reagent grade tetrahydrofuran (THF) eluted through an activated alumina column was refluxed with powdered $LiAlH_4$, then distilled

from $LiAlH_4$ and stored over molecular sieve. Triethylamine (TEA) was distilled from calcium hydride and stored over molecular sieve. n-Alkanes (n-C_{20} to n-C_{40}) were purchased from Applied Science Labs (State College, Pennsylvania). Decafluorotriphenylphosphine was obtained from PCR Inc. (Gainesville, Florida). Nucleosides and steroids were commercial samples (Sigma Chemical Company, St.Louis, Missouri) except for the cyclopropano-steroid metabolites, which were obtained from Dr. J.F.Templeton, Faculty of Pharmacy, University of Manitoba.

The reagents N-trimethylsilyl imidazole (TMSiIm), Tri-Sil Z (1.2M TMSiIm in PYR), bis-trimethylsilyl trifluoroacetamide (with 1% trimethylchlorosilane)(BSTFA), acetyl imidazole (AcIm), trifluoroacetyl imidazole (TFAIm), and trifloroacetic anhydride (TFAA) were obtained from Pierce Chemical Co.(Rockford, Illinois). Reagent grade acetic anhydride (AcAnh) was distilled from phthalic anhydride and stored in the dark. Acetic anhydride-d_6 (99%D) was purchased from Stohler Isotope Chemicals and N,O-bis-trimethylsilylacetamide-d_{18} (BSA-d_{18}) was purchased from Merck, Sharpe and Dohme (Montreal, Canada).

SCTASi reagents

tert-Butyldimethylchlorosilane (TBDMSiCl), m.p. 121-125°, was prepared according to the method of Sommer and Taylor (108); it is also available commercially from Willow Brooks Labs (Waukesha, Wisconsin). The mass spectrum is given in Figure 11c. cyclo-Tetramethylene-iso-propylchlorosilane (TMIPSiCl), b.p. 165-175°, and cyclo-tetramethylene-tert-butylchlorosilane (TMTBSiCl), b.p. 182-184°, were prepared by reacting iso-propyllithium and tert-butyllithium (Alpha Products, Ventron Corp., Danvers, Mass.), respectively, under nitrogen with freshly distilled cyclo-tetramethylenedichlorosilane (PCR Inc., Gainesville, Florida),

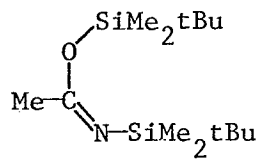
b.p. 138-139°, dissolved in pentane containing 10% THF, followed by fractional distillation. The preparation of these and other chlorosilane reagents is described in detail in the literature (69,83). The mass spectra of TMTBSiCl and TMIPSiCl are given in Figures 11a and 11b respectively.

tert-Butylmethyldichlorosilane ($TBMSiCl_2$) was prepared by reacting 1 equiv. tert-butyllithium with 1 equiv. methyltrichlorosilane (PCR Inc.) in pentane. After filtration and evaporation of pentane, the white solid product was used without further purification. The major significant peaks in the mass spectrum are: m/e 170/172/174, M^+; m/e 155/157/159, $(M-CH_3)^+$; m/e 135/137, $(M-Cl)^+$; m/e 113/115/117, $(M-C_4H_9)^+$; m/e 93/95, $C_2H_6SiCl^+$; m/e 63/65; m/e 57; m/e 56; m/e 41.

N-tert-butyldimethylsilylimidazole (TBDMSiIm) was prepared by refluxing TBDMSiCl (7.5g, 0.05 mole) with imidazole (3.4g, 0.05 mole) in TEA (150 ml) under nitrogen for 3h. The precipitate of TEA-HCl was filtered off under nitrogen and the filtrate was fractionally distilled to give a clear, viscous liquid, b.p. 150-155° at 77 torr. GC analysis showed the purity to be approximately 95%. The mass spectrum is given in Figure 12a. Recently, a similar preparation has been reported in the literature (109): b.p. 82.5-83.5° at 0.9 torr.

N,O-bis-tert-butyldimethylsilylacetamide ($TBDMSi_2Ac$) was prepared by refluxing TBDMSiCl (15.0g, 0.10 mole) and acetamide (1.2g, 0.02 mole) in TEA (100ml) under nitrogen for 3h. The precipitate of TEA-HCl was filtered off under nitrogen and the filtrate was fractionally distilled to give a clear liquid, b.p. 135° at 35 torr. GC analysis showed the purity to be approximately 95%. The mass spectrum is given in Figure 12b. There has been some debate on the structure of bis-silyl derivatives of

amides (110). This reagent was assigned as the N,O-bis-silyl derivative:



on the basis of the temperature dependence of its NMR spectrum in the light of the earlier studies. At 40°, the NMR spectrum showed δ,0.01 and 0.17ppm, two 6 proton singlets (two $SiMe_2$ groups); δ,0.84ppm, 18 proton singlet (two coincident t-Bu groups); δ,1.90ppm, 3 proton singlet (CH_3CO). With increasing temperature the two $SiMe_2$ resonances collapsed to a single resonance. The coalescence temperature, T_c, was not precisely determined but was estimated to be 90±10°. At this temperature, the value of $\Delta\nu_c$, extrapolated from the slow exchange values, was close to 9.0 Hz. Thus, from $k_c = \pi\Delta\nu_c/\sqrt{2}$ and $k_c = (kT_c/h)\exp(-\Delta G^{\ddagger}/RT_c)$, the free energy of activation, $\Delta G^{\ddagger}$, for the exchange of silyl groups was estimated to be 19.2±0.5 kcal/mole. ($\Delta\nu_c$ is the chemical shift difference at the coalescence temperature, k_c is the rate constant, and in the Eyring equation for the rate constant the transmission coefficient has been taken as unity, for consistency with the earlier work (110).) Comparison of this value of $\Delta G^{\ddagger}$ with the values for other amides, strongly supports the structure given above, since this value is in the range expected for N,O-bis-silyl amides, and is several kcal/mole higher than values obtained for N,N-bis-silyl amides (110). The preparation of this reagent has been described recently (109): b.p. 117.5-118.5° at 8 torr, with an identical NMR spectrum at 40°.

GC/MS of SCTASi reagents

In the synthesis of the above reagents (and other chlorosilanes now

reported in the literature (69,83)) GC/MS proved invaluable for optimizing yields, detecting side-products, and confirming product identities. The chromatographic and mass spectral properties of these reagents have not, as yet, been discussed in the literature. Therefore they are presented briefly in this section. Chromatograms in Figures 8 to 10 illustrate the chromatographic behavior of various reagents and their mass spectra are given in Figures 11 and 12.

Figure 9 demonstrates the value of GC/MS in analyzing reagent preparations. In this example, a synthesis of TMIPSiCl was determined to be a failure due to an impure batch of iso-propyllithium (from a commercial source). Fractional distillation did not purify the TMIPSiCl sufficiently. GC was also used to ensure that the appropriate amount of alkyllithium was added in these reactions. Thus, in this chromatogram a peak representing unreacted starting material is still present.

It should be noted that it is very important, especially for analytical derivatization work, to ensure that there are no impurities with Si-Cl bonds in the chlorosilane reagents. In a reaction, these would lead to non-quantitative yields of the desired derivative (particularly if the impurity chlorosilane was less sterically crowded than the reagent and therefore could react faster with substrates). With GC/MS it is quite easy to spot such impurities due to the characteristic Cl-35/37 doublets in the mass spectra.

Di-tert-butyltetramethylene-disiloxane (Figures 10 (peak 3) and 13b) is a side-product commonly observed in the synthesis of TBDMSiIm and $TBDMSi_2Ac$, and in the reactions of all TBDMSi reagents.

N-TBDMSi-acetamide (Figures 10(peak 2) and 13a) is a product in the reactions of $TBDMSi_2Ac$.

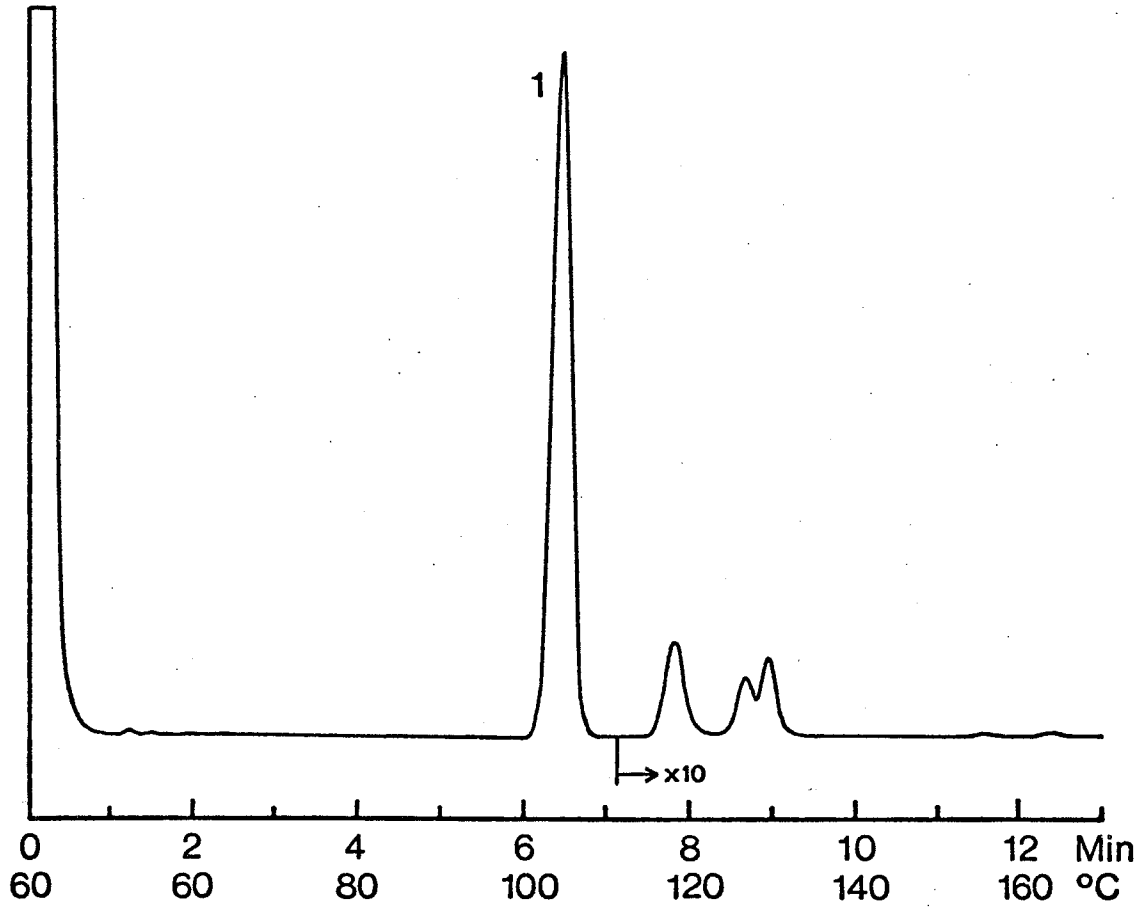


Figure 8. Gas chromatogram of a good preparation of cyclo-tetramethylene-tert-butylchlorosilane (TMTBSiCl, 1), indicating complete reaction of cyclo-tetramethylenedichlorosilane (retention time = 2 min) and low concentrations of side-products (not identified, but mass spectra indicated that no chlorines were present). Conditions: column D (10% OV-1); temperature program: 60° for 2 min, then 10°/min 60-220°; helium carrier gas at 40 ml/min.

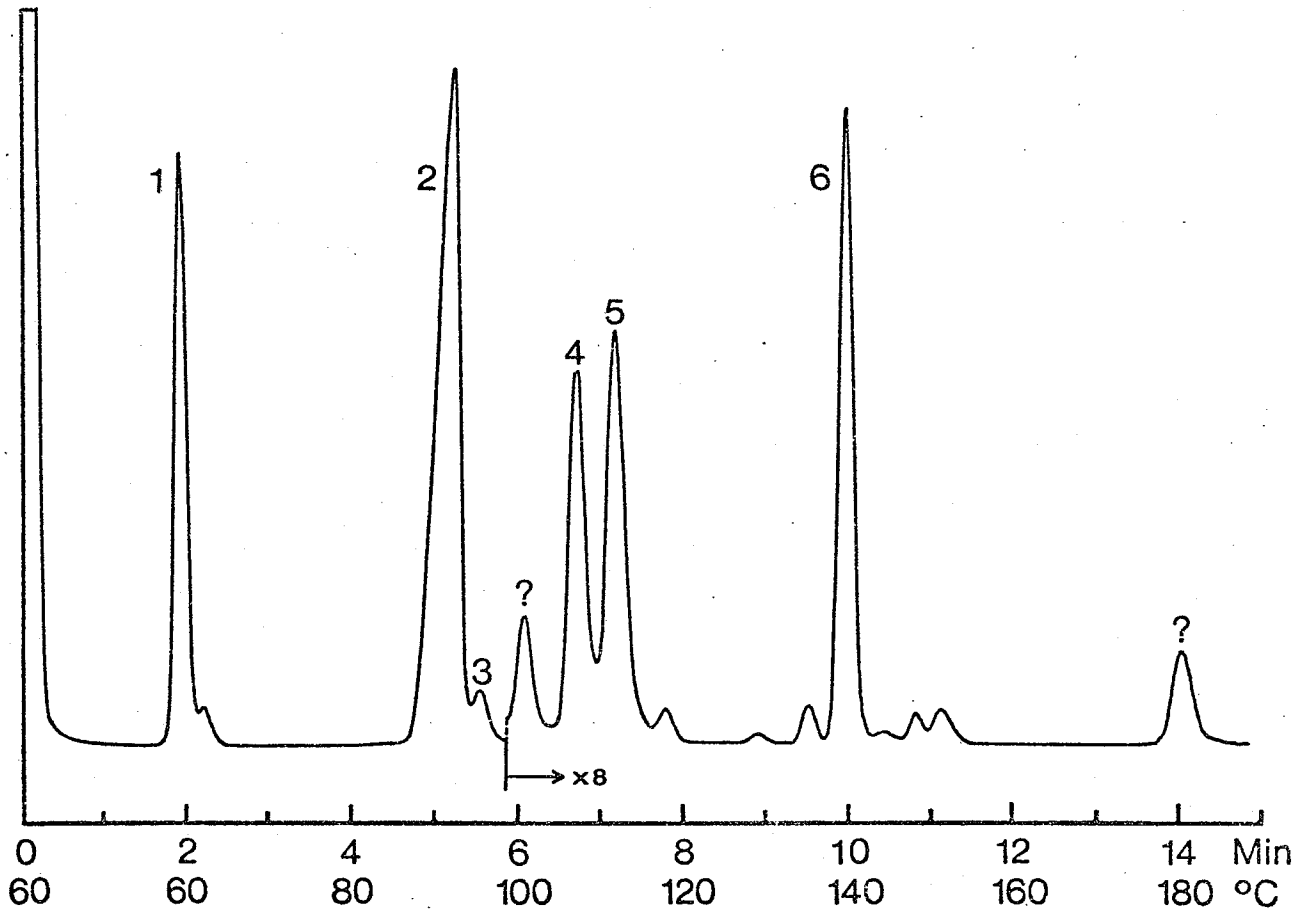
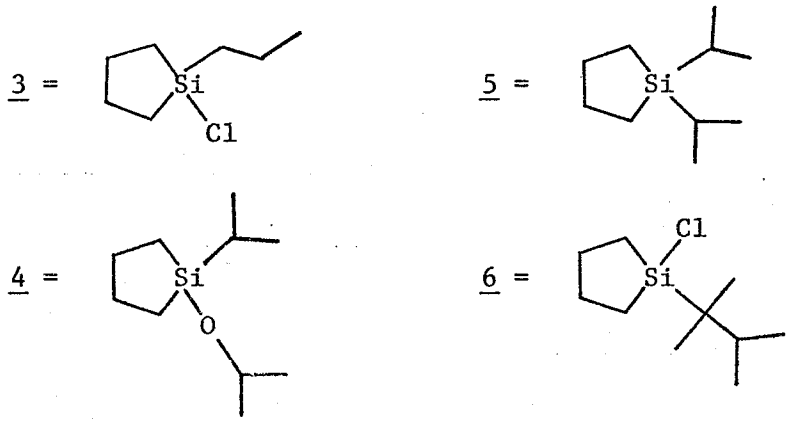


Figure 9. Gas chromatogram of a very poor preparation of cyclo-tetramethylene-iso-propylchlorosilane (TMIPSiCl, 2) due to an impure batch of iso-propyllithium, as well as incomplete conversion of cyclo-tetramethylenedichlorosilane (1). Same conditions as in Figure 8. Tentative identities of other peaks assigned by GC retention time and mass spectra:



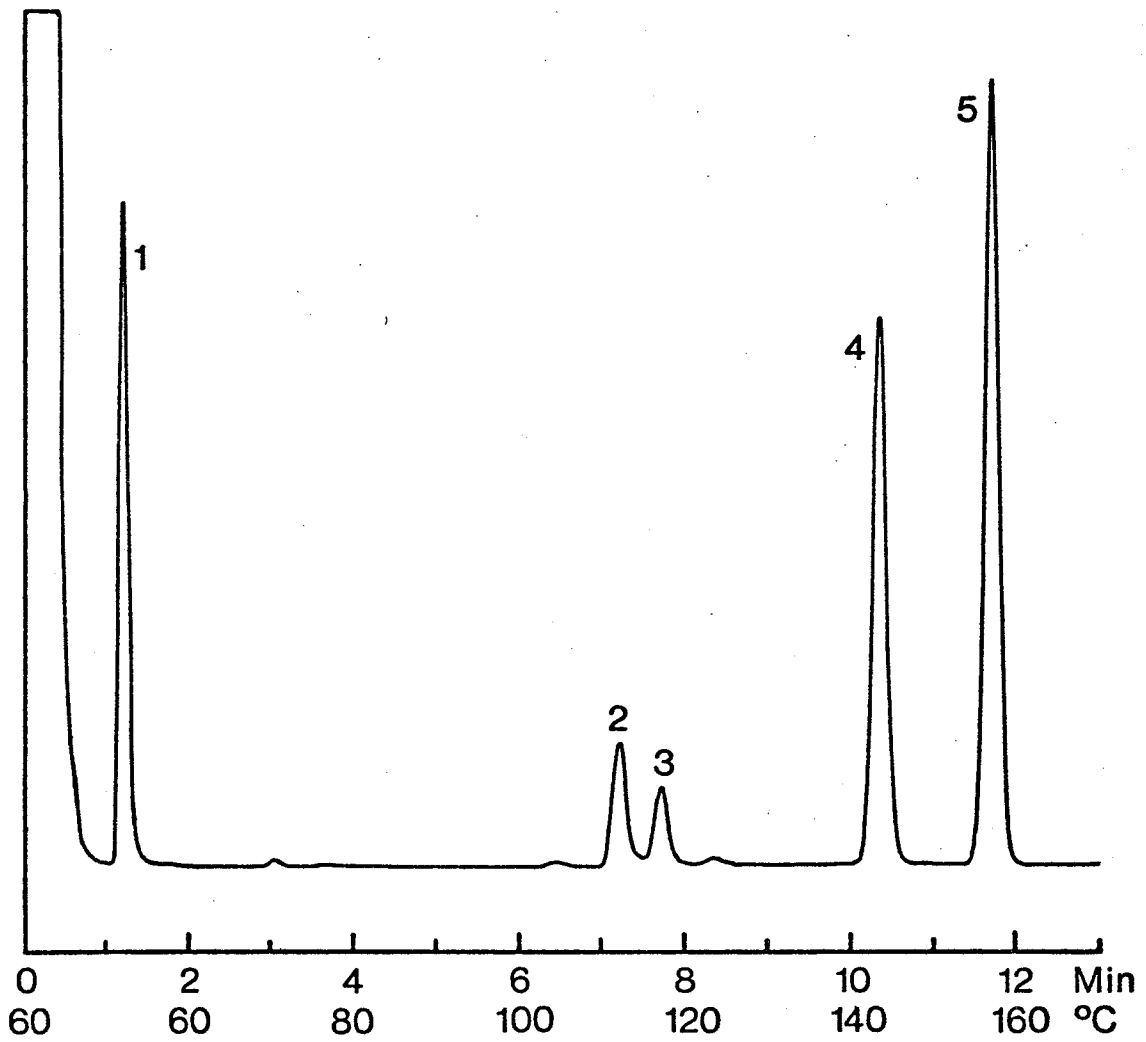


Figure 10. Gas chromatogram of a mixture of TBDMSi reagents and some side-products that occurred in their preparation. Same conditions as Figure 8. Peak indentities: 1 = TBDMSiCl; 2 = N-TBDMSi-acetamide; 3 = di-*tert*-butyltetramethyl-disiloxane; 4 = TBDMSiIm; 5 = $TBDMSi_2Ac$.

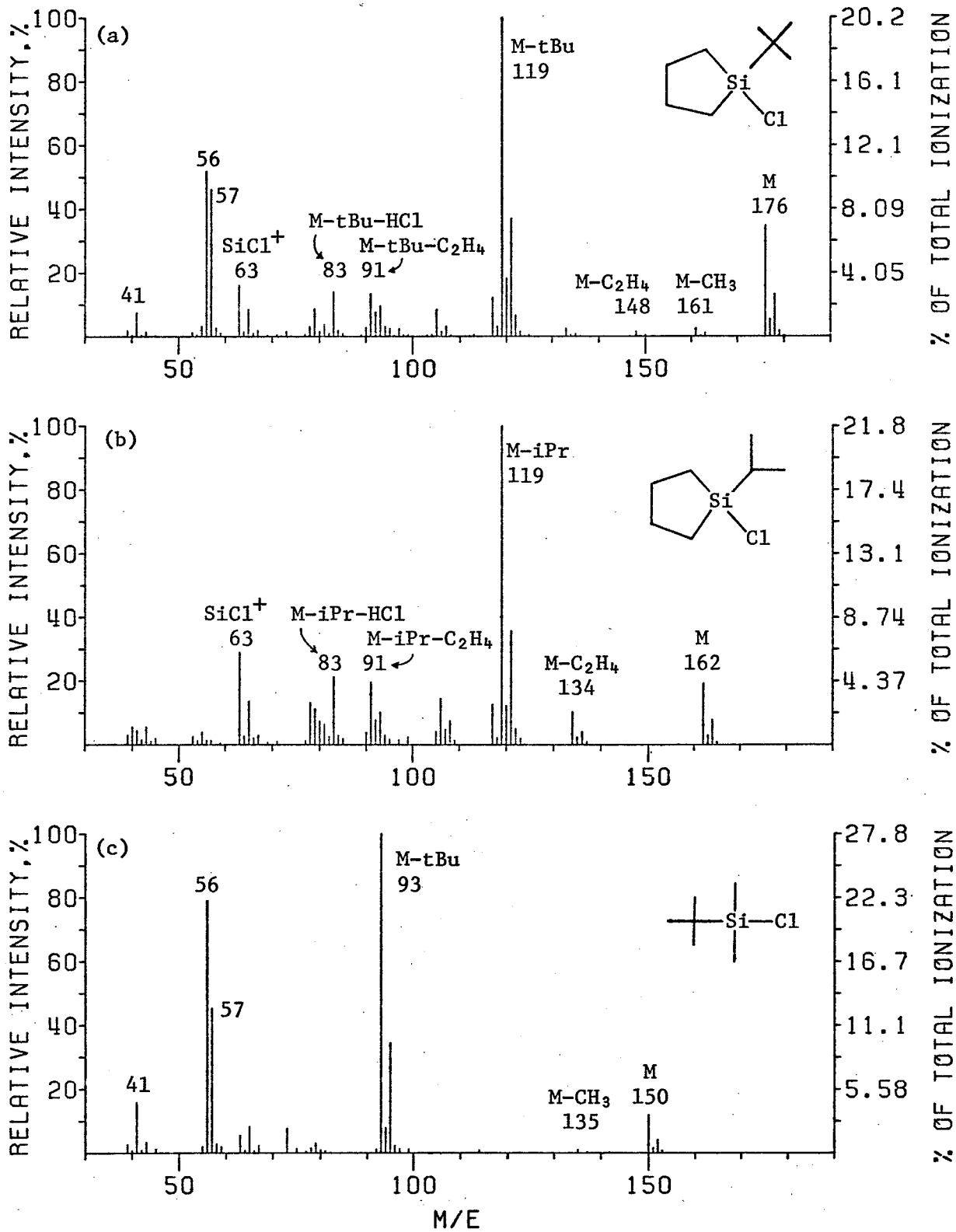


Figure 11. Mass spectra of chlorosilane reagents: (a) TMTBSiCl, (b) TMIPSiCl, and (c) TBDMSiCl. Finnigan MS, 70eV.

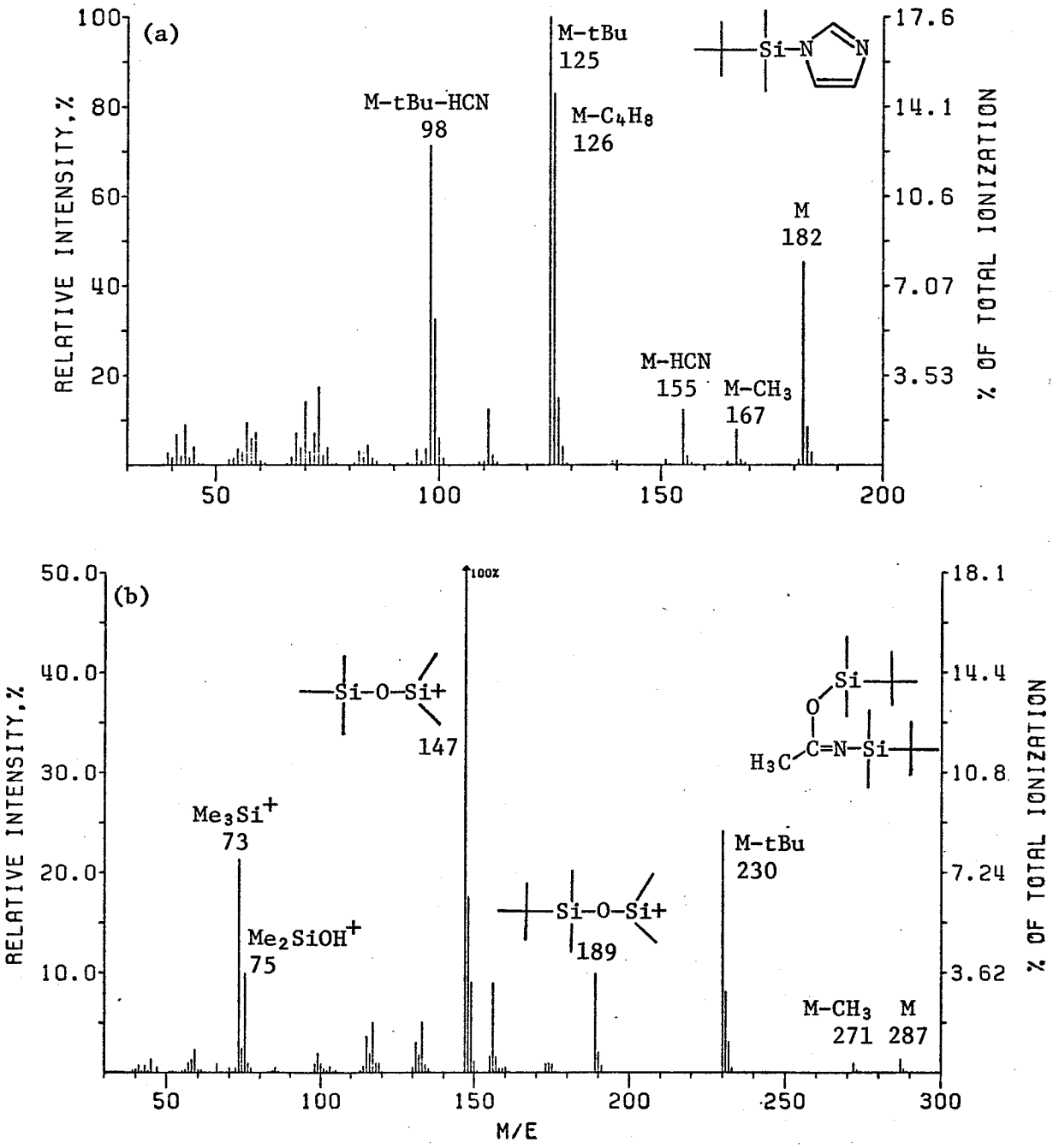


Figure 12. Mass spectra of TBDMSi reagents: (a) N-TBDMSi-imidazole and (b) N,O-bis-TBDMSi-acetamide. Finnigan MS, 70eV.

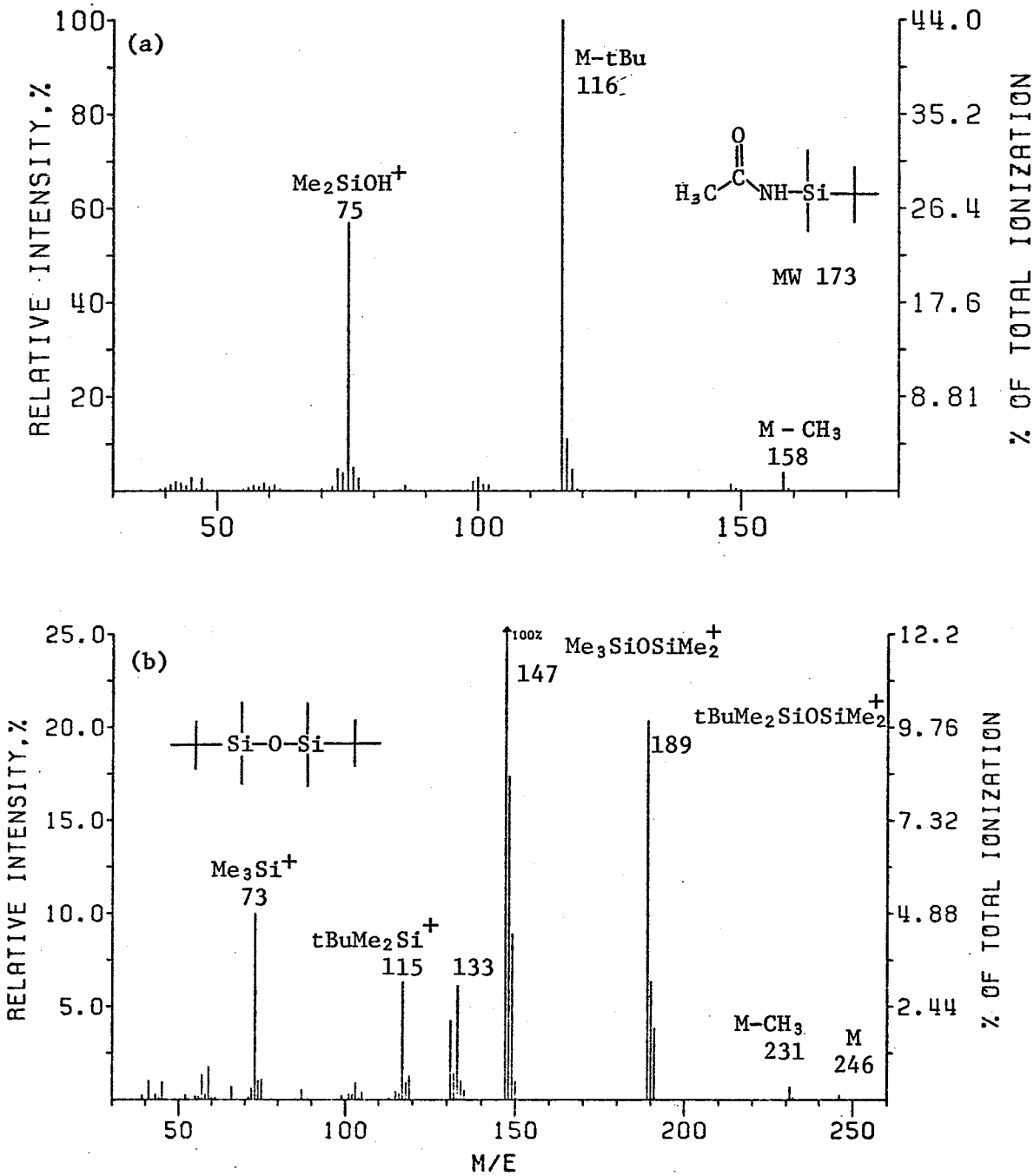


Figure 13. Mass spectra of side-products in the synthesis and reactions of TBDMSi reagents: (a) N-TBDMSi-acetamide and (b) di-<u>tert</u>-butyltetramethyldisiloxane. Finnigan MS, 70eV.

E. Preparation of Analytical Derivatives

Derivatizations were performed in dry, teflon-lined septum-capped vials (usually 0.3 ml screw-top "Reacti-Vials" from Pierce Chemical Co.). If required, vials could be inserted into a controlled-temperature aluminum block for reactions at temperatures other than ambient. One to 20 μmoles of substrate was weighed into the vials and reagent added so that the resultant substrate concentration was from 0.01 to 0.1 M with a reagent/substrate ratio between 10 and 100. Vials were then shaken to dissolve substrates.

Acetylations and trifluoroacetylations were executed with reagent systems of AcAnh/PYR (1:2,v/v) and TFAIm/PYR (1.5M), respectively. Acetyl-d_3 derivatives were prepared with AcAnh-d_6/PYR (1:2,v/v). Reactions were complete within 30 min at room temperature as confirmed by TLC or GC analyses, and could be analyzed directly by GC/MS. However, for quantitative studies involving acetylations, it was found that prior to GC analysis it was best to evaporate an aliquot of the reaction mixture *in vacuo* (to remove reagent and acetic acid side-product) and then dissolve the residue in a solvent such as dichloromethane.

Most trimethylsilylations were executed with the reagent Tri-Sil Z (1.2M TMSiIm in PYR). Primary and secondary hydroxyls (but not amines and enolizable ketones) were converted to TMSi ethers within 10 min at room temperature (determined by GC). N,O-per-trimethylsilylations (e.g., for deoxyadenosine, adenosine, and their O-SCTASi derivatives) were accomplished with BSTFA/PYR (5:1,v/v) at 90° for 1h. $TMSi_4$-uridine (VIeeee) was prepared by reacting uridine with neat BSTFA at 90° for 2h. For the complete silylation of 5β-pregnane-3α,17α,20α-triol, the reagent TMSiIm (neat) was used with overnight reaction at 80°. TMSi-d_9 derivatives were prepared with BSA-d_{18}/PYR (1.5M).

For SCTASi derivatizations, the following reagents were prepared, using the appropriate chlorosilane (TBDMSiCl,TMIPSiCl, or TMTBSiCl), and stored in teflon-lined, septum-capped hypo-vials (Pierce Chemical Co.):

A = 1M chlorosilane and 2M imidazole in DMF

B = 1M chlorosilane and 2M imidazole in PYR

C = 1M chlorosilane in PYR

D = 1M TBDMSiIm in PYR

E = 1M $TBDMSi_2Ac$ in PYR

Reactions of these reagents with various substrates are discussed in detail from an analytical viewpoint in Chapter V, and from a synthetic viewpoint in the literature (69,83). Silylations of primary and sterically unhindered secondary hydroxyls with Reagents A and B were usually complete within an hour at room temperature. Reagent A was used for most silylations, although it gave a precipitate of the derivatives of some steroids. This could be redissolved by adding dichloromethane to the reaction solution before analysis by GC/MS. Reagent B retained the derivatives in solution and was generally more suitable for quantitative GC of steroids.

All the partial O-SCTASi derivatives of nucleosides (and a few fully-O-silylated derivatives) were prepared by Drs. K.K.Ogilvie, E.A.Thompson, and K.L.Sadana and are described in the literature (64-69, 83,85).

5'-O-TBDMSi-2',3'-tert-butylmethylsilyldioxy-uridine(X) was prepared by reacting 5'-O-TBDMSi-uridine(VIbaa) with 1M tert-butylmethyldichlorosilane in THF/TEA(5:1,v/v) for one hour at room temperature, followed by direct GC/MS analysis.

SCTASi derivatives of steroids isolated for use as standards

were prepared by larger scale reactions using magnetic stirring and dry conditions. Derivatives that precipitated from Reagent A were isolated by filtration, followed by a methanol wash. Purification could be achieved by TLC, recrystallizations, and/or vacuum sublimations. Other compounds that did not precipitate during reaction could be isolated by evaporation of DMF or PYR, followed by extraction with hexane and purification by the above techniques. Sephadex LH-20 column chromatography was also useful for removing DMF or PYR, in addition to imidazole and its hydrochloride salt (Im·HCl).

F. Deuterations

The free hydroxyls and amines of SCTASi derivatives of nucleosides were deuterated on a micro-scale by dissolving 1-5 mg of a compound in 250 µl of D_2O/1,4-dioxane (1:1,v/v) and heating at 60° for 2h. This solution was then analyzed by GC/MS, after the GC column and the MS had been flushed with D_2O. Complete deuteration was not achieved, although it was sufficient for interpretation work (approximate yields: Iba, 70%d_2, 30%d_1; Iab, 50%d_2, 40%d_1; Ibb, 60%d_1).

G. Quantitative GC Procedures

Rates of silylations of nucleosides or hydrolyses of silyl derivatives of nucleosides were studied by quantitative GC. Special procedures were required to prevent injector reactions from causing errors in the analyses. Reactions were performed in dry, teflon-lined septum-capped vials (typically, 3ml screw-top vials from Pierce Chemical Co.) containing small teflon-covered magnetic stirring bars (approx. 2mm X 8mm).

A typical study of silylation rate was performed by weighing into the vial approximately 50 µmoles of substrate, adding in the re

amount of solvent (usually PYR or DMF, to give about 0.1M in substrate) containing the internal standard (pyrene or triphenylene) by syringe, sealing the vial, and then dissolving the substrate by stirring. If required, the vial could be snugly fitted into a controlled-temperature aluminum block for rate studies at temperatures other than ambient. The appropriate quantity of reagent was then added by syringe through the septum (amount as determined by the molar proportion of reagent to substrate desired). Timing was started when the reagent had mixed in. The reactions were then monitored at timed intervals by either Method 1 or 2 as appropriate.

Method 1. A 10 μl aliquot of the reaction solution was withdrawn from the vial by syringe. This was added to 20 μl of methanol in a small dry culture tube (50 X 6mm-OD) to quench the silylation reaction and allowed to stand for 5 min. Volatiles were then removed by the combination of a dry nitrogen stream and low pressure, or else by placing on a vacuum rack. The residue was dissolved in 20 μl of dichloromethane and then 0.5 to 2.0 μl portions were subjected to GC analysis.

Method 2. A 10 μl aliquot of the reaction solution was withdrawn from the vial with a syringe and placed in a dry culture tube fitted with a rubber 5mm NMR sample tube septum cap (Kontes Glass Company). To this was added 20 μl of one of BSTFA, TMSiIm, TFAIm, TFAA, or AcAnh reagents to rapidly convert unreacted substrate to a volatile derivative. The mixture was then mixed by vibration and allowed to stand for 10 min or longer. Except for the solutions containing acyl anhydrides, 0.5 to 2.0 μl portions were analyzed directly by GC. When anhydrides were present, the volatiles were removed *in vacuo*, the residue was dissolved in 20 μl of dichloromethane, and then analyzed by GC. Complete derivatization was

usually achieved within 10 min. Incomplete derivatization could be detected from the chromatograms.

Hydrolyses of silyl derivatives by aqueous acetic acid on a small scale were studied in a similar way except that the internal standard was not added until a later stage because of its limited solubility in the hydrolysis media. (The internal standard is still required because it serves to prevent a major source of error, i.e. variability of injection volume.) Reactions were set up by weighing accurately into a vial about 100 μmoles of silyl derivative followed by addition of 80% aqueous acetic acid to the appropriate concentration (typically 0.05M). Reactions were usually performed at room temperature, but other temperatures could be used. Stirring and timing were then started. The reactions were monitored at timed intervals by Method 3a or 3b.

Method 3. An accurately measured 25 μl aliquot of the reaction solution was withdrawn with a syringe (or a micropipette could be used) and placed in a dry culture tube. Volatiles were removed *in vacuo* and the residue was dissolved in 25 μl of pyridine containing the internal standard. Then this solution was treated in one of two ways: (a) 0.5 to 2.0 μl portions were subjected to GC analysis, or (b) 25 μl portions of one of BSTFA, TMSiIm, TFAIm, TFAA, or AcAnh reagents were added and the solution was allowed to stand for 10 min or longer. Except for the solutions containing the acyl anhydrides, portions of the solution were analyzed directly by GC. When acyl anhydrides were present , the volatiles were removed *in vacuo*, the residue dissolved in 25 μl of dichloromethane, and then portions were subjected to GC analysis.

GC analysis and calibration

For all injections the "solvent wash" technique was used, i.e. all

the sample was flushed into the chromatograph by a plug of a suitable solvent (e.g., dichloromethane) separated in the syringe from the sample solution by an air gap.

The co-injection experiments that were used to study injector port reactions were performed by drawing into the syringe the required volumes of the solutions under test, ensuring that there was an air gap between the plugs of the two solutions. In this way no reaction could occur until the sample had reached the injector port. In the case of studies of hydrolysis in the injector port, 1.0 μl of 80% aqueous acetic acid was co-injected with varying amounts of silylated nucleoside to imitate 1.0 μl portions of the hydrolysis medium in the desilylation experiments.

Areas of GC peaks were measured by triangulation or disc integration. Relative molar concentrations were determined from relative peak areas, the known concentration and molar response of the internal standard, and relative molar response (RMR) values for different compounds. The latter were obtained from a response curve derived by injecting different volumes of calibration solutions containing the compounds of interest and the internal standard in known concentrations.

The relative molar response plots for the cholesterol derivatives (Chapter V) were obtained by injecting a number of different volumes of a series of calibration solutions containing the derivatives of interest and triphenylbenzene as internal standard. Such solutions (5,10 and 25mM for each derivative) were prepared by treating aliquots of one standard solution of cholesterol (50mM) and triphenylbenzene (30mM) in pyridine with TMSi, TBDMSi, and TMTBSi reagents (Tri-Sil Z for TMSi; system B for TBDMSi and TMTBSi). Relative molar responses were determined from peak areas relative to that of the internal standard. The peak area of the latter was used to determine the exact amount of derivative injected.

III. CHROMATOGRAPHY OF NUCLEOSIDE DERIVATIVES

A. Introduction

There is considerable interest in the separation and analysis of nucleic acid components (bases, nucleosides, and nucleotides) and their analogs, especially due to their biological importance. Methods that are routinely used include paper chromatography, electrophoresis, ion-exchange chromatography (111-114), and thin-layer chromatography (115-118). In recent years, high performance liquid chromatography (HPLC) has emerged as a very powerful tool for nucleic acid analysis (119-122) and has tremendous potential for future development. Despite the availability of these established methods, the application of gas chromatography is of interest not only as a complementary technique but because of its speed, sensitivity, accuracy in quantitative determinations, and the qualitative information that it can supply (especially in conjunction with mass spectrometry).

Although the GC of nucleic acid bases is relatively straightforward (123-132), nucleosides and nucleotides present one of the most difficult and interesting challenges for gas chromatography. This is due to their multifunctional nature and consequent polarity, low volatility, and thermal lability. Conversion to volatile derivatives is essential for the successful analysis of these compounds. Acetylation (133) and alkylation (133,134) have been examined for some nucleosides, but the most successful method has been trimethylsilylation. Not only are TMSi derivatives of most nucleosides amenable to GC (128-132,135-142) but they are also the preferred derivatives for mass spectrometry (see Chapter IV). Several studies have shown that TMSi derivatives are suitable

for quantitative GC analysis (130-132,140-142). However, there are some problems with cytidine and deoxycytidine. The TMSi cytidine derivative exhibits more severe peak broadening and tailing than other TMSi-nucleosides, while TMSi-deoxycytidine has not as yet been chromatographed. The formation of TMSi/methoxime derivatives has been investigated as a possible solution (137). Preliminary studies have also shown that TMSi derivatives of some nucleotides (nucleoside monophosphates) can be analyzed by GC (131,135,143).

The main concern in this project is to analyze nucleosides which are already derivatized, either partially or fully, with SCTASi groups. The objective of the work reported in this chapter was to investigate chromatographic methods for this purpose, especially ones which could separate isomeric substituted derivatives and provide quantitative information.

There are very few examples of this type of problem in the GC literature. The closest one is the preliminary investigation of isomeric ribonucleoside monophosphates by Hashizume and Sasaki (143). In that case, the separation of 5'-nucleotides from 2'(3')-nucleotides was achieved using TMSi derivatives, but problems were encountered with 2' $\leftrightarrow$ 3' migration of a 2'- or 3'-phosphate group during trimethylsilylation. The separation of isomeric partially methylated sugar derivatives is another example of a similar problem. For these, conversion to mixed TMSi/Me derivatives has been useful for GC/MS studies (144-147). Finally, the preparation of partial TMSi derivatives of various types of compounds (sugars, steroids, etc.) followed by GC analysis has been proposed as a method for "fingerprinting" compounds or investigating their structure (148-151).

At the beginning of this project, it was anticipated that the SCTASi derivatives of at least some nucleosides should be amenable to GC, since the successful gas phase analysis of TBDMSi-prostaglandins had already been demonstrated (63), and it was expected that SCTASi derivatives should not behave drastically different from TMSi derivatives. Also, the derivatives were expected to have good liquid chromatographic properties due to the low polarity of silyl ethers and the stability of SCTASi ethers towards hydrolysis. In addition, Ogilvie (65) initially demonstrated that the TBDMSi derivatives of nucleosides were suitable for TLC.

The results below demonstrate the suitability of both liquid and gas chromatographic methods for this problem. Only a preliminary investigation of HPLC techniques has been made, and the TLC data for a wider range of compounds are presented in the reports dealing with synthetic aspects (69,83,85). The main emphasis in this chapter is on gas phase methods, but it should be noted that LC methods play an important complementary role in gas phase analytical schemes (see Figure 1).

B. Results and Discussion

1. Deoxynucleoside Derivatives

The chromatographic data for the various deoxynucleoside derivatives examined are presented in Table III. Some of these derivatives are of interest for analytical applications of SCTASi derivatization and are discussed further in Chapter V.

a) Liquid Chromatography

All the SCTASi derivatives of deoxynucleosides were suitable for TLC analysis. The derivatives of the less polar nucleosides (thymidine, deoxyuridine, and deoxyadenosine) are quite mobile, allowing the use of low polarity, volatile solvents such as diethyl ether. This is a definite

TABLE III: Numbers, names, melting points, and chromatographic data for 2'-deoxynucleoside derivatives.

Compound[a] Number	Compound Name[a]	Melting Point (°C)	R_f TLC[b] A	B	C	GC DATA[c] $I^{230°}_{OV-1}$	$I^{260°}_{OV-1}$	$I^{290°}_{OV-1}$	Isomer Pairs' α
Partial O-SCTASi Derivatives									
Iab	3'-O-TBDMSi-thymidine	83-84	0.52	0.69	0.79	2587			1.104
Iba	5'-O-TBDMSi-thymidine	198-199	0.36	0.60	0.74	2621			
Iad	3'-O-TMIPSi-thymidine	57-64	0.52	0.69		2851			1.077
Ida	5'-O-TMIPSi-thymidine	139-140	0.36	0.60		2876			
Iac	3'-O-TMTBSi-thymidine	72-73	0.52	0.69		2872			1.112
Ica	5'-O-TMTBSi-thymidine	182-183	0.36	0.60		2909			
IIab	3'-O-TBDMSi-deoxyuridine		0.41	0.59		2558			1.080
IIba	5'-O-TBDMSi-deoxyuridine		0.38	0.61		2584			
IIIab	3'-O-TBDMSi-deoxycytidine	163-165	0.00	0.00	0.40	d			
IIIba	5'-O-TBDMSi-deoxycytidine	240-242	0.00	0.00	0.46	d			
IVab	3'-O-TBDMSi-deoxyguanosine	82-84	0.00	0.00	0.50	d			
IVba	5'-O-TBDMSi-deoxyguanosine	dec>230	0.00	0.00	0.30	d			
Vaba	3'-O-TBDMSi-deoxyadenosine	216-217		0.36	0.76	2731			1.022
Vbaa	5'-O-TBDMSi-deoxyadenosine	165-166		0.23	0.70	2739			
Mixed Ac/SCTASi Derivatives									
Igb	3'-O-TBDMSi-5'-O-Ac-thymidine		0.49			2644			1.006
Ibg	3'-O-Ac-5'-O-TBDMSi-thymidine		0.52			2646			
Igd	3'-O-TMIPSi-5'-O-Ac-thymidine		0.49			2897			1.015
Idg	3'-O-Ac-5'-O-TMIPSi-thymidine		0.52			2892			

continued...

TABLE III (continued)

		R_f TLC [b]			GC DATA[c]			
		A	B	C	$I^{230°}_{OV-1}$	$I^{260°}_{OV-1}$	$I^{290°}_{OV-1}$	Isomer Pairs' α
Igc	3'-O-TMTBSi-5'-O-Ac-thymidine	0.49			2917			1.033
Icg	3'-O-Ac-5'-O-TMTBSi-thymidine	0.52			2928			
Vgba	3'-O-TBDMSi-5'-O-Ac-deoxyadenosine	0.17			2798			1.009
Vbga	3'-O-Ac-5'-O-TBDMSi-deoxyadenosine	0.21			2795			
Vgbg	3'-O-TBDMSi-5'-O-Ac-N6-Ac-deoxyadenosine	0.40			3079			1.063
Vbgg	3'-O-Ac-5'-O-TBDMSi-N6-Ac-deoxyadenosine	0.43			3058			
Mixed TFA/SCTASi Derivatives								
Iib	3'-O-TBDMSi-5'-O-TFA-thymidine	h			2437			1.108
Ibi	3'-O-TFA-5'-O-TBDMSi-thymidine	h			2403			
Iid	3'-O-TMIPSi-5'-O-TFA-thymidine	h			2681			1.099
Idi	3'-O-TFA-5'-O-TMIPSi-thymidine	h			2649			
Iic	3'-O-TMTBSi-5'-O-TFA-thymidine	h			2710			1.082
Ici	3'-O-TFA-5'-O-TMTBSi-thymidine	h			2684			
Vibi	3'-O-TBDMSi-5'-O-TFA-N6-TFA-deoxyadenosine	h			2666			1.312
Vbii	3'-O-TFA-5'-O-TBDMSi-N6-TFA-deoxyadenosine	h			2574			
Mixed TMSi/SCTASi Derivatives								
Ieb	3'-O-TBDMSi-5'-O-TMSi-thymidine	0.74h			2616			1.014
Ibe	3'-O-TMSi-5'-O-TBDMSi-thymidine	0.74			2621			
Ied	3'-O-TMIPSi-5'-O-TMSi-thymidine	0.74h			2870			1.037
Ide	3'-O-TMSi-5'-O-TMIPSi-thymidine	0.74			2858			
Iec	3'-O-TMTBSi-5'-O-TMSi-thymidine	0.74h			2881			1.031
Ice	3'-O-TMSi-5'-O-TMTBSi-thymidine	0.74			2892			

continued...

	TABLE III (continued)	Melting Point(°C)	R_f TLC[b] A	B	C	GC DATA[c] $I^{230°}_{OV-1}$	$I^{260°}_{OV-1}$	$I^{290°}_{OV-1}$	Isomer Pairs' α
Veba	3'-O-TBDMSi-5'-O-TMSi-deoxyadenosine		0.39[h]			2734			1.010
Vbea	3'-O-TMSi-5'-O-TBDMSi-deoxyadenosine		0.39			2737			
Vebe	3'-O-TBDMSi-5'-O-TMSi-N6-TMSi-deoxyadenosine		h			2841			1.028
Vbee	3'-O-TMSi-5'-O-TBDMSi-N6-TMSi-deoxyadenosine		h			2832			
Mixed SCTASi Derivatives									
Idb	3'-O-TBDMSi-5'-O-TMIPSi-thymidine		0.74				3107		1.017
Ibd	3'-O-TMIPSi-5'-O-TBDMSi-thymidine		0.74				3114		
Ibc	3'-O-TMTBSi-5'-O-TBDMSi-thymidine		0.74				3140		1.014
Icb	3'-O-TBDMSi-5'-O-TMTBSi-thymidine		0.74				3146		
Idc	3'-O-TMTBSi-5'-O-TMIPSi-thymidine		0.74				3386		1.025
Icd	3'-O-TMIPSi-5'-O-TMTBSi-thymidine		0.74				3396		
bis-O-Acyl Derivatives									
Igg	3',5'bis-O-Ac-thymidine					2439			
Iii	3',5'bis-O-TFA-thymidine					2032(205°)			
bis-O-TMSi Derivatives									
Iee	3',5'bis-O-TMSi-thymidine		0.74[h]			2382			
IIee	3',5'bis-O-TMSi-deoxyuridine		0.71[h]			2336			
Veea	3',5'bis-O-TMSi-deoxyadenosine		0.39[h]			2496			
bis-O-SCTASi Derivatives									
Ibb	3',5'bis-O-TBDMSi-thymidine	144-145	0.74	0.85	0.90	2837	2866		
Idd	3',5'bis-O-TMIPSi-thymidine		0.74				3356		
Icc	3',5'bis-O-TMTBSi-thymidine	113-114	0.74				3416		

continued...

	TABLE III (continued)	Melting Point(°C)	R_f TLC [b] A	B	C	GC DATA[c] $I^{230°}_{OV-1}$	$I^{260°}_{OV-1}$	$I^{290°}_{OV-1}$	Isomer Pairs' α
IIbb	3',5'bis-O-TBDMSi-deoxyuridine		0.71	0.83		2799	2826		
IIIbb	3',5'bis-O-TBDMSi-deoxycytidine	188-189		0.12	0.64	d			
IVbb	3',5'bis-O-TBDMSi-deoxyguanosine	dec>265		0.07	0.56	d			
Vbba	3',5'bis-O-TBDMSi-deoxyadenosine	132.5-133	0.39	0.55	0.77	2954	2985		
Vdda	3',5'bis-O-TMIPSi-deoxyadenosine		0.39					3520	
Vcca	3',5'bis-O-TMTBSi-deoxyadenosine		0.39					3594	
N,O-perSilyl Derivatives of V									
Veee	3',5'bis-O-TMSi-N6-TMSi-deoxyadenosine		h			2607			
Vbbe	3',5'bis-O-TBDMSi-N6-TMSi-deoxyadenosine		h				3076		
Vbbb	3',5'bis-O-TBDMSi-N6-TBDMSi-deoxyadenosine		0.70			3205	3278		
Vbbc	3',5'bis-O-TBDMSi-N6-TMTBSi-deoxyadenosine		0.70				3598		
Vddd	3',5'bis-O-TMIPSi-N6-TMIPSi-deoxyadenosine		0.70					4070	
Vccc	3',5'bis-O-TMTBSi-N6-TMTBSi-deoxyadenosine		0.70					4183	

[a] Refer to Scheme 3 for numbering system and structures.

[b] TLC developing solvent system: A = diethyl ether; B = ethyl acetate; C = chloroform/ethanol (8:2,v/v).

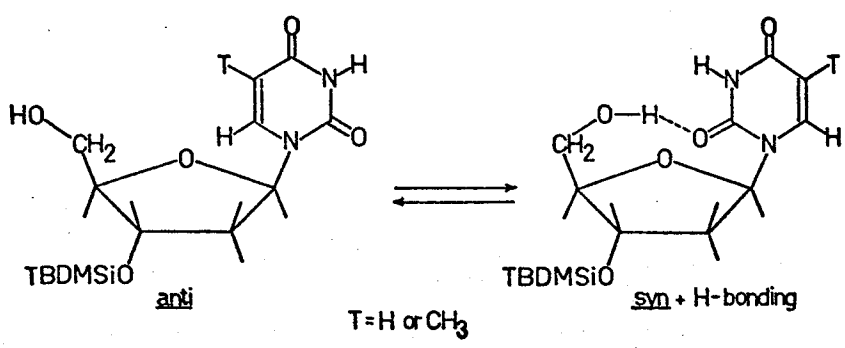
[c] I = Kovats' retention index; α = separation factors for isomer pairs ($\geqslant 1$, always)

[d] Decomposition during GC.

[h] Hydrolysis occurs during TLC (complete if no R_f value given also)

advantage for both preparative and analytical work. The results reported in Table III indicate that the type of silyl group has no detectable influence on the relative mobility. However, the number of silyl groups and the position of substitution in partial derivatives have important effects on R_f values. Thus, the fully protected compounds have the greatest mobility, while for the partially protected derivatives, the 3'-O-SCTASi compounds usually move faster than their 5'-O-SCTASi isomers. The exceptions are deoxycytidine and deoxyuridine, where the order of partial derivative mobility is reversed.

It is interesting that a change in the base structure (especially in the case of thymidine(I) → deoxyuridine(II), where only a change of CH_3 → H occurs) should influence the separation of sugar substituted isomers so dramatically. Examination of the data reveals that the 5'-O-TBDMSi derivatives of I and II have similar R_f values (0.36 and 0.38, resp.), while 3'-O-TBDMSi-II moves much slower (i.e., is more "polar") (R_f = 0.41) than 3'-O-TBDMSi-I (R_f = 0.52). One possible theory to explain this phenomenon is that the degree of intramolecular H-bonding between the 5'-OH and the C_2=O of the base in the 3'-O-silyl derivatives (and therefore the "polarity" and subsequent TLC mobility) is influenced by the C5 substituent (T). Such H-bonding (in the <u>syn</u> conformation) has been demonstrated for pyrimidine nucleosides in organic solvents (152). A



methyl group at C_5 may enhance this H-bonding (thus reducing the polarity and increasing the mobility) by either increasing the proportion of syn/anti conformations or by some inductive effect. Another theory is that the orientations of isomeric molecules on the silica surface are different and the contribution of the base to the binding to the surface (via its carbonyl groups) may be reduced when the C_5 methyl group is present in the case of the 3'-O-silyl isomers.

TLC was very useful in this project for the preparative isolation of crystalline samples of most derivatives, as a preliminary chromatographic step prior to GC, and for fast analysis of samples to confirm GC results. It was also possible to perform TLC/MS analyses successfully (see Experimental).

HPLC was investigated only very briefly because an instrument was not available until near the end of this project. Figure 14 illustrates the successful HPLC analysis of a mixture of bis- and mono-TBDMSi derivatives of thymidine using a silica column and ethyl acetate as a mobile phase. As expected, the order of relative mobilities was the same as that observed with TLC. Measurement of peak areas by disc integration indicated that the method gives excellent quantitative results.

b) Gas Chromatography

The GC retention data for deoxynucleoside derivatives are reported in Table III, using the isothermal Kovats' retention index system (101). Kovats' retention indices, or I values, express the retention times of compounds with respect to the retention times of a series of even-numbered n-alkane (C_zH_{2z+2}) standards, the indices of which are defined as 100z. Since the logarithms of the retention times of n-alkanes increase linearly with chain length, the I value of any substance is calculated by

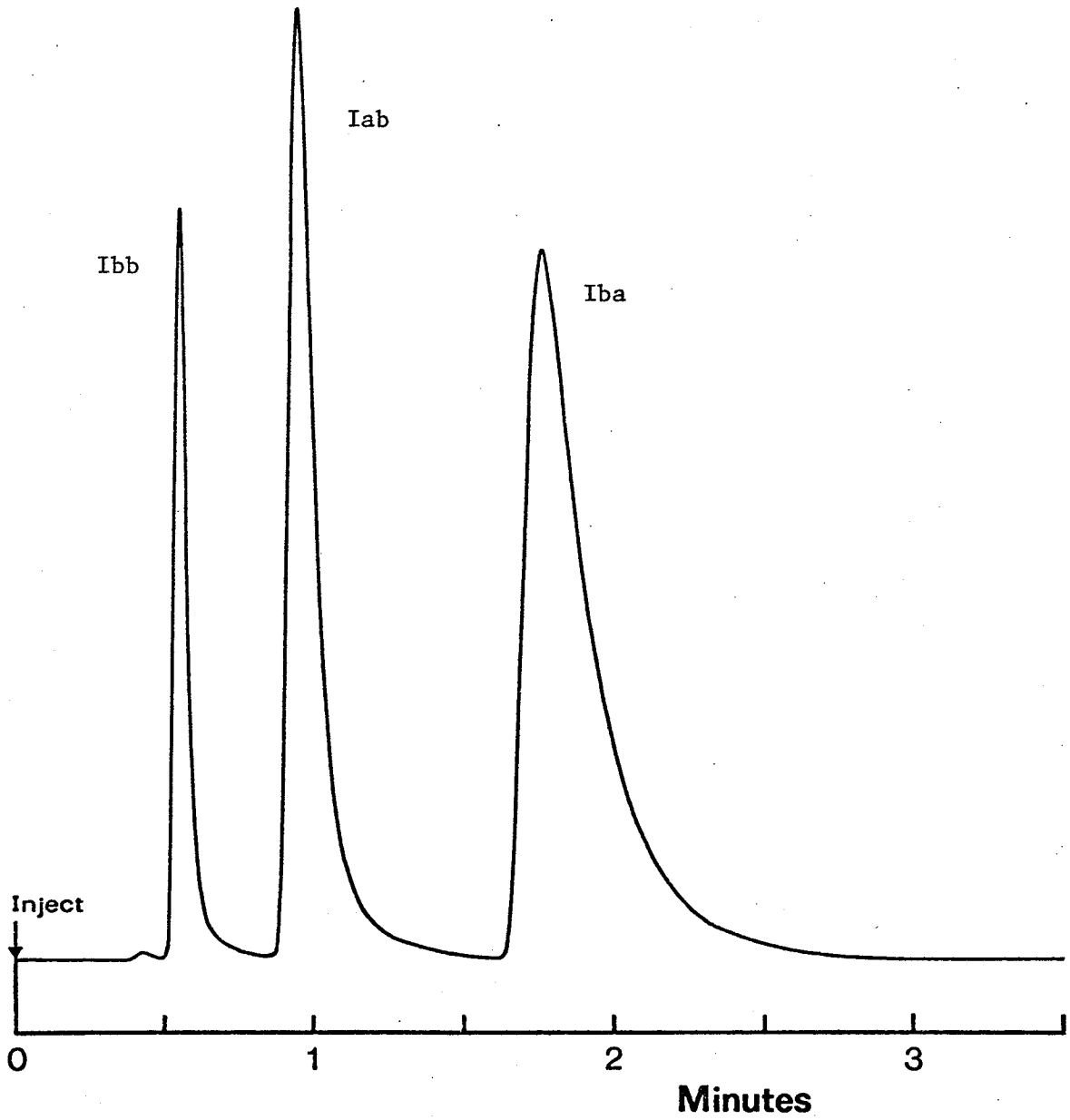


Figure 14. High performance liquid chromatogram of a mixture of 3',5'-bis-O-TBDMSi-thymidine (Ibb, 1.6 μg), 3'-O-TBDMSi-thymidine (Iab, 2.5 μg), and 5'-O-TBDMSi-thymidine (Iba, 4.8 μg). Conditions: 3mm-ID X 0.25 m column packed with 5μ Spherisorb Silica; 3.2 ml/min ethylacetate mobile phase; UV detection at 280 nm.

a linear interpolation of the plot of log t_r' (t_r' = retention time adjusted for gas hold-up time) versus 100z determined for the alkane standards under identical conditions. Each compound has a specific I value for a certain stationary liquid phase. Retention indices show very good inter-laboratory reproducibility, since they are almost independent of operating conditions and column dimensions. In addition, they are very valuable because their use can reveal a great deal of structural information (153). The analogous programmed temperature methylene unit (MU) indices (I ≃ 100MU) have been used previously for TMSi derivatives of nucleosides (129,139).

It should be mentioned here, that for all compounds studied, mass spectra were run on the eluted GC peaks to ensure that no alteration in structure had occurred during chromatography. This was done by trapping eluted substances in glass capillary traps, using the splitter/collector system described in Experimental. This system also allowed TLC analysis on collected material, or else further derivatization followed by a second GC analysis.

It should also be noted that columns with 10% stationary liquid phase loading were used. Columns with a lower percentage of liquid phase (1-3%), or else capillary columns, should have been investigated since they would have allowed lower analysis temperatures (and higher resolution). However, in this project, GC was primarily a tool for fast exploratory analyses and for isolating samples for further studies (MS, TLC, derivatization, etc.). The higher capacity of packed 10% phase columns was an advantage for the latter.

The results in Table III show that all the partially and fully silylated derivatives of thymidine, deoxyuridine, and deoxyadenosine could

be chromatographed on columns packed with 10%OV-1 (a nonpolar liquid phase) coated on Gas Chrom Q support (columns A or C). Other liquid phases were not examined in any detail for the nucleoside derivatives. Initial experiments on the partial O-SCTASi derivatives with column B (10% OV-17, a medium polarity phase) did not appear promising: compounds such as Iba and Iab did not chromatograph under any conditions studied. However, both OV-1 and OV-17 columns were used for SCTASi derivatives of steroids, as reported in Chapter V.

With the OV-1 column, isomeric partial O-SCTASi derivatives were separable from each other as well as from the bis-O-SCTASi derivatives. This is illustrated for compounds Iab, Iba, and Ibb in Figure 15. The order of elution for all deoxynucleoside derivatives was 3'-O-SCTASi < 5'-O-SCTASi < 3',5'bis-O-SCTASi. The separation factors (α) for the isomeric partial derivatives (see Table III) were large enough for separation on medium resolution columns. The behavior of the partial derivatives was not ideal however, since they suffered a slight amount of thermal decomposition during chromatography (vide infra) and exhibited bad tailing when injected at trace levels. The fully protected derivatives behaved well, and in Chapter V, they are proposed as useful "analytical" derivatives.

No success was achieved with the O-SCTASi derivatives of deoxycytidine(III) and deoxyguanosine(IV). This was not surprising since these nucleosides are difficult to analyze by GC even as N,O-perTMSi derivatives. Further derivatization of the SCTASi derivatives of III and IV should be examined as a possible solution. This has not yet been studied; TLC/MS techniques were sufficient for the objectives of this project.

However, the further derivatization of the partial O-SCTASi

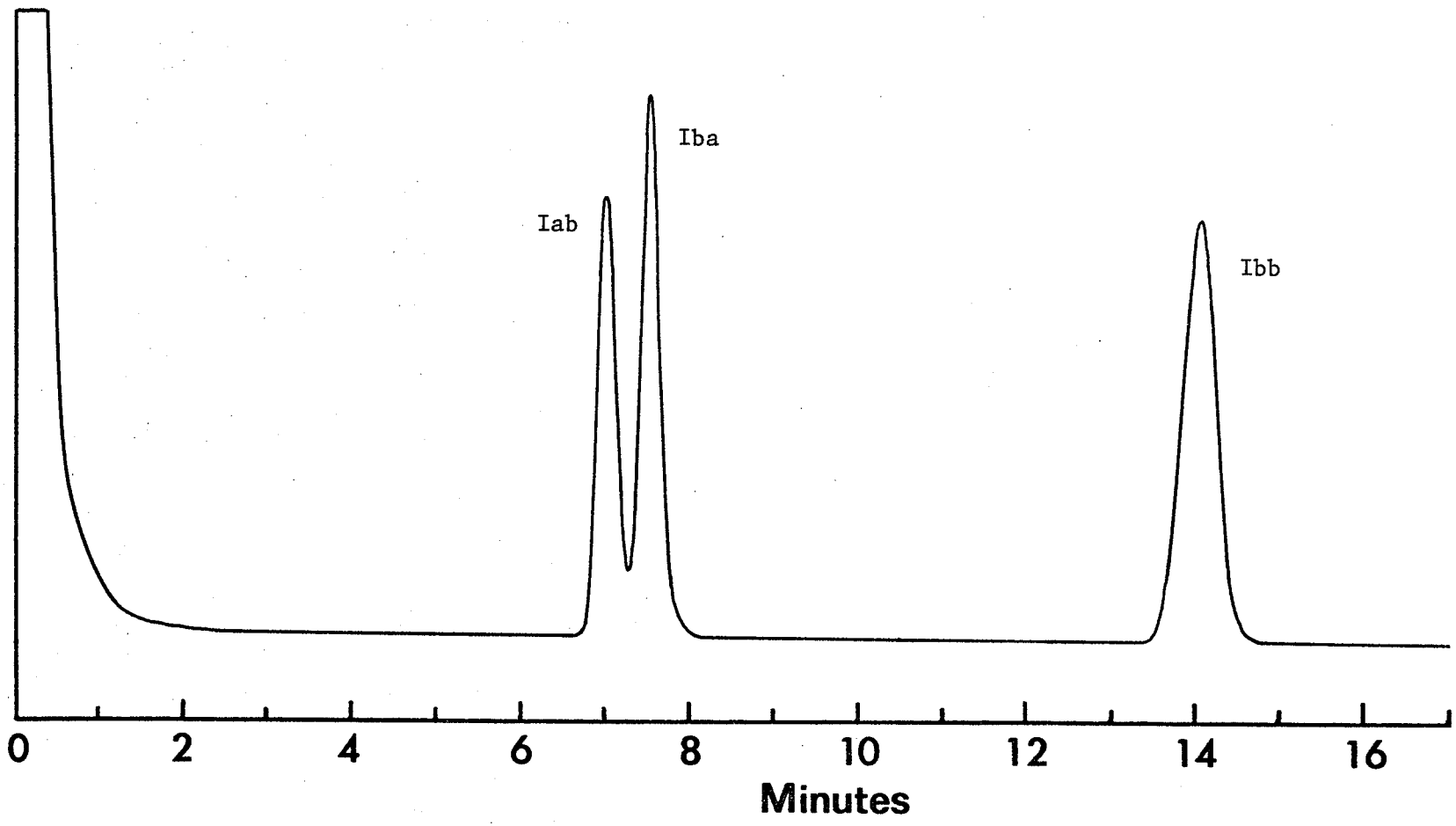


Figure 15. Gas chromatogram of a mixture of 3'-O-TBDMSi-thymidine (Iab), 5'-O-TBDMSi-thymidine (Iba), and 3',5'bis-O-TBDMSi-thymidine (Ibb). Conditions: column C (10% OV-1, 3.75m X 2.4mm-ID), 280°, 30 ml/min N_2 carrier.

derivatives of thymidine and deoxyadenosine was examined in detail. This should lay the groundwork for future studies with the more labile nucleosides. Acetylation, trifluoroacetylation, and trimethylsilylation (to prepare the mixed Ac/SCTASi, TFA/SCTASi, and TMSi/SCTASi derivatives) were studied in the hope that GC properties (peak symmetry, volatility, and thermal stability) could be improved, while still allowing separation of isomers. These procedures would also allow the simultaneous analysis of underivatized nucleosides that might be present in samples (e.g., silylation reaction solutions). Furthermore, it was of interest to examine the mass spectral fragmentations of such mixed derivatives (see Chapter IV). Table III also lists the retention data for the mixed SCTASi derivatives of thymidine (prepared by further SCTASi derivatization of partial O-SCTASi derivatives), and a series of N6-protected deoxyadenosine derivatives. The former are of interest for mass spectral studies, while the latter are valuable for quantitative GC studies where complete N,O-derivatization is essential.

Figure 16 presents a diagrammatic summary of the results for the mixed derivatives of thymidine. The relative retention times of pairs of isomeric substituted derivatives are plotted as a function of the 3'- and 5'-hydroxyl protecting groups. There appears to be a certain amount of correlation between the steric bulk of silyl groups and the degree of separation. Such an effect has been observed more clearly for the SCTASi derivatives of isomeric steroids and is discussed in Chapter V. From these results, it is obvious that trifluoroacetylation is the only derivatization procedure that allows sufficient separation of isomers. The most dramatic separation is that achieved for the N,O-bis-TFA derivatives of isomeric mono-TBDMSi-deoxyadenosines, Vbii and Vibi (α = 1.31). Also,

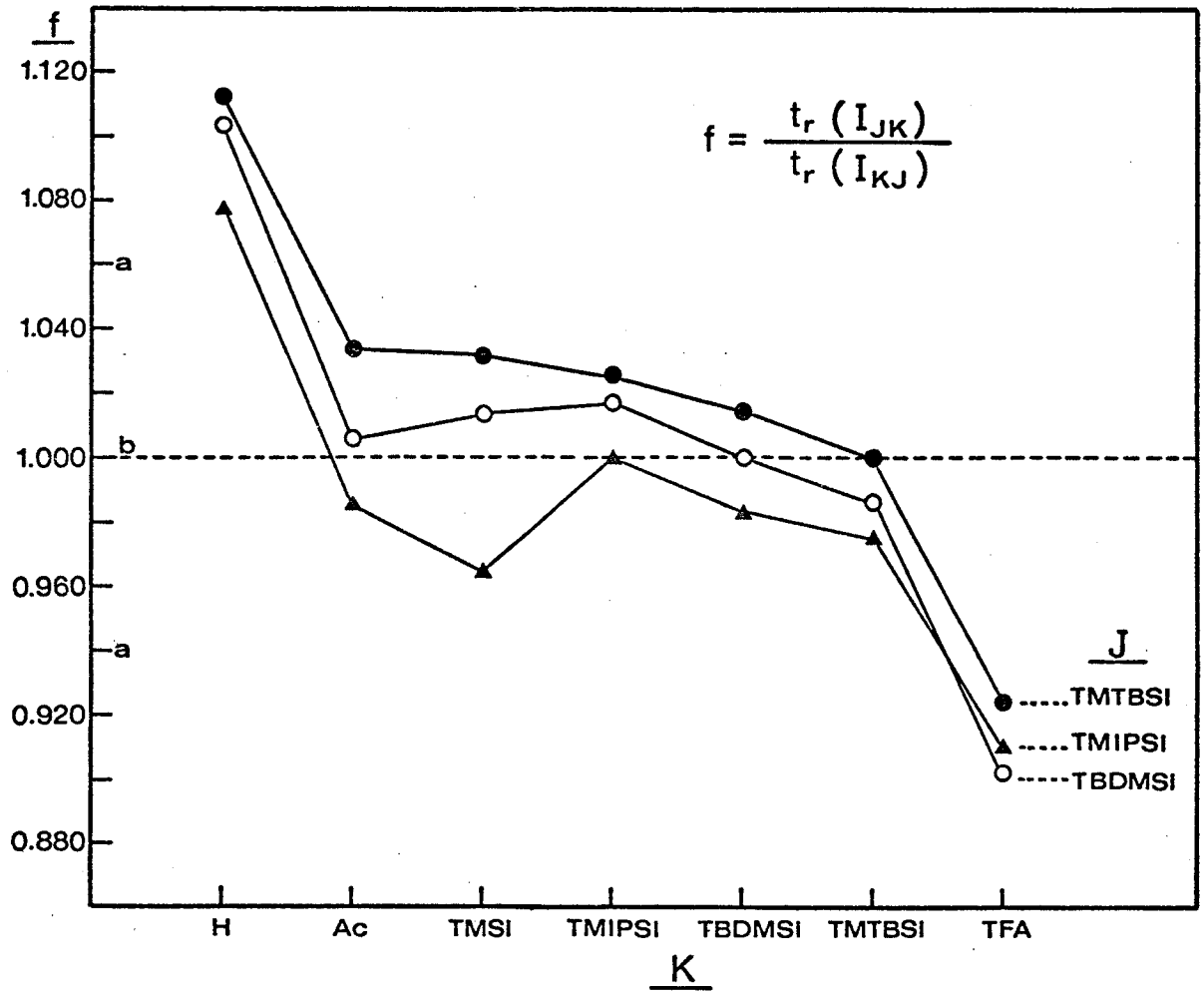


Figure 16. Diagrammatic summary of the relative retention times of pairs of isomeric substituted thymidine derivatives as a function of the hydroxyl protecting groups, J and K. I_{JK} = 5'J - 3'K - thymidine; I_{KJ} = 5'K - 3'J - thymidine; t_r = retention time; f = relative retention time of I_{JK} with respect to I_{KJ}; b = no separation possible; a = 98% resolution of isomer pair on a column of 5000 plates (typically, a good 2.5m X 2mm-ID column).

the mixed TFA/SCTASi derivatives are the most volatile derivatives examined and, interestingly, the isomers are eluted in an order reversed from that of the partial O-SCTASi isomers. Although the other isomeric mixed derivatives do not separate easily, they may still be valuable for analytical work. Since their mass spectra are quite different, it should be possible to perform GC/MS-SIR analyses. Unfortunately, the necessary instrumentation for this technique was not avilable for this project.

Another interesting aspect of the GC data for the SCTASi derivatives of nucleosides is the relationship between retention indices and substituent groups. Figure 17 illustrates this relationship for various derivatives of thymidine, deoxyadenosine, and uridine (a ribonucleoside: see Table VIII for data). Although there is no linear relationship, the retention index increases with the mass of the silyl group (or the molecular weight of the derivative). It is also observed from this figure that the I value is very dependent upon the number of SCTASi groups. On the other hand, examination of the data in Table III reveals that the retention index is relatively insensitive to the number of TMSi groups. For example, trimethylsilylation of OH and/or NH functions in partial O-SCTASi derivatives leads to only small increases in I values (e.g., for Vbaa, Vbea, and Vbee, the I_{OV-1} values are 2739, 2737, and 2832, respectively).

From the data in Table III (and Table VIII) it was possible to determine the average retention index increments (δI^{OV-1}_{J-K}) that occur with the change of a single silyl group (K → J). These values, which are listed in Table IV, can be used to predict the retention indices of new derivatives and are valuable for determining structural information from the measured retention indices of unknown compounds (e.g., the number of

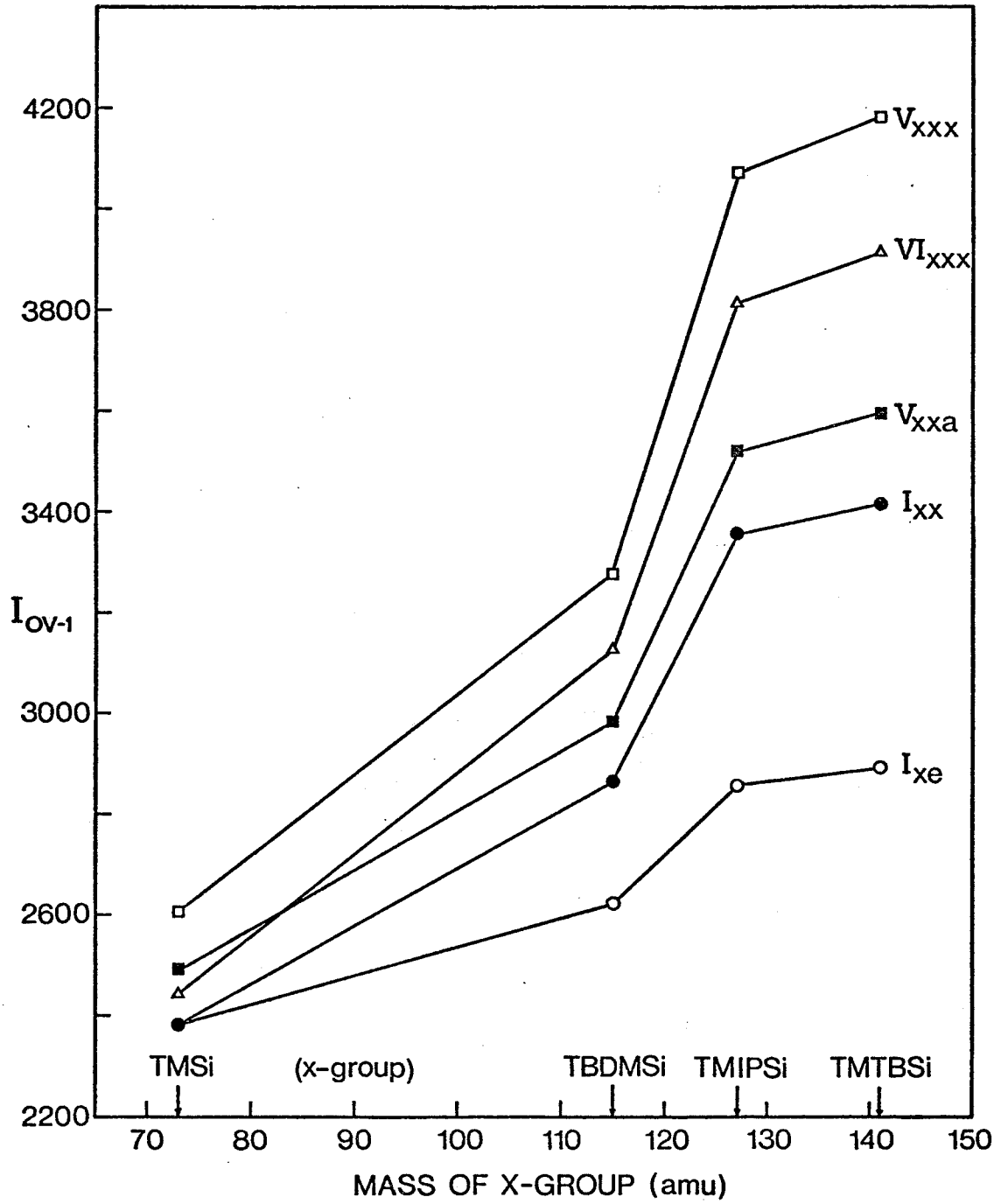


Figure 17. Retention indices of a number of nucleoside derivatives plotted as a function of silyl substituent mass: Ixe = 3'-O-TMSi-5'-O-X-thymidine; Ixx = 3',5'bis-O-X-thymidine; Vxxa = 3',5'bis-O-X-deoxyadenosine; VIxxx = 2',3',5'tris-O-X-uridine; Vxxx = 3',5',N6-tris-X-deoxyadenosine. The relative slopes of the curves are proportional to the number of SCTASi groups in the compounds.

TABLE IV. Average values for the retention index increment (δI^{OV-1}_{J-K}) due to the change of one silyl group ($K \rightarrow J$) in nucleoside derivatives.

	δI^{OV-1}_{J-K}			
K J =	TMSi	TBDMSi	TMIPSi	TMTBSi
TMSi	0	225	470	500
TBDMSi	-225	0	250	280
TMIPSi	-470	-250	0	30
TMTBSi	-500	-280	-30	0

silyl groups). Since the δI values are additive for the change of more than one silyl group, the following equation can be used to calculate the approximate retention indices (±50) of various silylated nucleosides:

$$I^{230°}_{OV-1} = N + 225b + 500c + 470d$$

where b, c, and d are the number of TBDMSi, TMTBSi, and TMIPSi groups respectively, and N is the retention index at 230° for the corresponding TMSi derivative. N values that can be used are: Iee = 2382, IIee = 2336, Veea = 2496, Veee = 2607, VIeee = 2444, VIIeee = 2481, VIIIeeea = 2573, and VIIIeeee = 2647. (This equation is not suitable for mixed acyl/SCTASi and partial SCTASi derivatives. The indices of such compounds are best calculated by single increment analysis (i.e., using δI values (Table IV) and I values of closely related compounds already reported in Tables III and VIII).) It should be noted that minor variations between calculated and measured values do occur due to isomeric differences, and compensation must also be made for different column temperatures. Retention indices and δI values are discussed further in Chapter V for the SCTASi derivatives of steroids.

c) Quantitative Analysis by GC

Another aspect of gas chromatography that has been investigated in this study is the application of quantitative methods to SCTASi derivatives of nucleosides. Although the theory and techniques of quantitative analysis by GC have been reviewed recently by Novak (154), there are a number of problems, peculiar to the analysis of complex compounds of biological interest, that should be discussed here. One of the most common sources of error is the decomposition or transformation of a compound being analyzed into another substance. Usually this involves a thermally-induced reaction in the injector port and/or on the column. A number of studies (155-167) have reported such problems for a variety of compounds. For example, partial decomposition to a non-volatile product can lead to the loss of a certain amount of a compound during chromatography. It is a serious error to assume that the area of a GC peak accurately represents the quantity of substance injected into the GC. Only a response curve (peak area vs. amount injected) for the compound being quantitated will determine if the response is linear and if there is any decomposition. A confusing situation can arise if intermolecular reactions or intra-molecular rearrangements take place in the injector port. It is important that tests be made for such occurrences and that the identities of all peaks being quantitated be confirmed by methods such as GC/MS. Furthermore, when analyses involve a derivatization step, it is important that only single products are formed and in quantitative yield. Multiple products due to incomplete derivatization or side-reactions have been sources of error and confusion in several studies (168-170). For this preliminary study of SCTASi derivatives of nucleosides, the main emphasis was on the detection of practical problems such as these and the development of

appropriate solutions.

For the deoxynucleoside derivatives, all GC peaks were analyzed by MS. No rearrangements were observed.
However, as shown later, injector port intermolecular reactions were observed under certain conditions and intramolecular rearrangements occur for some ribonucleoside derivatives.

The response curves were measured for a number of derivatives to assess their suitability for quantitation. Figure 18 illustrates the results for 3',5'bis-O-TBDMSi-thymidine(Ibb) and 5'-O-TBDMSi-thymidine (Iba) determined by internal standardization experiments (see Experimental). As shown by the straight line plots, both the partially and fully silylated derivatives give a linear response. However, the mono-TBDMSi-thymidines (both 3' and 5' isomers) suffered a slight amount of thermal decomposition in the injector port and/or on the column (indicated by the curve for Iba which passes through the x-axis rather than the origin) and tailed badly at trace levels. The fully protected thymidines, on the other hand, behaved well during chromatography (symmetrical peaks and no detectable decomposition). It is still possible to perform quantitative analysis on the partial derivatives, since the decomposition is only slight and the response is linear, but further derivatization appears to be a more favorable approach. It should be noted that for all derivatives, it was necessary to use an all-glass system (glass column and glass inserts in the injector and detector) to avoid decomposition due to contact with heated metallic parts. This effect has been observed for the TMSi derivatives of other compounds (155,156). Also, the column was treated periodically with silylating reagents ("Silyl-8", Pierce Chemical Co.) to block any active sites that could cause irreversible adsorption and tailing

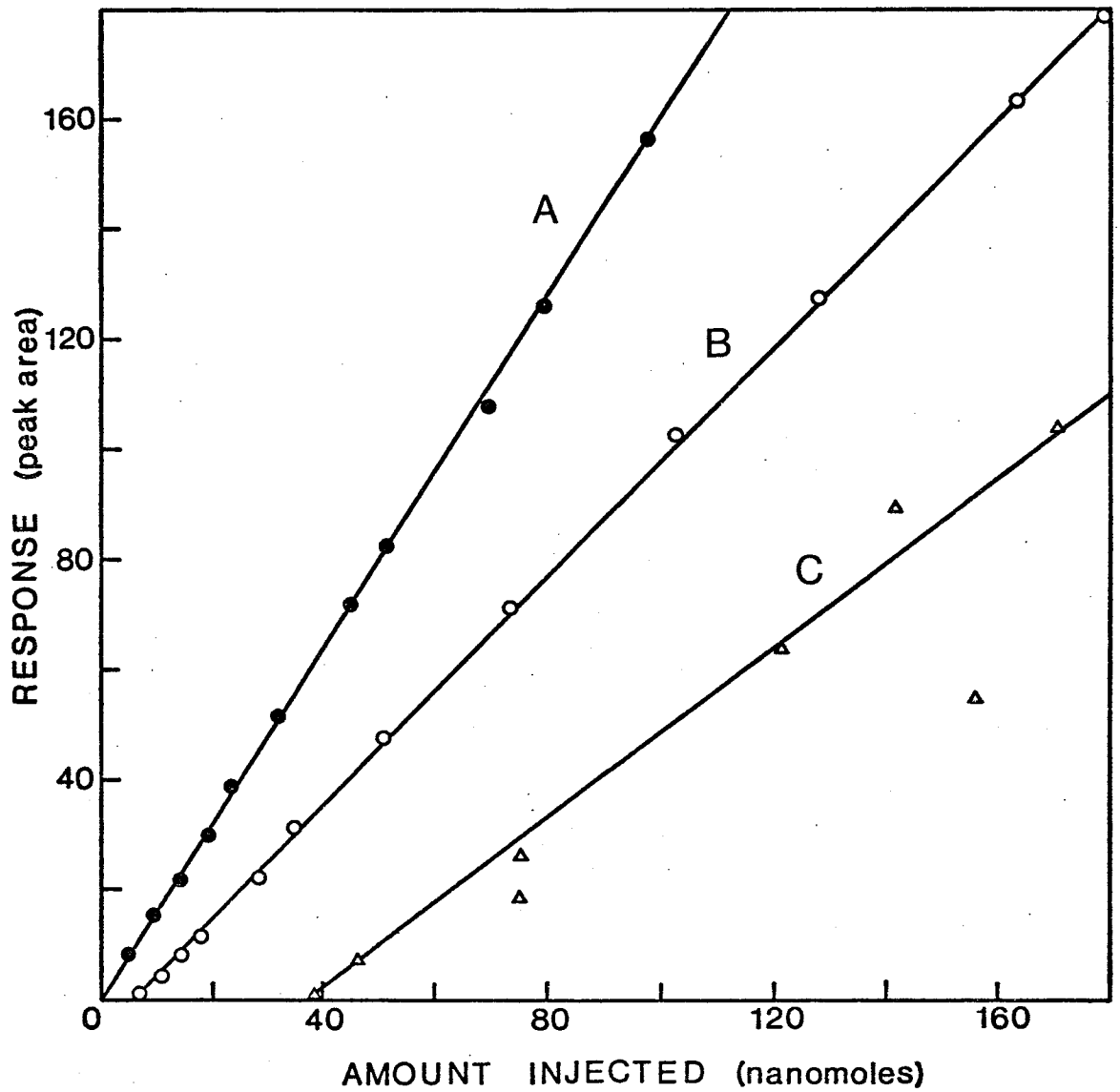


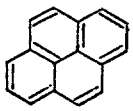
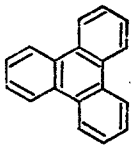
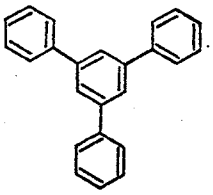
Figure 18. Detector response curves for deoxynucleoside derivatives: (A) 3',5'bis-O-TBDMSi-thymidine, Ibb; (B) 5'-O-TBDMSi-thymidine, Iba. The exact amount injected was determined by internal standardization. The relative molar response (RMR) of Ibb with respect to Iba is 1.56 ± 0.05 as determined from the ratio of the slopes of A and B.

(C) results from the co-injection of varying amounts of Iba with 1.0 µl of 80% aqueous acetic acid and demonstrates the effect of the presence of acid upon the quantitation of silylated nucleosides.

of these polar compounds.

For the accurate determination of concentrations of compounds in a sample, the measurement of relative molar response (RMR) values and the use of internal standards are essential. For this study, RMR values were determined from the relative slopes of their response curves (which were measured relative to an internal standard). Thus, the RMR of Ibb with respect to Iba was 1.56±0.05 which, as expected, is close to that calculated by taking the ratio of C-H bonds in the two compounds (1.58). Table V lists the internal standards used in this study. They were selected according to the following requirements of an internal standard: (a) it must yield a completely resolved peak, (b) should not be present in the original samples, (c) must not react chemically with the sample components, (d) must not decompose to any extent, should give a linear response, and should remain in solution, (e) should elute close to the component(s) being measured, and (f) should be present in about the same concentrations as the component(s) being measured.

TABLE V. Internal standards for quantitative GC analysis.

		I_{OV-1}	
		230°	260°
	Pyrene	2114	
	Triphenylene	2411	
	Triphenylbenzene	2829	2872

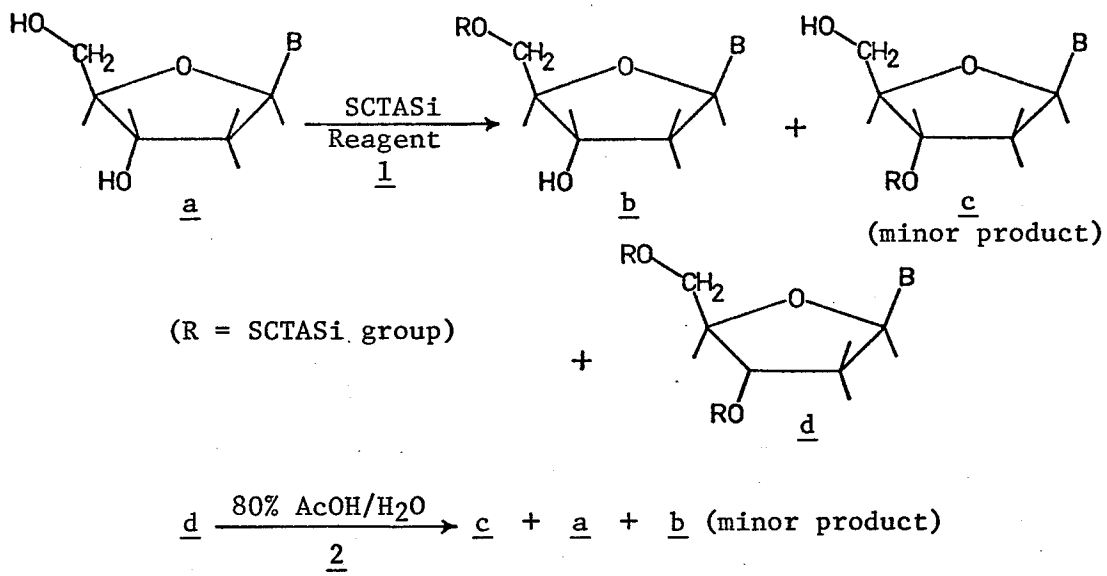
i) Methods

The main interest in developing quantitative methods for SCTASi derivatives of nucleosides was to apply them to monitoring the progress of silylation and desilylation reactions (such as those in Scheme 6) for the purpose of optimizing reaction conditions and yields, as well as to study reaction rates. However, the direct monitoring of such reactions is complicated by interfering intermolecular reactions in the GC injector port.

Silylation Reactions

Normally, analytical silylation reactions are rapid and the compounds under investigation are arranged to be completely derivatized before GC analysis is performed. The reaction solution containing the derivatized compound and excess silyl donor is simply injected into the GC and the analysis is straightforward. However, with some SCTASi reagents

Scheme 6



(e.g., Reagents C, D, and E), or for compounds with sterically hindered hydroxyls, complete derivatization may take several hours. When attempting to determine the rates of SCTASi derivatization (e.g., Scheme 6.1) by GC it was found that an accelerated reaction between underivatized substrates and excess silyl donor can occur in the hot injector port, thus leading to errors in quantitation. This effect was not totally unexpected, since adventitious trimethylsilylation has been observed for phenols injected onto a column previously used for TMSi reagents (171) and injector port silylation of hydroxylated compounds has been reported as a fast method of analysis (172-175). The effect was proven by a "coinjection" of separate solutions of reagent and substrate, being careful to avoid any mixing of solutions until they had entered the injector port. (A rather similar technique, called "sandwich" injection, has been proposed by other investigators (176) but no injector port silylation effect was observed.) The extent of injector port derivatization was erratic but was in the order of 50% conversion to silylated products. For thymidine, the amounts of b, c and d were in an approximately 1:1:10 mole ratio, respectively. The possibility of a "ghost effect" was also investigated. Nucleosides that were injected in earlier analyses could remain in the injector port and then be derivatized by the excess reagent in successive injections. To test this, a series of injections of thymidine alone was made, followed by silylating reagent alone. No ghost peaks corresponding to silylated thymidine derivatives were observed. Presumably, the thymidine decomposed in the injector port.

The injector port silylations can be counteracted by chemical methods directed towards deactivating either (1) the excess silyl donor or (2) the substrates. The method of choice will depend upon the system under

investigation and the kind of information required.

Method 1. Deactivation of excess silyl donor.

With this approach, a large excess of methanol was used to quench the excess reagent. An aliquot of the reaction mixture was mixed into a larger volume of methanol and allowed to react for a short time. The methanol reacts with the reagent much faster than does the nucleoside. Direct analysis of this mixture could not be made, however, as it was found that, in the injector port, hydrolysis of the silylated nucleoside by the excess methanol occurred to a small extent. This was confirmed by coinjection of silylated nucleoside and methanol. Thus, to obtain a reliable analysis, it was first necessary to remove excess methanol and other volatiles *in vacuo*, redissolve the residue in a suitable solvent (such as dichloromethane), and then analyze by GC, relating all peak areas to that of an internal standard present in the original reaction mixture. Nucleoside derivatives have a very low vapor pressure at room temperature, which allows the use of vacuum to remove volatiles. The method is obviously not suited to very volatile derivatives. In the study of reaction 1 (Scheme 6) this procedure allows a precise analysis for b, c, and d. The non-volatile nucleoside (a) does not chromatograph.

Method 2. Deactivation of substrates.

The substrates were deactivated by treatment with a reagent which reacts rapidly with the unreacted substrate hydroxyls to give derivatives which are amenable to GC analysis and prevent the injector port silylation effect. Methods explored included trimethylsilylation, acetylation, and trifluoroacetylation. As indicated earlier for the deoxynucleosides, all three of these methods give derivatives which are amenable to GC analysis. However, only trifluoroacetylation yields derivatives of the 3'- and 5'-O-

SCTASi isomers that are readily separable to allow GC determination of b and c. (Of course GC/MS-SIR should be feasible for the other derivatives, but has not yet been investigated.)

The necessary requirements for the reagent which deactivates the substrate are that it reacts rapidly to stop the original silylation reaction, gives a reliable 100% derivatization, and gives a single derivative that is suitable for quantitative GC analysis (i.e., linear detector response with no significant decomposition on column). The reagents that have been studied are: BSTFA, TMSiIm, TFAA, TFAIm, AcAnh, and AcIm. In the procedure used, an aliquot of the reaction solution to be monitored was treated with one of these reagents, as described in Experimental. Then the sample was analyzed directly, except in the case of the acyl anhydride reagents, for which the GC column is activated by injection of the reagents' acidic side products. In this case, the reagents and volatiles must be removed *in vacuo* after derivatization is complete and the residue dissolved in a suitable solvent.

For trimethylsilylations the preferred reagent may depend upon the substrate. Both TMSiIm and BSTFA react very rapidly with hydroxyls, but the latter reacts also with amines and enols though more slowly and erratically. With thymidine, BSTFA gave a mixture of two derivatives (bis- and tris-TMSi-thymidine), depending upon the extent of trimethylsilylation of the base functions. These are incompletely separated by GC, a shoulder (due to tris-TMSi-thymidine) appearing on the trailing edge of the main GC peak. This can lead to problems in quantitation since it is desirable to have a single derivative rather than a mixture. Similar problems have been noted previously (130,131). TMSiIm, on the other hand, gives a single derivative (Iee) and a well-shaped GC peak.

Trifluoroacetylations are more conveniently performed with TFAIm than with TFAA, since with the former reagent the reaction solution can be directly injected into the GC while, with the latter, the excess anhydride and trifluoroacetic acid must be removed first.

For acetylations, it has been found that AcAnh is the only suitable reagent. AcIm reacts too slowly (usually requires heating for 100% reaction) and would therefore not stop the original reaction fast enough.

Overall, for most applications, trimethylsilylation by TMSiIm is preferable since it has all the requirements cited above. However, in particular cases such as the studies with deoxynucleosides, trifluoroacetylation can be more useful since it allows for analysis of a, b, c, and d, while trimethylsilylation allows for only a, (b + c), and d (see Scheme 6).

Desilylation Reactions

A related problem arises in the monitoring of the hydrolysis of compounds such as d (Scheme 6, reaction 2) by 80% aqueous acetic acid. Desilylation of silylated nucleosides by excess acetic acid occurs in the hot injector port. There is also activation of the column by the acid, interfering with precise quantitative work. These results are not unexpected since it is well known that acidic solutions should not be injected onto columns being used for analysis of TMSi derivatives (157) and that the hydrolysis reaction is accelerated at higher temperatures. The effect is substantiated by the results in Figure 18 (curve C) from another "co-injection" experiment designed to closely resemble conditions for direct analysis of the hydrolysis reactions. Co-injection of a fixed amount of acetic acid with variable amounts of Iba gave erratic results and a response curve that shows that extensive decomposition occurs in

the injector port and/or on the column.

Method 3.

Thus, to obtain an accurate analysis, it was necessary to remove *in vacuo* the acetic acid and water from an aliquot of the reaction mixture. The residue could be treated in one of two ways: (a) dissolution in a measured amount of solvent containing an internal standard of known concentration, followed by GC analysis of *b*, *c*, and *d*; or (b) dissolution and reaction in pyridine containing either TMSiIm or TFAIm in excess and the internal standard at known concentration, followed by GC analysis to give a determination of *a*, *b*, *c*, and *d*.

ii) Studies on the formation and hydrolysis of SCTASi derivatives of thymidine.

Methods *1*, *2*, and *3* were successfully applied to the determination of the rates of silylation and hydrolysis of SCTASi derivatives of deoxynucleosides. Some of the results obtained for TBDMSi derivatives of thymidine are presented below.

The reaction of thymidine with the reagent N-*tert*-butyl-dimethylsilylimidazole in pyridine solvent (Reagent D) was monitored by Method *1*, the results being shown in Figure 19. This is a weak reagent compared to Reagents A and B, but is useful for synthetic work since it gives a controllable reaction with high selectivity for primary hydroxyls. Significant concentrations of Iab (3'-O-TBDMSi-thymidine) are never obtained (<1% by GC analysis) because this can readily react further to give the bis-silyl derivative, Ibb. In the later stages of the reaction, the concentration of Ibb grows at the expense of Iba (5'-O-TBDMSi-thymidine). If a full-scale reaction to produce Iba is being monitored, it can be stopped and worked up at any stage. The maximum analytical yield of Iba by GC analysis was 92 mole % compared with 91 mole % isolated

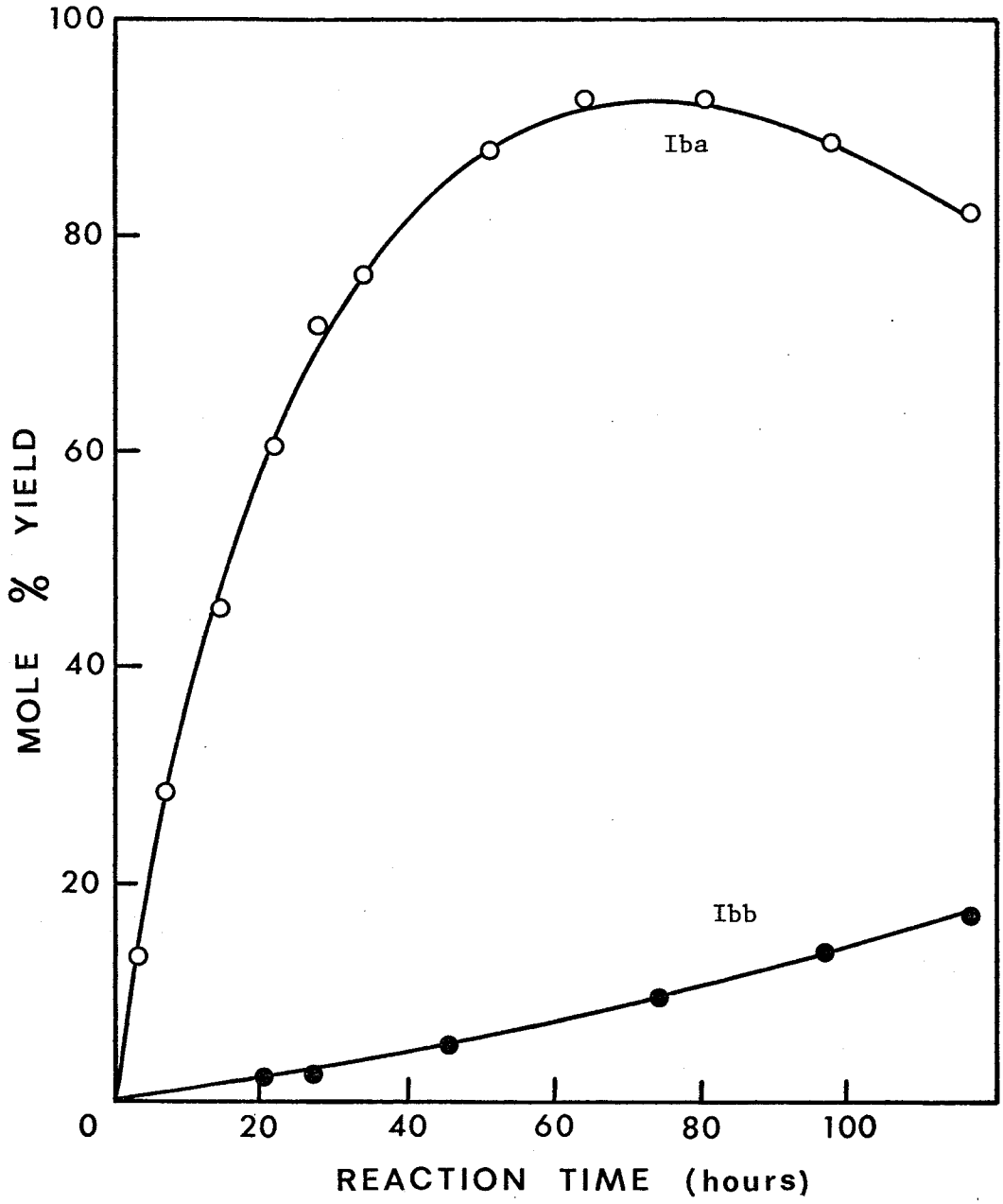


Figure 19. Quantitative GC results for the reaction of thymidine (32 μmoles, 0.145M) with TBDMSiIm (190 μmoles, 0.863M) in pyridine containing internal standard (pyrene, 7 μmoles) with a total volume of 220 μl, at room temperature (22°). Reaction products measured: -O- = 5'-O-TBDMSi-thymidine (Iba); -●- = 3',5'bis-O-TBDMSi-thymidine (Ibb).

yield on work-up of the preparative-scale reaction.

Upon hydrolysis by 80% aqueous acetic acid, the 5'-O-SCTASi group is removed much more readily than the 3'-O-SCTASi group. Figure 20, in which the analytical results were obtained by Method 3a shows that Iab can be obtained from Ibb in good yield (≃60 mole %; <5% of Iba and ≃1% of a side-product, 5'-O-Ac-3'-O-TBDMSi-thymidine (Igb), were also obtained).

iii) Evaluation of the analytical methods

The relative merits of the analytical procedures for minimizing injector port effects in the study of silylation reactions are summarized in Table VI. Some of the comments are generally applicable while others relate to specific problems. An additional advantage of the techniques developed is that during the monitoring of a reaction, a particular sampling at a certain time can be re-analyzed by GC; there is sufficient sample taken from the reaction and quenched that at least ten analyses could be performed. This allows the determination of the precision of each point in a rate curve, and the problem that sometimes arises when a chromatogram is unsatisfactory (e.g., poor injection, too little or too much sample injected, incorrect attenuation setting, etc.) is also avoided. One of the disadvantages (though not a serious one) is that larger samplings are required at each timed analysis. Thus, the reaction has to be kept on a slightly larger scale than would be allowed by direct analysis (if that were possible). The relative merits of Method 1 and 2 apply also to Methods 3a and 3b for the study of hydrolysis reactions. However, Method 3 is unsuitable for the determination of very volatile compounds that would be lost *in vacuo*.

These methods can be generalized to the study of many reactions by GC, in which the presence of an excess reagent or solvent, which will react

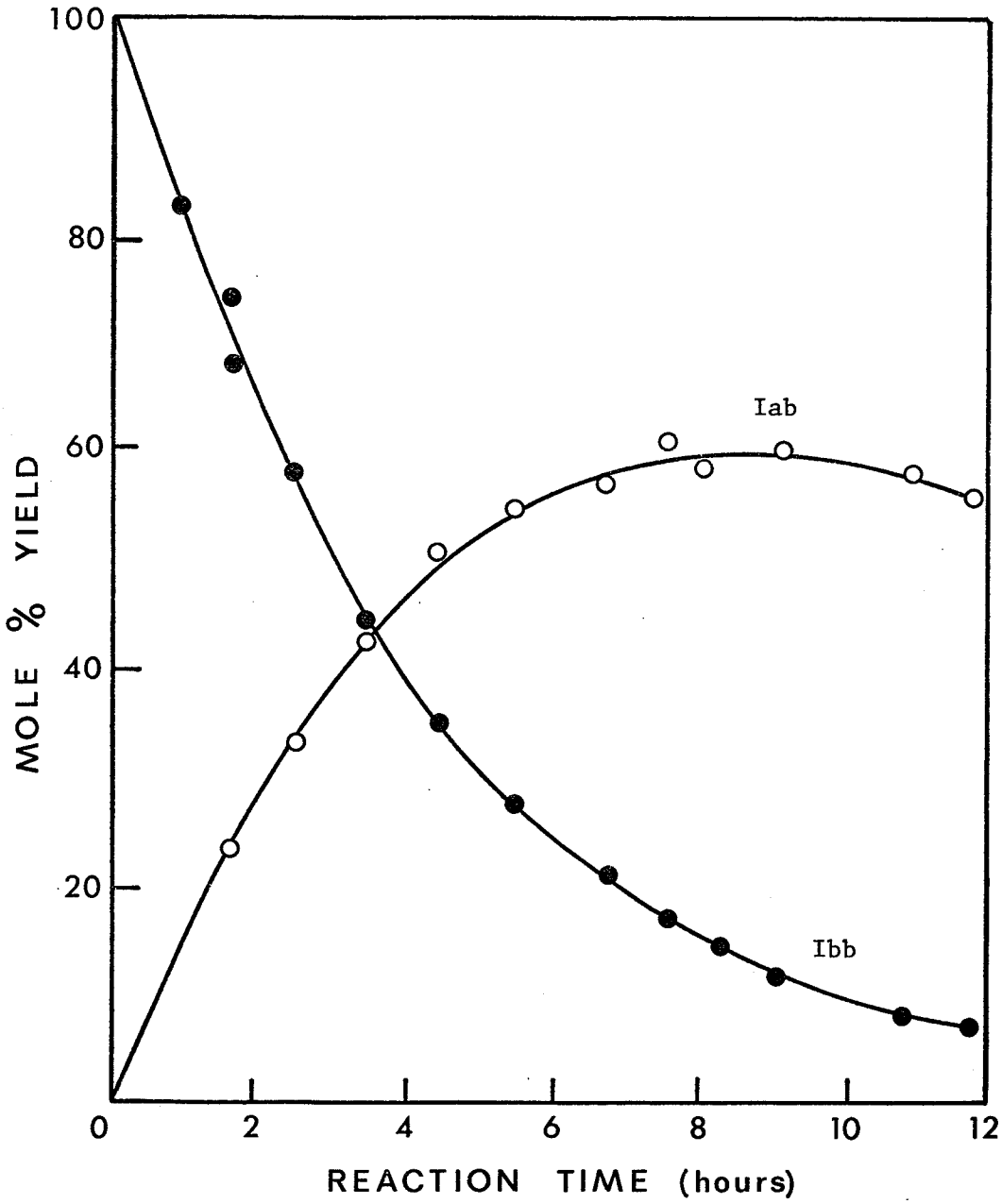


Figure 20. Quantitative GC results for the hydrolysis of 3',5'bis-O-TBDMSi-thymidine, Ibb (-●-), (with an initial concentration of 0.01M) in 80% aqueous acetic acid at room temperature (22°). Reaction product measured: -○- = 3'-O-TBDMSi-thymidine (Iab).

TABLE VI. Relative merits of the experimental procedures.

Procedure	Advantages	Disadvantages
Method 1: Deactivation of silyl reagent by methanol and evaporation of volatiles.	No concern over 100% derivatization at second step as in Method 2.	(a) Cannot determine underivatized non-volatile substrates. (b) Volatile substrates and derivatives lost during evaporation. (c) Evaporation procedure is inconvenient.
Method 2: Deactivation of substrate by further derivatization by:		
BSTFA, TMSIm, TFAIm, TFAA, AcAnh	Can determine substrate.	May not get 100% derivatization at second step.
BSTFA, TMSIm, TFAIm	No loss of volatile compounds.	
TFAA, AcAnh		Reagent and acidic side products must be removed; inconvenient and does not allow determination of volatile substrates and derivatives.
TFAIm, TFAA	Separation of 3'- and 5'-O-SCTASi deoxynucleosides.	
BSTFA, TMSIm, AcAnh		Does not separate 3'- and 5'-O-SCTASi deoxynucleosides.
AcIm		Derivatization is too slow.

at an accelerated rate with the compound being analyzed during flash evaporation in the hot injector port, can lead to serious errors in quantitation. Undoubtedly, problems similar to the ones encountered in this study will arise in other situations and analysts should examine their analytical methods by using the coinjection technique.

2. Ribonucleoside Derivatives

Tables VII and VIII present the chromatographic data for the ribonucleoside derivatives studied in this project.

a) Liquid Chromatography

As for the deoxynucleoside series, TLC was a useful method for the separation of SCTASi derivatives of ribonucleosides. However, the situation is more complex and not all the 6 partial silyl derivatives are separable at one time. The important isomers, VIaba and VIaab, present a particularly difficult problem since no solvent system has been found for their TLC separation.

HPLC appears very promising for the ribonucleoside derivatives. With its higher resolution, it is possible to separate all the partial TBDMSi derivatives of uridine (see Table VII). However, long retention times and severe tailing are encountered for the mono-TBDMSi derivatives, expecially 5'-O-TBDMSi-uridine (VIbaa). The solvent system and conditions still need to be optimized for these three compounds. A feasible separation of the tris- and the three bis-TBDMSi-uridines is illustrated in Figure 21.

TABLE VII. HPLC retention data for TBDMSi derivatives of uridine (same conditions as Figure 21).

Compound	#	Relative Retention Time[a]
2',3',5'tris-TBDMSi-uridine	VIbbb	1.000
2',5'bis-TBDMSi-uridine	VIbab	1.081
2',3'bis-TBDMSi-uridine	VIabb	1.494
3',5'bis-TBDMSi-uridine	VIbba	1.722
2'-TBDMSi-uridine	VIaab	4.23[b]
3'-TBDMSi-uridine	VIaba	4.71[b]
5'-TBDMSi-uridine	VIbaa	26.94[c]

a = not corrected for dead volume; b = tailing; c = severe tailing

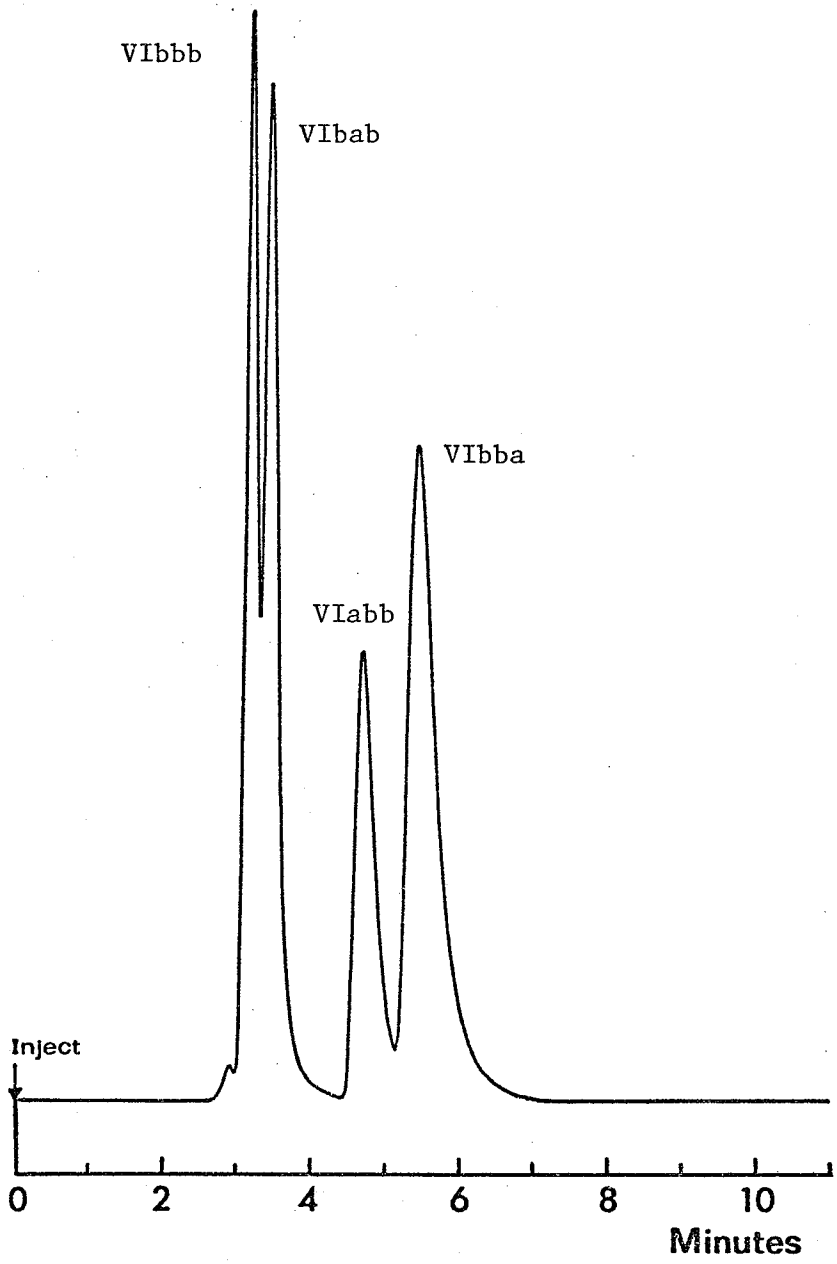


Figure 21. High performance liquid chromatogram of a mixture of 2',3', 5'tris-O-TBDMSi-uridine (VIbbb, 2.1 μg), 2',5'bis-O-TBDMSi-uridine (VIbab, 1.8 μg), 2',3'bis-O-TBDMSi-uridine (VIabb, 1.8 μg), and 3',5'bis-O-TBDMSi-uridine (VIbba, 3.0 μg).

Conditions: 3mm-ID X 0.25m column packed with 5μ Spherisorb Silica; 0.4 ml/min ethyl acetate mobile phase; UV detection at 280 nm.

b) Gas chromatography

Although the GC of ribonucleoside derivatives has not been investigated very extensively, a number of interesting observations have been made and are described below. These serve to suggest many future experiments in this area.

Table VIII gives the retention data for the various derivatives that have been examined. It should be noted that the previous discussion on retention indices for deoxynucleosides also applies to ribonucleosides. The tris-O-SCTASi derivatives of uridine (VI), 5-methyluridine (VII), and adenosine (VIII), and the N,O-perSCTASi derivatives of adenosine displayed good GC behavior although their retention indices are rather high compared to TMSi derivatives. Figure 22 illustrates the excellent peak shapes for some adenosine derivatives and also demonstrates the retention increment effect. Substitution of one hydrogen of the N6-amino group of VIIIbbba by a TMSi group to give VIIIbbbe leads to a rather small increase in retention time ($\delta I = 37$) compared to that for substitution with TBDMSi to give VIIIbbbb ($\delta I = 244$). The number of silyl groups in a SCTASi derivative is far easier to recognize by GC than with TMSi derivatives.

The partial O-SCTASi derivatives of ribonucleosides present an interesting problem. It was found that direct analysis by GC was complicated by intramolecular rearrangements. Only the TBDMSi derivatives of uridine have been examined in this respect, but their behavior should be representative for this entire class of derivative.

The GC behavior of VIbaa and VIabb on OV-1 columns was straightforward. Figures 23a and 24a show that a single peak was obtained in each case. MS and TLC of trapped eluates verified that the derivatives passed through the column undecomposed. The remaining partial derivatives gave

TABLE VIII: Numbers, names, and chromatographic data for ribonucleoside derivatives.

Compound[a] Number	Compound Name[a]	R_f TLC[b] A	R_f TLC[b] D	GC DATA[c] $I^{230°}_{OV-1}$	GC DATA[c] $I^{260°}_{OV-1}$	GC DATA[c] $I^{290°}_{OV-1}$	GC DATA[c] Important Isomer Pairs' α
Partial O-TBDMSi Derivatives							
VIbaa	5'-O-TBDMSi-uridine	0.05	0.02	2576			
VIaba	3'-O-TBDMSi-uridine	0.26	0.04	2628[r]			1.227
VIaab	2'-O-TBDMSi-uridine	0.27	0.04	2559[r]			
VIbba	3',5'bis-O-TBDMSi-uridine	0.59	0.12	2884[r]			1.128
VIbab	2',5'bis-O-TBDMSi-uridine	0.77	0.25	2843[r]			
VIabb	2',3'bis-O-TBDMSi-uridine	0.57	0.21	2866			
IX	5'-O-TBDMSi-2',3'-tert-butylmethylsilyl-dioxy-uridine	0.78		2767			
TMSi/TBDMSi Derivatives							
VIeee	2',3',5'tris-TMSi-uridine	0.80[h]		2444			
VIbee	2',3'bis-O-TMSi-5'-O-TBDMSi-uridine	0.80		2682			
VIebe	2',5'bis-O-TMSi-3'-O-TBDMSi-uridine	0.80[h]		2660			1.026
VIeeb	3',5'bis-O-TMSi-2'-O-TBDMSi-uridine	0.80[h]		2669			
VIbbe	2'-O-TMSi-3',5'bis-O-TBDMSi-uridine	0.80		2891			1.023
VIbeb	3'-O-TMSi-2',5'bis-O-TBDMSi-uridine	0.80		2899			
VIebb	5'-O-TMSi-2',3'bis-O-TBDMSi-uridine	0.80[h]		2879			
VIbbb	2',3',5'tris-O-TBDMSi-uridine	0.80	0.54	3102	3128		
Ac/TBDMSi Derivatives							
VIggg	2',3',5'tris-O-Ac-uridine			2509			

continued...

TABLE VIII (continued)

		R_f TLC[b]		GC DATA[c]			
		A	D	$I^{230°}_{OV-1}$	$I^{260°}_{OV-1}$	$I^{290°}_{OV-1}$	Important Isomer Pairs' α
VIbgg	2',3'bis-O-Ac-5'-O-TBDMSi-uridine			2706			
VIgbg	2',5'bis-O-Ac-3'-O-TBDMSi-uridine	0.47		2718			1.089
VIggb	3',5'bis-O-Ac-2'-O-TBDMSi-uridine	0.55		2689			
VIbbg	2'-O-Ac-3',5'bis-O-TBDMSi-uridine			2919			1.041
VIbgb	3'-O-Ac-2',5'bis-O-TBDMSi-uridine			2905			
VIgbb	5'-O-Ac-2',3'bis-O-TBDMSi-uridine			2905			
VIbbb	2',3',5'tris-O-TBDMSi-uridine			3102	3128		
TFA/TBDMSi Derivatives							
VIiii	2',3',5'tris-O-TFA-uridine			d			
VIbii	2',3'bis-O-TFA-5'-O-TBDMSi-uridine			d			
VIibi	2',5'bis-O-TFA-3'-O-TBDMSi-uridine			d			
VIiib	3',5'bis-O-TFA-2'-O-TBDMSi-uridine			2272			
VIbbi	2'-O-TFA-3',5'bis-TBDMSi-uridine			d			
VIbib	3'-O-TFA-2',5'bis-TBDMSi-uridine			2690			
VIibb	5'-O-TFA-2',3'bis-TBDMSi-uridine			2688			
Miscellaneous Derivatives							
VIeee	2',3',5'tris-O-TMSi-uridine	0.80[h]		2444			
VIeeee	2',3',5',4tetrakis-O-TMSi-uridine	h		2449			
VIIeee	2',3',5'tris-O-TMSi-5-methyluridine	0.85[h]		2481			
VIIIeeea	2',3',5'tris-O-TMSi-adenosine	0.56[h]		2573			
VIIIeeee	2',3',5'tris-O-TMSi-N6-TMSi-adenosine	h		2647			

continued...

TABLE VIII (continued)

		R_f TLC [b]		GC DATA[c]			
		A	D	$I^{230°}_{OV-1}$	$I^{260°}_{OV-1}$	$I^{290°}_{OV-1}$	Important Isomer Pairs' α
VIggg	2',3',5'tris-O-Ac-uridine			2509			
VIiii	2',3',5'tris-O-TFA-uridine			d			
VIbbb	2',3',5'tris-O-TBDMSi-uridine	0.80	0.54	3102	3128		
VIddd	2',3',5'tris-O-TMIPSi-uridine	0.80				3815	
VIccc	2',3',5'tris-O-TMTBSi-uridine	0.80				3916	
VIIbbb	2',3',5'tris-O-TBDMSi-5-methyluridine	0.85			3133		
VIIIbbba	2',3',5'tris-O-TBDMSi-adenosine	0.56		3203	3237	3273	
VIIIbbbe	2',3',5'tris-O-TBDMSi-N6-TMSi-adenosine	h			3274		
VIIIbbbb	2',3',5'tris-O-TBDMSi-N6-TBDMSi-adenosine	0.79			3481	3556	
VIIIbbbc	2',3',5'tris-O-TBDMSi-N6-TMTBSi-adenosine	0.79				3811	

[a] Refer to Scheme 4 for numbering system and structures.

[b] Solvent systems: A = diethyl ether; D = ether/hexane (2:1,v/v)

[c] I = Kovats' retention index; α = separation factors for isomer pairs (⩾1,always).

[d] Decomposition during GC.

[h] Partial hydrolysis occurs during TLC (complete if no R_f value given as well).

[r] Rearrangement occurs in hot injector port (and on-column at higher temperatures).

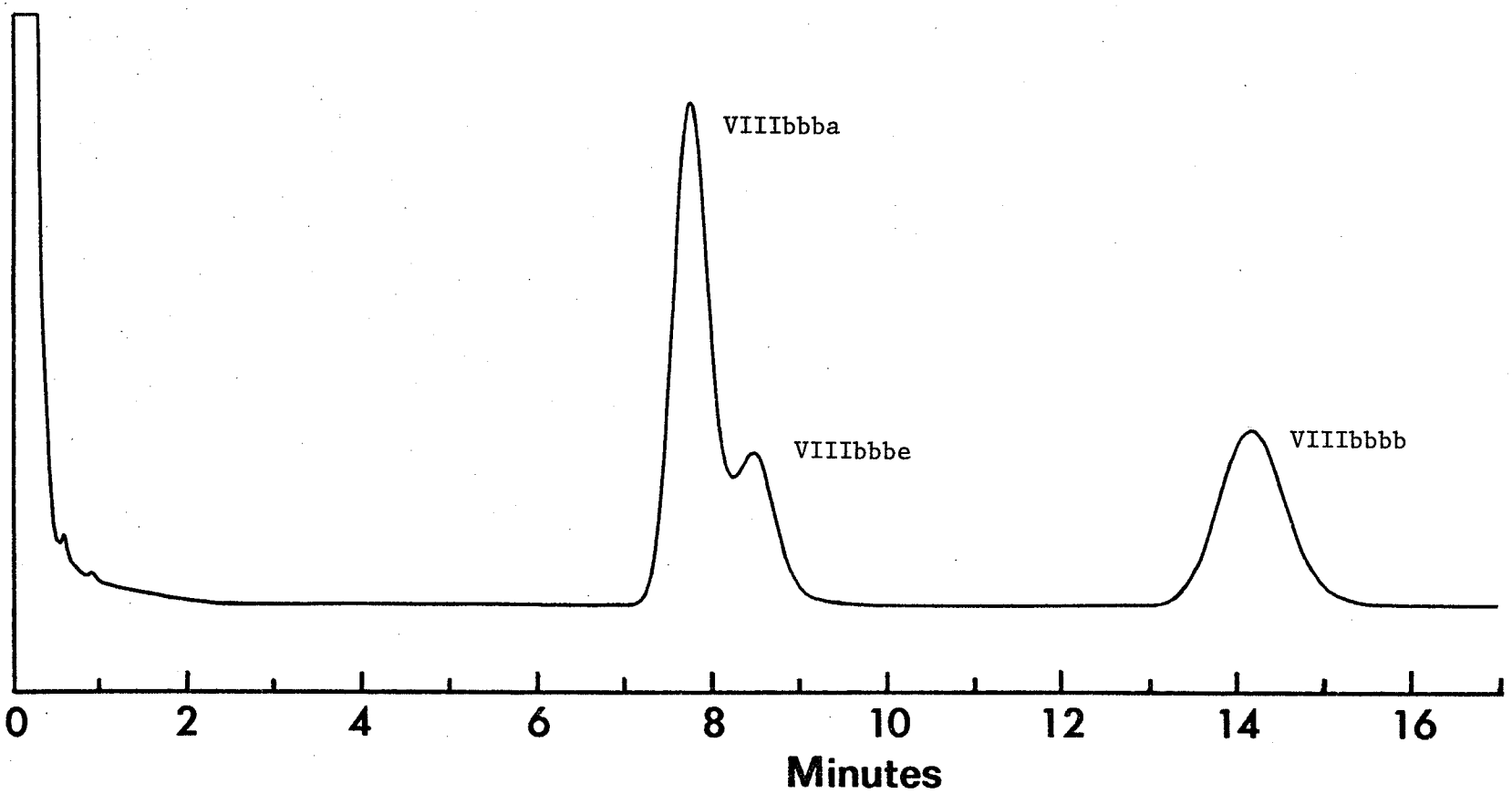


Figure 22. Gas chromatogram of a mixture of 2',3',5'tris-O-TBDMSi-adenosine (VIIIbbba), and its N6-TMSi (VIIIbbbe) and N6-TBDMSi (VIIIbbbb) derivatives. Conditions: column A (10% OV-1, 1m x 2mm-ID), 260°, 30 ml/min N_2 carrier gas.

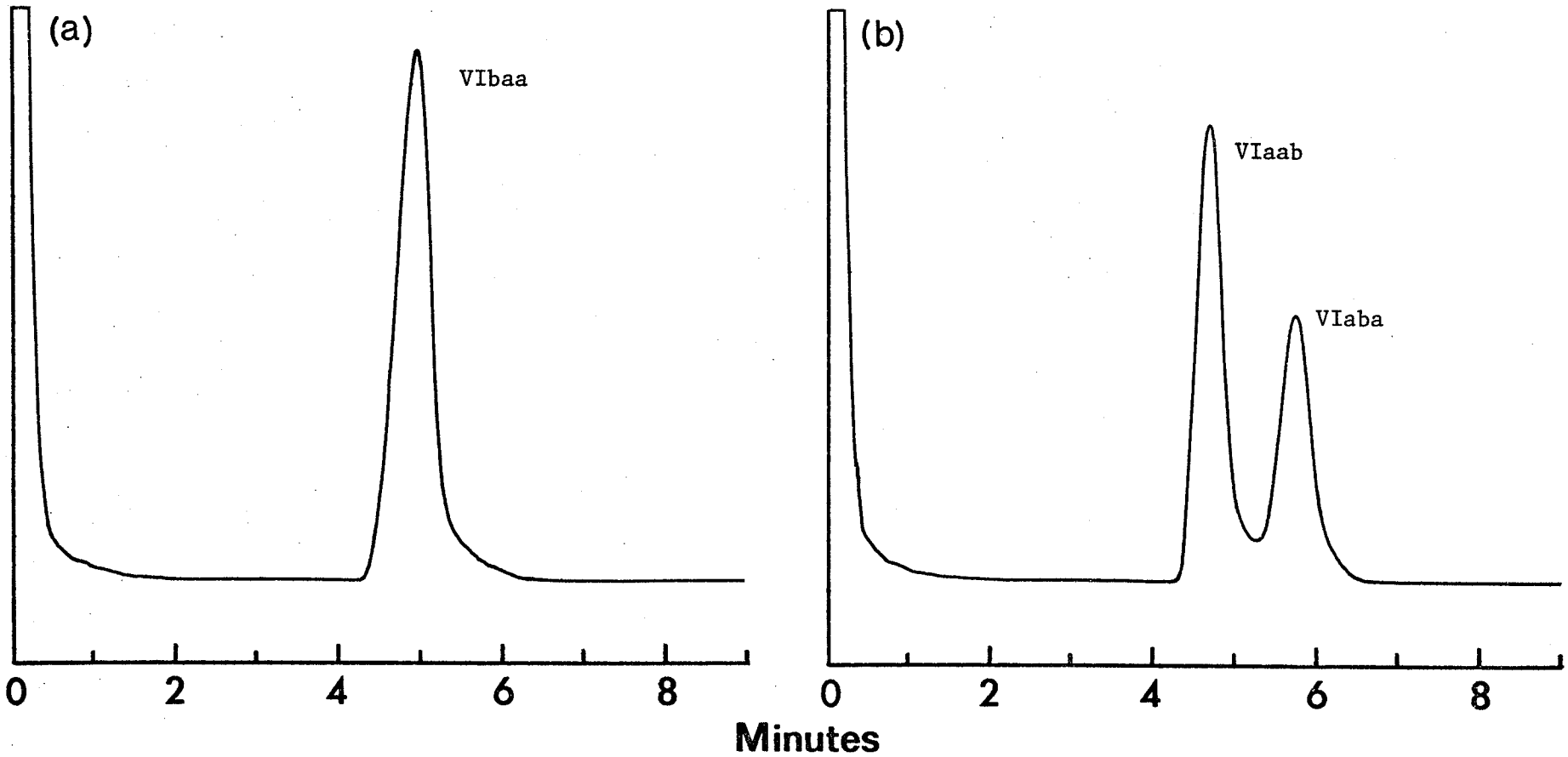


Figure 23. Gas chromatograms of mono-TBDMSi derivatives of uridine. (a) pure 5'-O-TBDMSi-uridine (VIbaa) was injected; (b) pure 3'-O-TBDMSi-uridine (VIaba) was injected, but an injector port rearrangement gave an additional peak corresponding to 2'-O-TBDMSi-uridine (VIaab). Injection of pure VIaab gave a chromatogram almost identical to (b). Conditions: column A (10% OV-1, 1m x 2mm-ID), 230°, 30ml/min N_2 carrier.

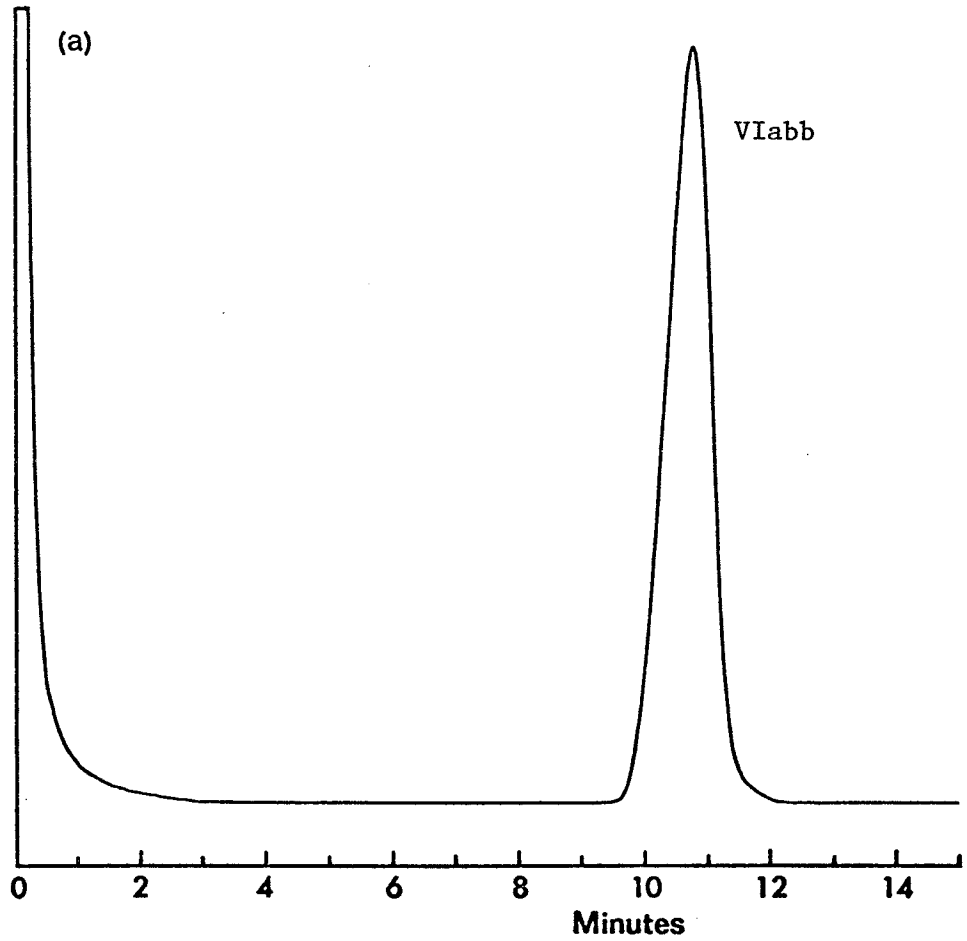


Figure 24. Gas chromatogram of bis-O-TBDMSi-uridine derivatives. (Same conditions as Figure 23)

(a) Injection of pure 2',3'bis-O-TBDMSi-uridine (VIabb) gave a single peak without rearrangement.

(on next page:)

(b) Injection of pure 2',5'bis-O-TBDMSi-uridine (VIbab) gave additional peaks due to rearrangement to 3',5'bis-O-TBDMSi-uridine (VIbba) and decomposition to 5'-O-TBDMSi-2',3'-tert-butylmethylsilyldioxy-uridine (IX). The mass spectrum and structure of the latter is given in Figure 26.

(c) Injection of pure VIbba also gave three peaks with the same identities as in (b).

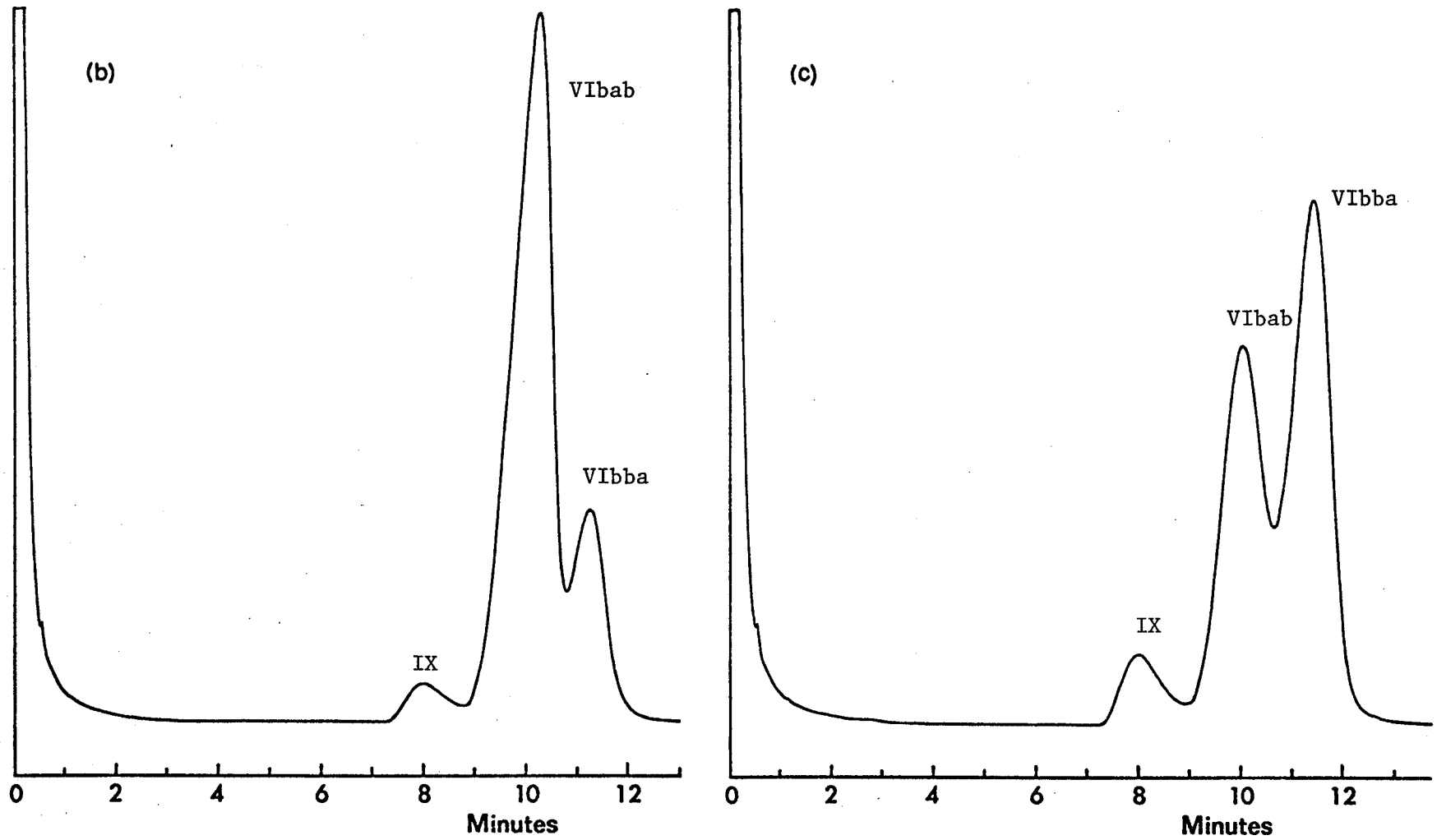


Figure 24 (continued)

some unusual results. When pure samples of the 2'- and 3'-mono-silyl derivatives (VIaab and VIaba) were injected, essentially identical chromatograms with two peaks in each were obtained (Figure 23b). Injection of the 2',5'- and 3',5'-bis-silyl derivatives (VIbab and VIbba) gave different chromatograms which contained three peaks with the same retention times (Figures 24b and 24c, respectively). When a longer column was used, with a consequently higher column temperature, the peaks became broader and less well-resolved in each case (Figure 25). It was established, by the evidence given below, that these effects are due to a thermally-induced intramolecular rearrangement of a silyl group between the 2' and 3' positions, together with, for compounds VIbab and VIbba, partial decomposition to compound IX (presumably by thermal elimination of methane). Scheme 7 illustrates the proposed reactions for the bis-silyl derivatives. The rearrangements occur primarily in the hot injector port (250°), but are also induced during elution by higher column temperatures. The latter results in a broadening and fusing of chromatographic bands. (It is interesting to note that the degree of rearrangement is different for the two bis-silyl isomers. This is probably due to a steric repulsion between the 5'- and 3'-O-TBDMSi groups in Ibba.) The 2' ↔ 3' rearrangement of silyl groups has also been observed in refluxing pyridine and under certain catalytic conditions at room temperatures (85).

Several pieces of evidence support the foregoing conclusions:

1) The eluted peaks were trapped in glass capillaries, dissolved in dichloromethane, and reinjected into the GC under identical conditions. Both the first and second peaks of Figure 23b when reinjected gave chromatograms identical to the one from which they were collected. The

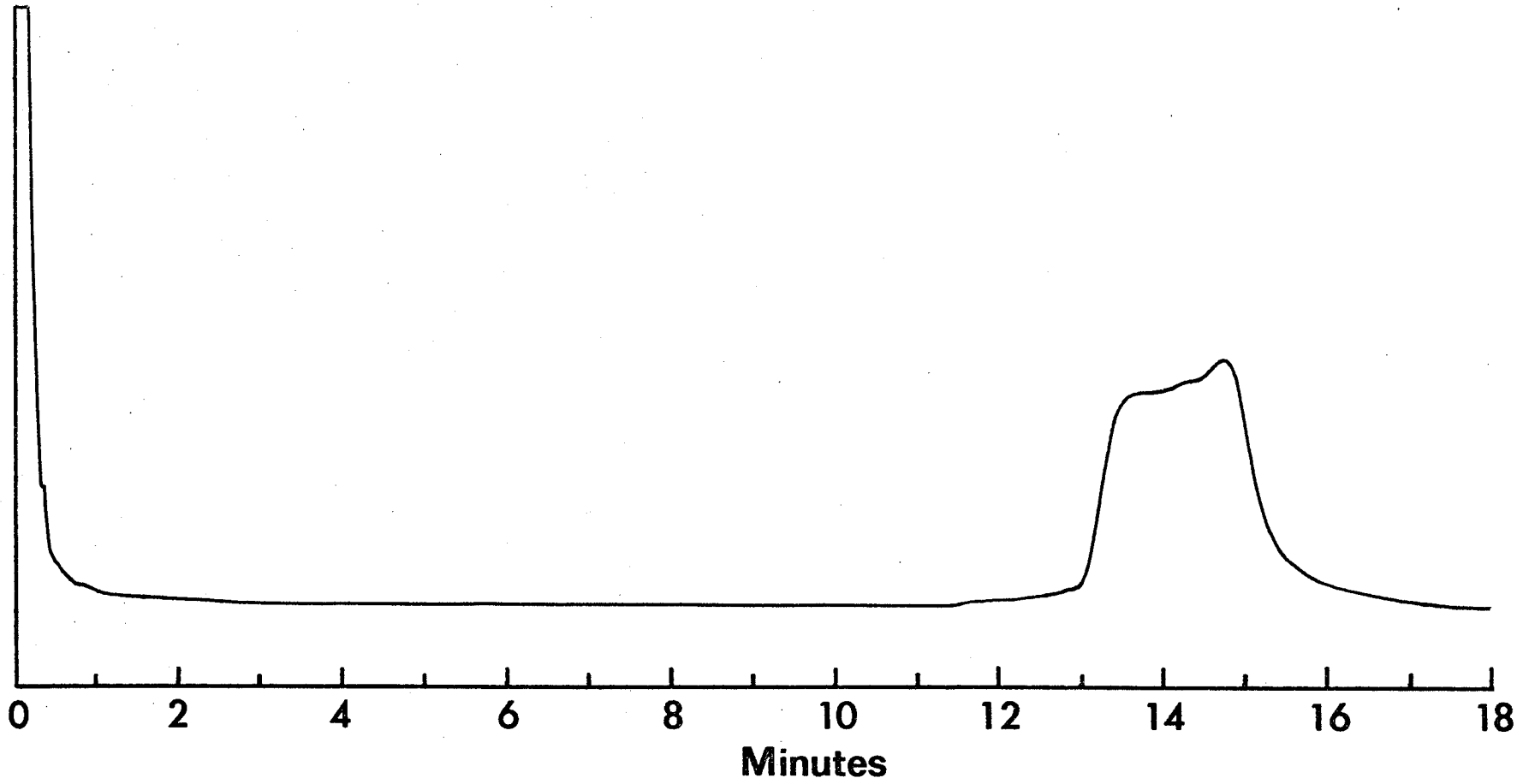
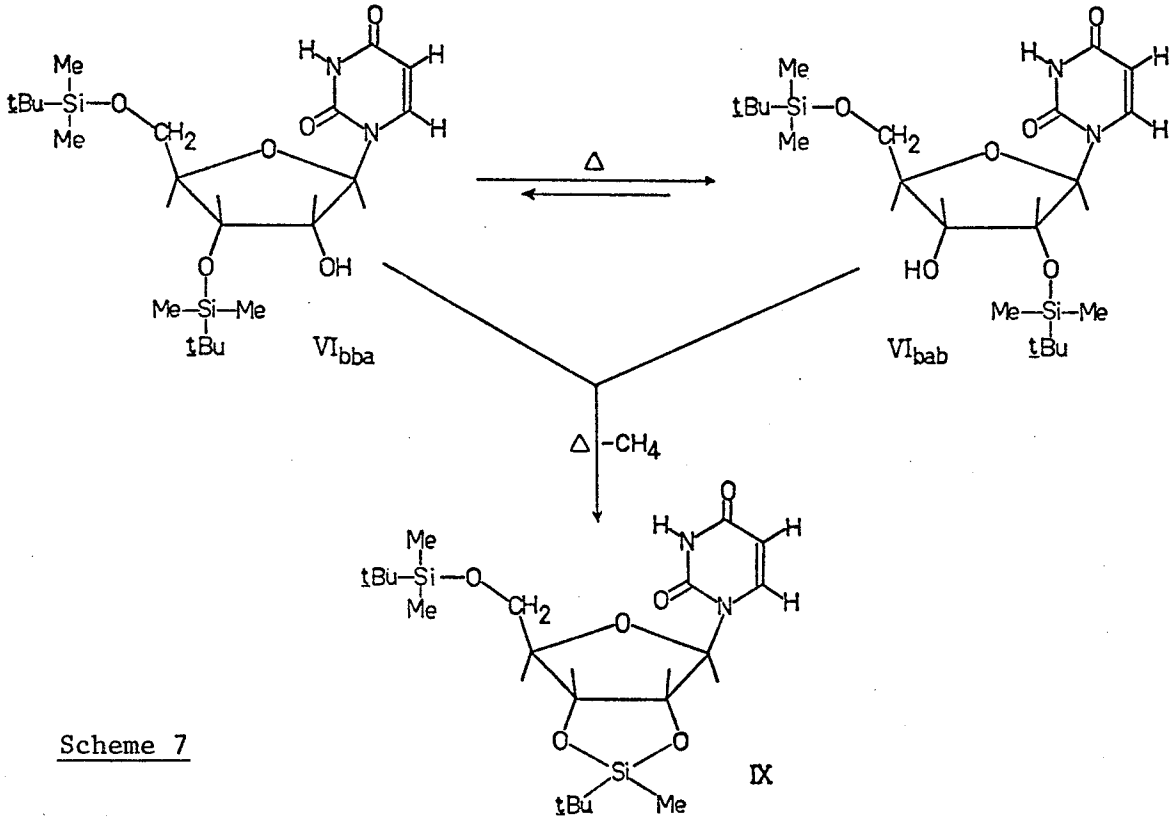


Figure 25. Gas chromatogram of pure 3',5'bis-O-TBDMSi-uridine (VIbba) showing extensive rearrangement on a long column at a high temperature.

Conditions: column C (10% OV-1, 3.75m x 2.4mm-ID), 280°, 30 ml/min N_2 carrier.



Scheme 7

first peak of Figure 24b (and 24c) gave a single peak at the same retention time, while the second and third peaks gave chromatograms identical to those in Figures 24b and 24c, respectively. These results confirm that rearrangement is actually occurring rather than that the starting compounds were impure.

2) Peak identities were confirmed by MS and TLC analyses on collected GC eluates.

3) Compound IX was synthesized independently by reacting 5'-O-TBDMSi-uridine (VIbaa) with a new reagent, tert-butylmethyldichlorosilane, to give 5'-O-TBDMSi-2',3'-O-tert-butylmethylsilyldioxy-uridine (IX). This had a mass spectrum identical to that obtained for the first peaks in Figures 24b and 24c (see Figure 26). It should be noted that this 2',3'-silyldioxy derivatization should have some valuable applications in both

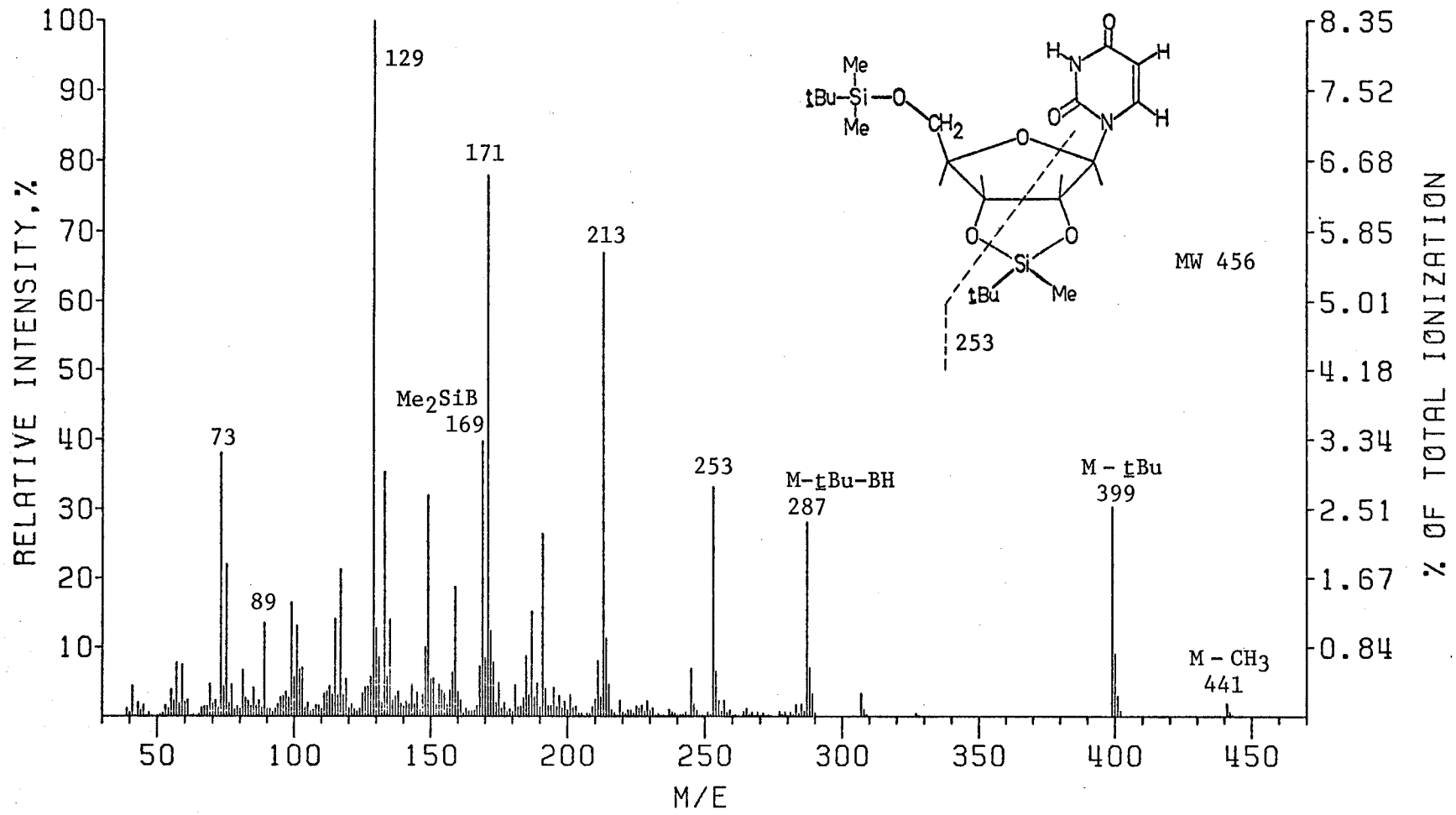


Figure 26. Mass spectrum of 5'-O-TBDMSi-2',3'-tert-butylmethylsilyldioxy-uridine (IX), prepared by reacting VIbaa with $TBMSiCl_2$. The spectra of the first GC peaks in Figures 24b and 24c were identical. Spectrum recorded on Finnigan instrument at 70eV.

synthesis and gas phase analytical chemistry. An analogous dimethylsilyldioxy derivative has been studied for the GC and MS of steroids (177-180).

4) Further evidence for the assignment of mono- and bis-silyl peak identities was obtained by micro-scale acetylation of trapped eluates, followed by either TLC analysis or reinjection into the GC with MS analysis of eluted peaks. The mixed Ac/TBDMSi derivatives have characteristic R_f and I values and unique mass spectra.

The decomposition and rearrangements just described are obviously undesirable for the gas phase analysis of partial O-SCTASi derivatives of ribonucleosides. Since variation of instrumental conditions did not produce a solution to the problem, the formation of fully protected derivatives by acetylation, trifluoroacetylation, and trimethylsilylation was investigated.

Acetylation with AcAnh was very successful. This gave, in quantitative yield, the mixed Ac/TBDMSi derivatives which are stable for isolation, have unique mass spectra, and do not rearrange during GC analysis. Figure 27 illustrates the chromatography of the Ac derivatives of 2'-O- and 2',5'bis-O-TBDMSi-uridines. Each Ac/TBDMSi derivative gives a single symmetrical peak. Furthermore, the derivatives of the synthetically important isomers, VIaab/VIaba and VIbab/VIbba, are separable as illustrated for VIggb and VIgbg in Figure 28. However, all six mixed derivatives were not separable. Combined techniques such as TLC/GC or GC/MS-SIR may be possible solutions to this problem.

Unexpectedly, trifluoroacetylation was found to be an unsuitable method. Mixed TFA/SCTASi derivatives of uridine with a TFA group in the 2' position (e.g., VIbii, VIibi, VIbbi) decomposed during GC analysis, although the derivatives with a 5'- or 3'-O-TFA function (e.g., VIiib,

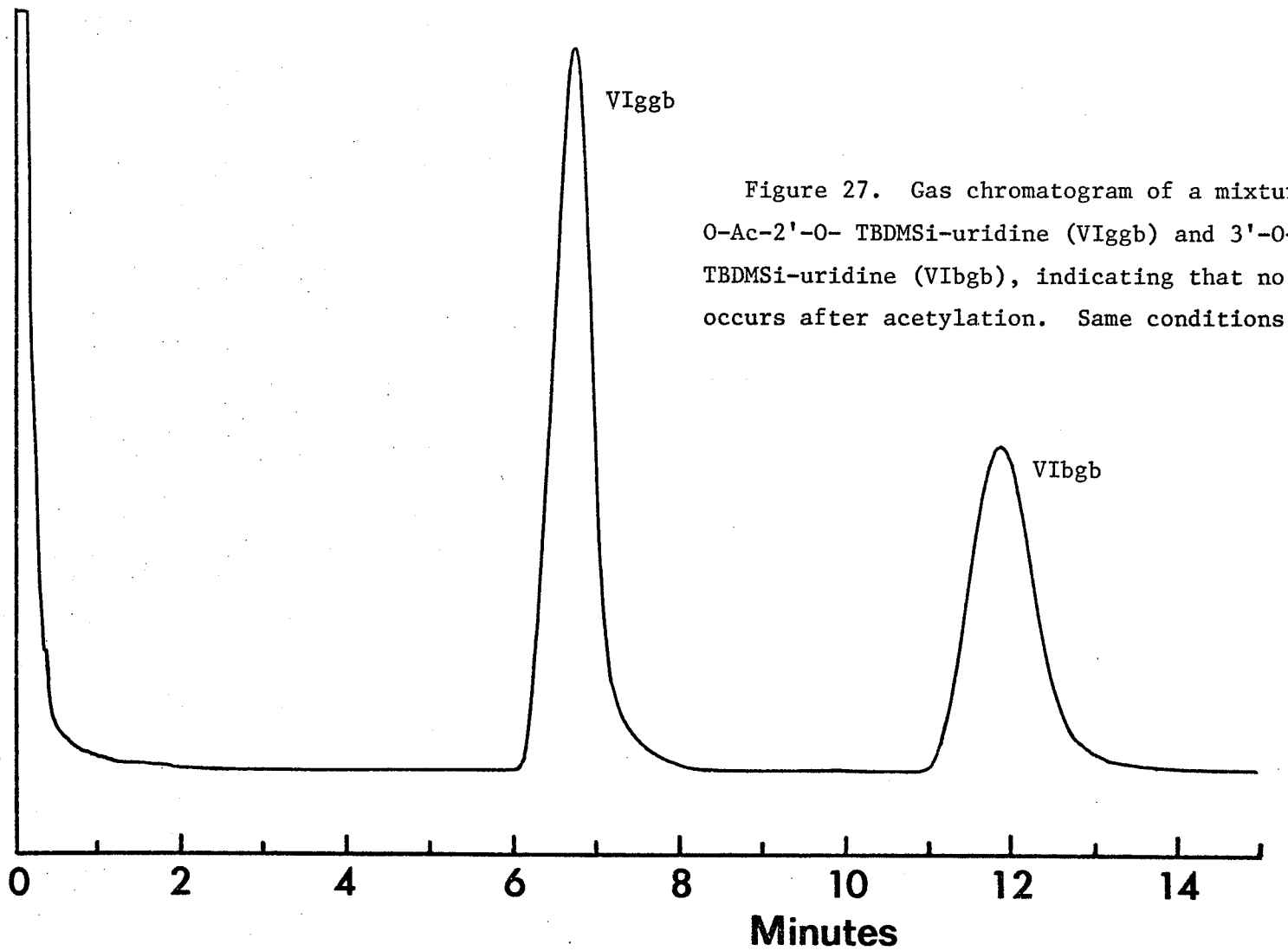


Figure 27. Gas chromatogram of a mixture of 3',5'bis-O-Ac-2'-O- TBDMSi-uridine (VIggb) and 3'-O-Ac-2',5'bis-O-TBDMSi-uridine (VIbgb), indicating that no rearrangement occurs after acetylation. Same conditions as Figure 23.

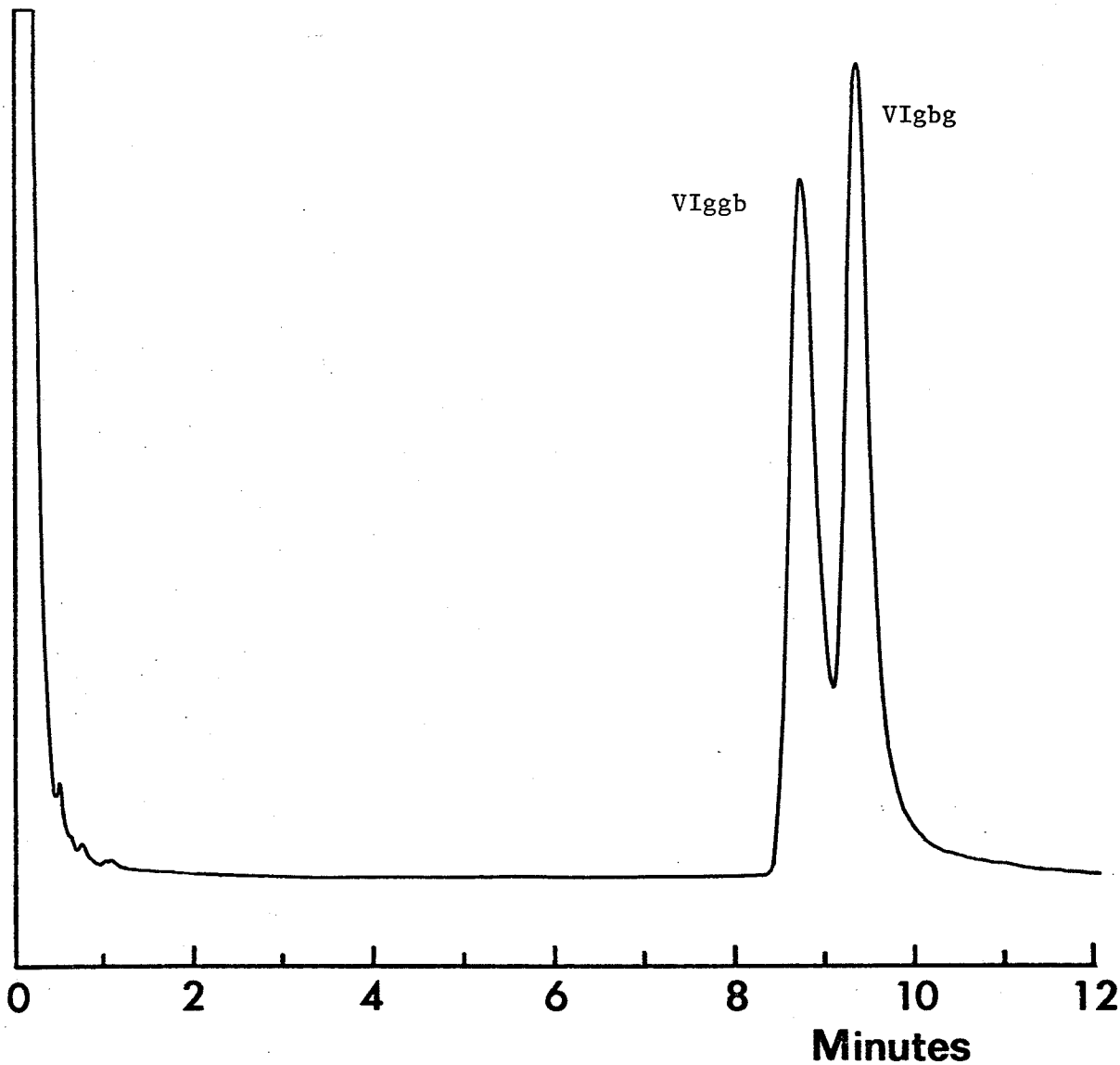


Figure 28. Gas chromatogram showing the separation of 3',5'bis-O-Ac-2'-O-TBDMSi-uridine (VIggb) and 2',5'bis-O-Ac-3'-O-TBDMSi-uridine (VIgbg). Conditions: column C (10% OV-1, 3.75m x 2.4mm-ID), 280°, 30ml/min N_2 carrier.

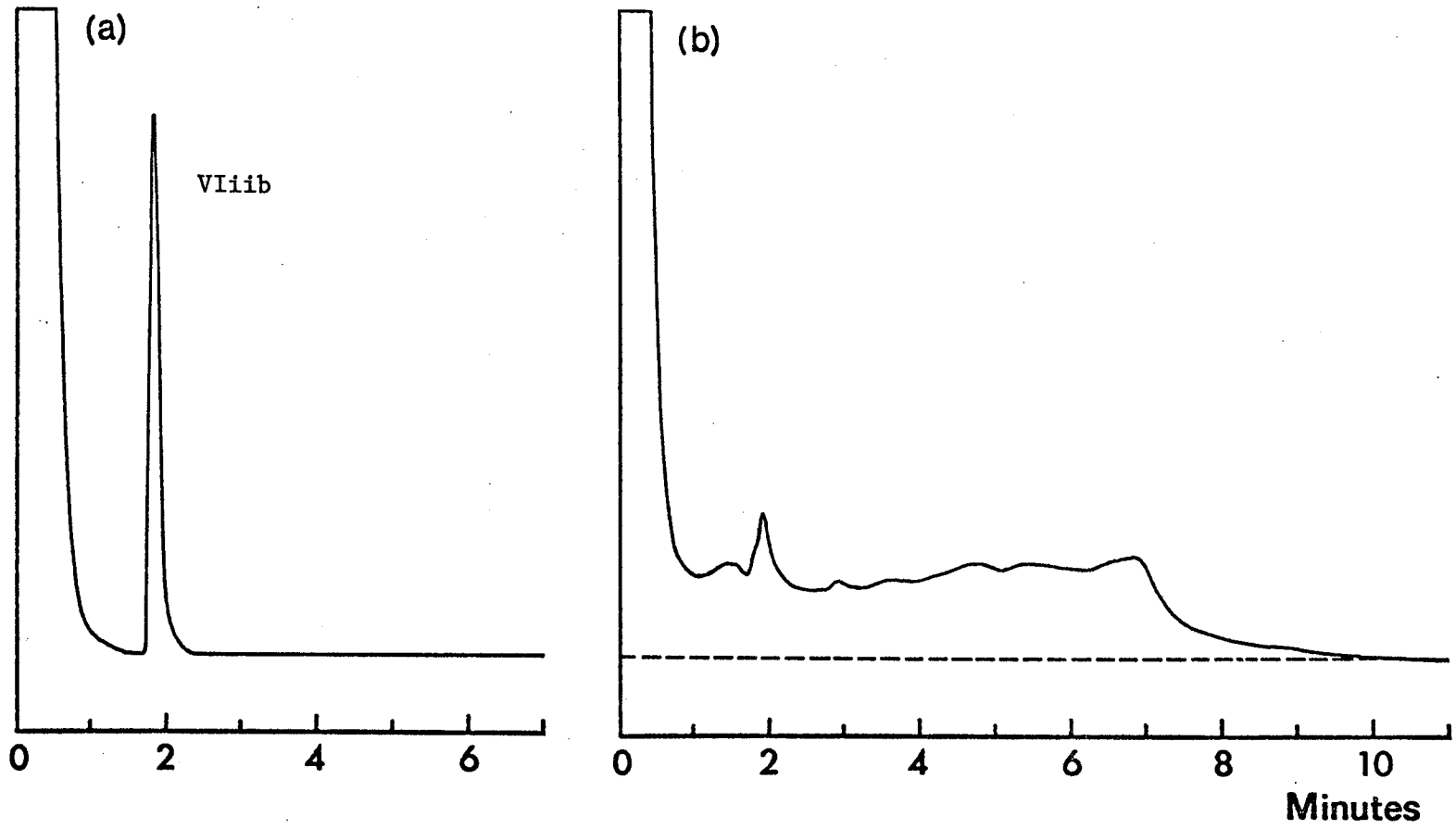


Figure 29. Gas chromatograms for (a) 3',5'bis-O-TFA-2'-O-TBDMSi-uridine (VIiib) and (b) 2',5'bis-O-TFA-3'-O-TBDMSi-uridine (VIibi) illustrating good behavior for the former but severe decomposition for the latter. Same conditions as Figure 23.

VIbib, VIibb) behaved well (see Figure 29). This phenomenon has not yet been explained and needs further investigation.

Trimethylsilylation of the partial O-TBDMSi derivatives of uridine produced mixed TMSi/TBDMSi derivatives which had excellent GC and MS behavior. Unfortunately, isomeric derivatives could not be separated. Figure 30 illustrates a chromatogram of a trimethylsilylated mixture of uridine and its mono-, bis-, and tris-TBDMSi derivatives. GC/MS-SIR would probably be a feasible method for determining all eight compounds in this chromatogram.

One concern in the above techniques was the possibility of 2' ↔ 3' rearrangement of the SCTASi group during further derivatization. However, evidence from TLC, GC, and MS experiments indicated that rearrangement did not occur during acetylation and trimethylsilylation of partial TBDMSi derivatives of uridine.

C. Summary

The results in this chapter have demonstrated that both liquid and gas phase chromatographic methods are useful for the separation and characterization of synthetically important SCTASi derivatives of nucleosides. TLC is valuable for fast qualitative analysis and preparative work, while HPLC appears to have tremendous potential for both the separation and quantitation of isomeric partial derivatives. With GC, analyses are fast and sensitive, and can be very accurate for quantitative work (if care is taken to prevent certain sources of error such as injector port reactions and rearrangements). However, foremost in the advantages of GC is the ability of this technique to distinguish between compounds on the basis of differences in molecular weight, stereochemistry, and functional groups (polarity). Liquid chromatographic methods are based almost entirely

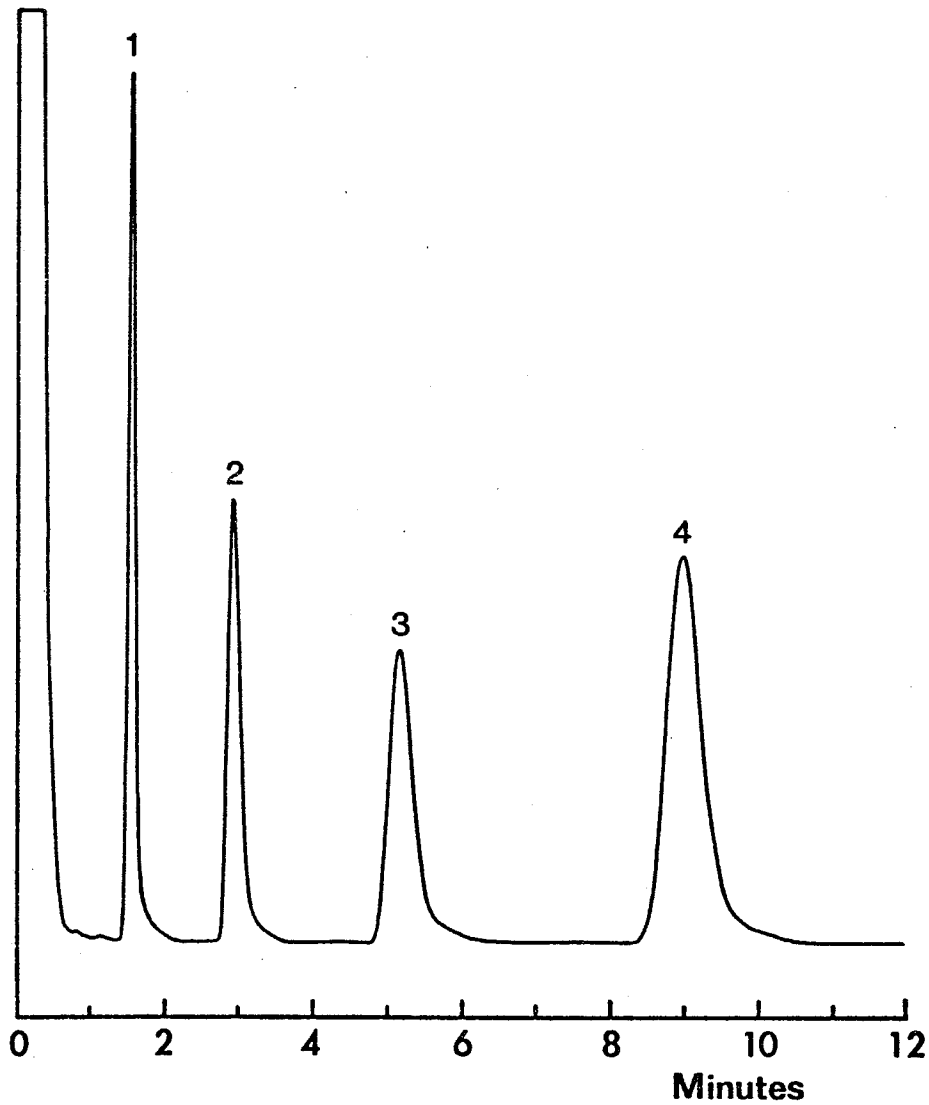


Figure 30. Gas chromatogram of a mixture of TMSi/TBDMSi derivatives of uridine:

1 = tris-TMSi (VIeee)

2 = bis-TMSi-mono-TBDMSi (VIbee + VIebe + VIeeb)

3 = mono-TMSi-bis-TBDMSi (VIbbe + VIbeb + VIebb)

4 = tris-TBDMSi (VIbbb)

Conditions: column A (10% OV-1, 1m x 2mm-ID), 250°, 30 ml/min N_2.

on the latter property. Thus, compounds differing only in the type of silyl group (i.e., MW difference) can be separated quite easily by GC, but not at all with LC. In addition, it is generally easier to find the optimum conditions for separation by GC than it is for LC, although the development of routine quantitative procedures may be easier with HPLC. HPLC does have the advantage that non-volatile and high molecular weight compounds, such as nucleotides, can be analyzed. When HPLC/MS techniques have been developed, HPLC may be a prime method in this area. Nevertheless, GC and HPLC should still be considered complementary, and research into either technique is always valuable.

In summary, the results of the investigation of GC methods have shown that the SCTASi derivatives of some nucleosides are amenable to GC analysis and that some partial derivatives can be separated. However, the ribonucleoside and labile deoxynucleoside derivatives require further derivatization to be chromatographed. Overall, the best general approach to this entire problem appears to be further derivatization for GC analysis. With some derivatives, it is still possible to achieve separation for isomers (e.g., TFA/SCTASi-deoxynucleosides and some Ac/TBDMSi-uridines), but for those that do not separate, it should be possible to use GC/MS-SIR techniques. The preparation of mixed TMSi/SCTASi derivatives combined with SIR appears to be the most promising method, since trimethylsilylation is readily performed with quantitative yields under mild conditions, the derivatives have the best chromatographic properties, and the mass spectra are sufficiently different for isomers (see Chapter IV).

Analytical aspects of SCTASi derivatization for chromatography are explored in Chapter V.

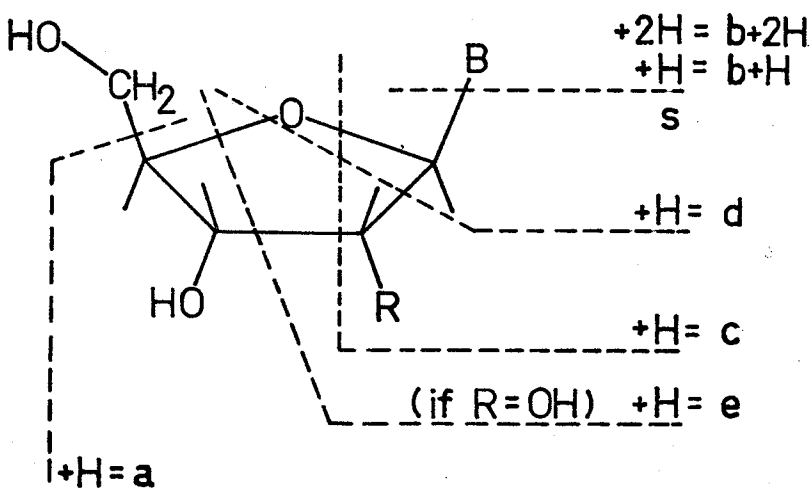
IV. MASS SPECTROMETRY OF NUCLEOSIDE DERIVATIVES

A. Introduction

1. Nucleosides

The application of mass spectrometry to nucleosides has rapidly expanded since the pioneering work of Biemann and McCloskey in 1962 (181). Many papers have proven the value of MS for identification of previously unknown, modified nucleosides as well as other components of nucleic acid: bases and nucleotides. Recently, the field of nucleic acid mass spectrometry has been reviewed in an excellent treatise by McCloskey (15).

Empirical studies with underivatized nucleosides of known structure have revealed that their electron-impact mass spectra contain a number of structurally informative fragment ions (illustrated in Scheme 8). The ions s, b+H, b+2H, and the molecular ion, M, help to identify the nucleoside, while ions a (M-30), d (b+30), c (M-89), and e allow elucidation of structural modifications in the sugar ring. The latter ion, e, is abundant only in ribonucleosides (R=OH), and its mass corresponds to



Scheme 8

R = H or OH

b+60.

There are some major difficulties in the application of MS to underivatized nucleosides in general. Their low volatility requires that samples be introduced via the solid probe inlet system. In some cases, such as guanosine, nucleosides may have such low thermal stability that extensive pyrolysis occurs, resulting in irreproducible and confusing spectra. In other cases, such as inosine and xanthosine, the molecular ion and valuable structural ions may not be present at all, even though pyrolysis does not occur.

New methods of ionization, such as chemical ionization (182,183), field ionization (184), field desorption (185) and recently, ^{252}Cf plasma desorption (186), can extend MS to the more labile nucleosides. These techniques offer more abundant molecular ions (or quasi-molecular ions) and less extensive fragmentation. However, the simplified spectra do not usually give as much structural information as do electron-impact spectra, and fewer points of correlation are available for "fingerprint" matching with reference spectra.

Chemical derivatization is a powerful solution to many of the above problems. The enhanced volatility and thermal stability allows the use of gas chromatography as an alternative means of sample introduction, which in turn extends MS to the analysis of complex mixtures. In addition, the structural inferences offered by GC can be of great complementary value. Moreover, certain types of derivatives may yield much improved electron-impact fragmentation behavior, such as an abundant molecular ion together with structurally valuable fragment ions. A number of derivatization procedures for nucleosides have been investigated: alkylation (187), acetylation (188), trifluoroacetylation (189), iso-propylidation

and boronation (190) for cis-diols of ribosides, and trimethylsilylation (15,191,192). Silylation has been the most valuable, particularly for GC/MS.

The basic fragmentation behavior of TMSi derivatives of nucleosides is summarized in Scheme 9 (adapted in part from refs. 15,191 and 192). The molecular weight is determined from M, M - CH_3(15), and M - TMSiOH(90). Structurally informative fragments include b+H, b+2H, b+H+TMSi, d, c, e, $TMSiOCH_2^+$ (m/e103), and a series of sugar fragments. Three specific fragments are characteristic of ribosides: f, m/e217, and m/e230; and the ubiquitous silicon-containing fragments, m/e73, 75, 89, and 147, are observed in all spectra.

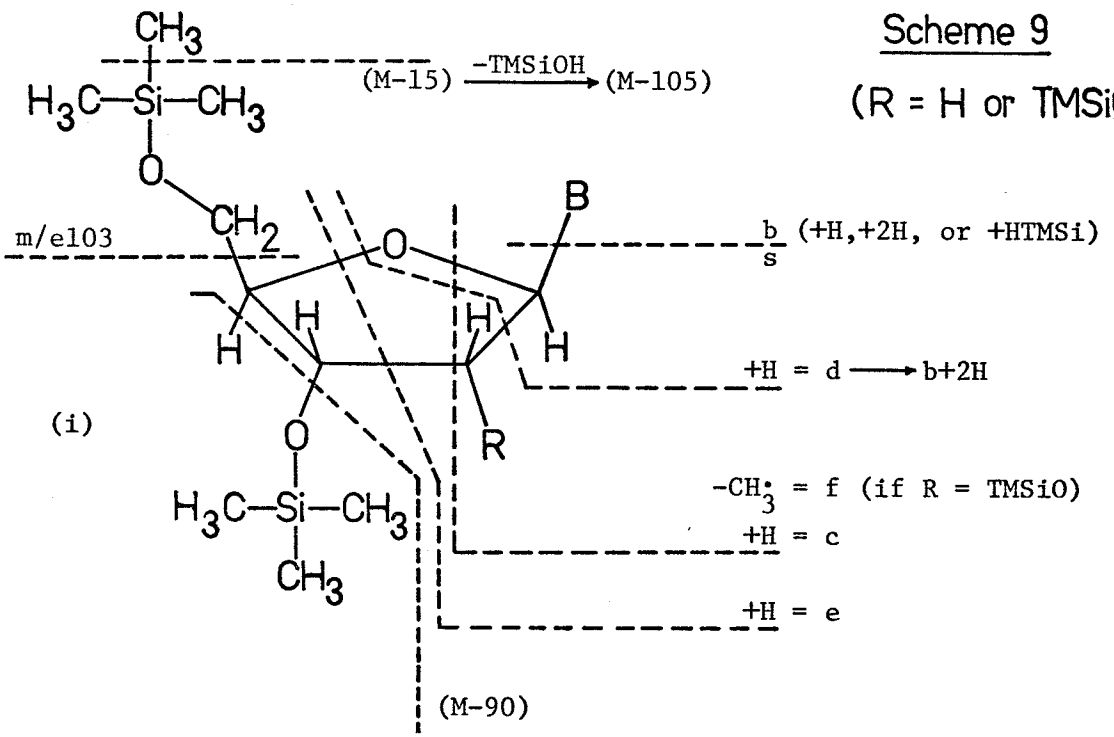
One important point should be noted: for TMSi derivatives of nucleosides, as well as many other compounds, fragments containing silicon usually dominate the spectra. The presence of silicon has an important influence on the fragmentation of a compound. Therefore, it is valuable to review at this time the behavior of silicon compounds under electron-impact.

2. Electron-Impact Behavior of Silicon Compounds

As the analytical applications of silylation developed, it became apparent that silicon imparted some unusual properties to molecules subjected to electron-impact, and that these properties could be very useful in structural studies if they were completely understood. Therefore, within the last few years, there have been many efforts directed towards elucidating the interesting behavior of silylated compounds under electron-impact. Two reviews have discussed the MS of "organometallic" silicon compounds (193,194), but there have been no comprehensive reviews of the fragmentations of silyl derivatives. The papers in this area now

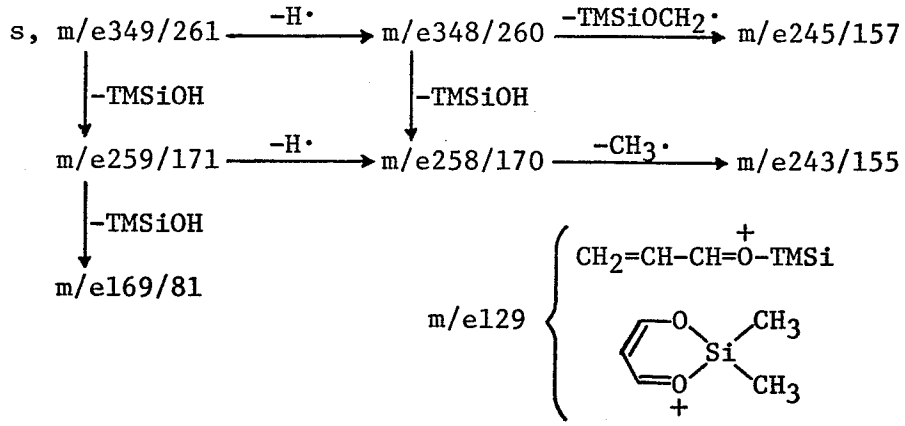
Scheme 9

(R = H or TMSiO)

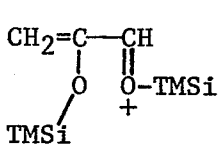


(i)

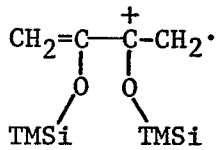
(ii) sugar fragments: (m/e riboside/deoxyriboside)



(iii) specific riboside fragments



m/e217



m/e230

(iv) other silicon fragments

$(CH_3)_3Si^+$	m/e73
$(CH_3)_2SiOH^+$	m/e75
$H(CH_3)_2SiOCH_2^+$	m/e89
$(CH_3)_3SiOSi(CH_3)_2^+$	m/e147

number in the hundreds.

a) Silicon Chemistry

In order to fully comprehend and be able to predict the behavior of silicon in MS, it is important to examine its chemistry (23,195-197), especially compared with that of carbon. Although carbon and silicon are in the same periodic group (IV), they differ greatly in their chemical and physical properties. These differences can be rationalized from the characteristics of their respective valence shells. Although both elements participate in sp^3 bonding, silicon also possesses vacant d-orbitals which can apparently participate in $(p \rightarrow d)_{\pi}$-bonding in Si-N, Si-O, and Si-X(X = F, Cl) bonded systems. On the other hand, while carbon participates readily in $(p \rightarrow p)_{\pi}$-bonding, silicon appears very reluctant to form double bonds. In addition, both the differences in electronegativity (Si = 1.8, H = 2.1, C = 2.5, N = 3.0, and O = 3.5 on Pauling's scale) and covalent radius (C = 0.77Å, Si = 1.17Å) (198,199) contribute to their bonding characteristics. Due to the large size of silicon, there is less steric strain within a trialkylsilyl group than within the corresponding alkyl group (e.g., TMSi vs. tBu), and because trialkylsilyl groups are larger, they can exert more important steric effects. In comparison with carbon, Si generally forms less stable bonds to C and H, but stronger bonds to N, S, O and Cl. Literature values for bond energies vary depending upon the method of measurement. Table IX gives the values determined for the dissociation of the $(CH_3)_3Si$-Y bond by electron-impact measurements (200). These values are useful in considering electron-impact fragmentations, but other factors are important for bond-breaking in solution reactions. Silicon is noted for its high migratory aptitude under a variety of reaction conditions (thermal, photochemical, ionic intermediates, etc.) (197,201).

TABLE IX. Bond energy values for the dissociation of $Me_3Si—Y$ as determined by electron impact studies (200).

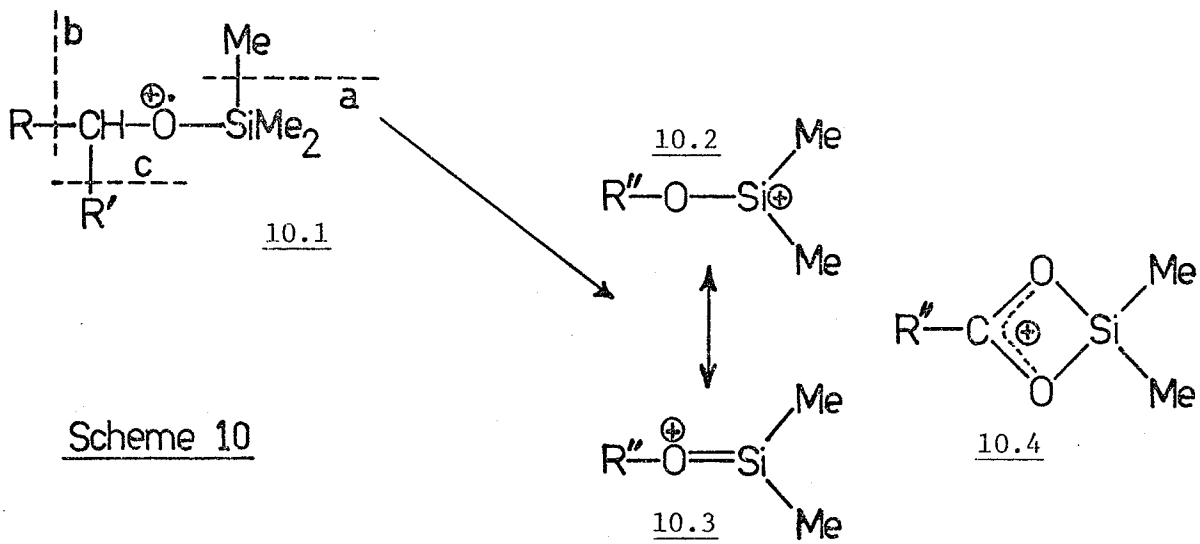
Y	Kcal/mole (±10)
H	88
Me	85
Et	83
$SiMe_3$	86
OMe	127
F	193
Cl	126
B	86
NEt_2	131

This is usually attributed to silicon's d-orbitals and its ability to expand its coordination number from 4 to 6.

b) Mass Spectral Fragmentations

The major fragmentations initiated by a silyl ether group can, in general, be explained by assuming initial charge localization on the ether oxygen (Scheme 10), followed by α-cleavage, either at b or c in the substrate skeleton, or at a with expulsion of an alkyl radical from the silyl group. Of course, another possibility is fragmentation of a precursor in which ionization occurs at a site removed from the silyloxy group. In the mass spectra of TMSi ethers, all three processes are usually important.

For TMSi derivatives, fission at a yields the $[M-15]^+$ ion. For straight chain compounds (R'=H), this is greatly preferred over fission at b. In some cases (especially TMSi esters), the formation of $[M-15]^+$ is so favorable that M^+ is very weak or even absent. With branched compounds, when R' = CH_3, about 20% of the $[M-15]^+$ ion is due to cleavage at c (202).



Scheme 10

There are several reasons for the abundant $[M-15]^+$ ion, namely: (a) decomposition of a molecular ion (odd electron) is most favored to occur by expulsion of an odd-electron (radical) fragment to yield an even-electron ion; (b) the high degree of branching and steric crowding at Si enhances fission at a; (c) since Si is more electropositive than the substituents attached to it, $R_4Si^{+\cdot} \rightarrow R\cdot + R_3Si^+$ is favored over $R_4Si^{+\cdot} \rightarrow R_3Si\cdot + R^+$; (d) the ternary ion R_3Si^+ should be particularly stable because it has an even number of electrons and the electron configuration is that of the corresponding (stable) Group III compound; and (e) for TMSi ethers, the resonance structure 10.3 could help to stabilize $[M-15]^+$ (as could 10.4 for TMSi esters). Also, $(p \rightarrow d)_\pi$-bonding may be very important in this regard. Although structure 10.3 is often used in the literature to represent the $[M-15]^+$ ion, current experimental evidence (*vide infra*) indicates that 10.2 is probably a more realistic representation of the siliconium cation. This also accords with the reluctance of Si to participate in $(p \rightarrow p)_\pi$-bonding.

Fragmentation at b or c in cyclic systems can often lead to characteristic fragment ions. An example of the "fragmentation directing"

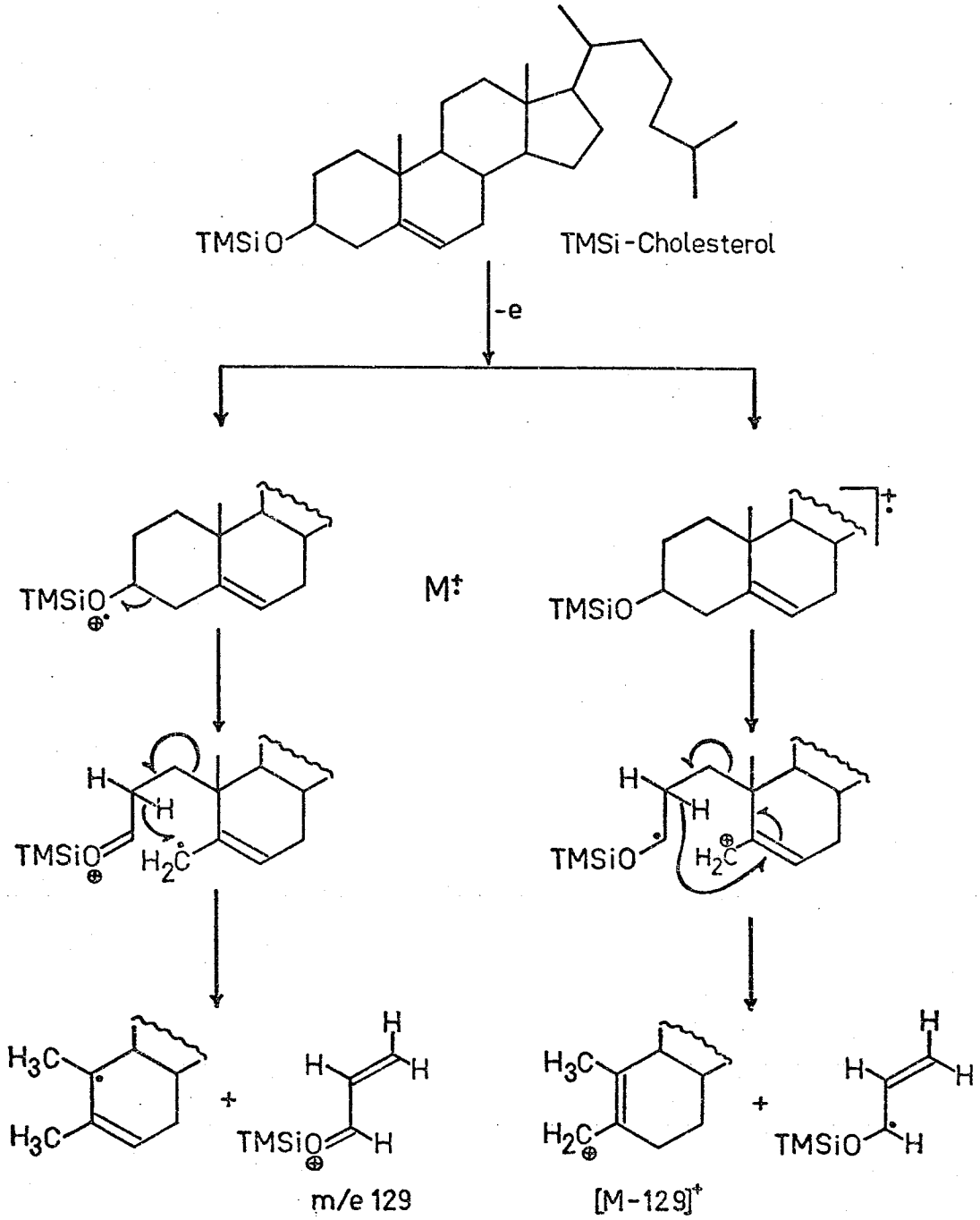
ability of the TMSiO group is given by the fragments m/e129 and M-129 in the spectra of TMSi derivatives of certain steroids (Scheme 11) (203).

Other decompositions of M and ions resulting from fission at a, b, or c can lead to several fragment ions commonly observed in the spectra of TMSi derivatives. These include m/e73, 75, 89, 103, 147, and ions due to loss of silanol (TMSiOH): M-90, M-105, etc.. These have been described previously in Scheme 9 for TMSi-nucleosides.

c) Electron-Impact Induced Rearrangements

Although commercial mass spectrometers became available in the mid-1940's for quantitative analysis, the development of MS as a tool for molecular structure elucidation progressed quite slowly. One of the main reasons for this was the early observation in some mass spectra of anomalous ions which could not arise from simple cleavage of bonds in the sample molecule. The formation of such rearranged ions cast doubt upon the possibility of correlating fragment ions with molecular structure. Unfortunately, the rearrangement processes first noted were mainly in the spectra of hydrocarbons, now well-known to give the most confusing and varied array of rearrangement ions. Such rearrangements have now been classified as "random". Eventually it became apparent that organic compounds containing functional groups undergo more characteristic rearrangements that yield simpler and more distinct ions. A tremendous research effort has since been directed towards a mechanistic understanding of such "specific" skeletal rearrangements. Now, those processes which are well-defined and predictable, such as the McLafferty rearrangement, are among the most valuable probes for structure elucidation by MS. Several reviews have dealt with electron-impact induced rearrangements in organic molecules (204-209).

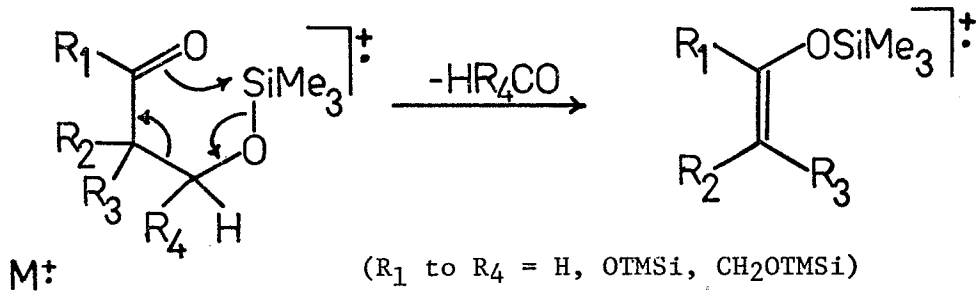
Scheme 11. Electron impact induced fragmentations of TMSi-cholesterol.



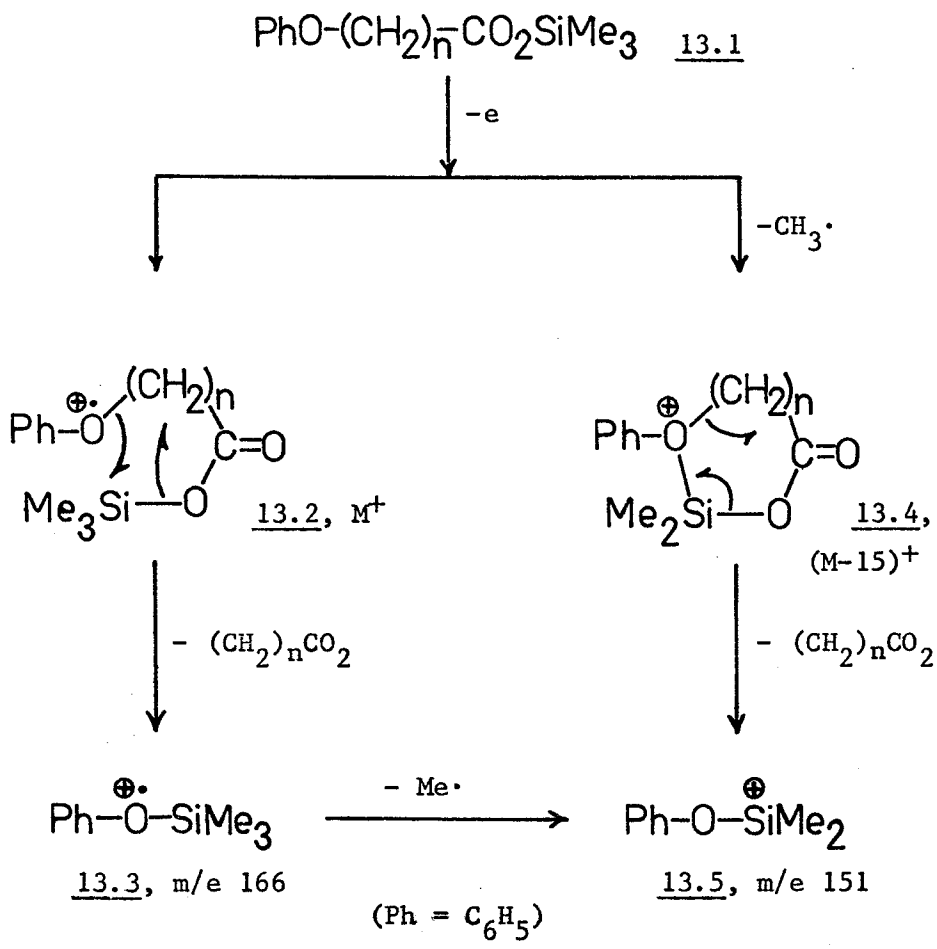
In recent years, it has become increasingly clear that silicon has a high aptitude for such "specific" rearrangements (194,210). Two major types of rearrangements involving silyl centers have been well studied, namely: (a) the migration of an intact TMSi group within an ion (usually the molecular ion) with concurrent fragmentation, and (b) the interaction of a siliconium ion center with electron dense functions in the molecule, often resulting in macrocyclic transition states, with subsequent rearrangement.

The migration of intact TMSi groups appears to proceed in a manner similar to certain types of hydrogen migrations frequently observed. Several authors have emphasized the remarkable similarity of behavior between the TMSi group and the H atom. Examples of TMSi migrations are given in Schemes 12 (211) and 13 (2 → 3) (212). The former has been termed a trimethylsilyl McLafferty rearrangement.

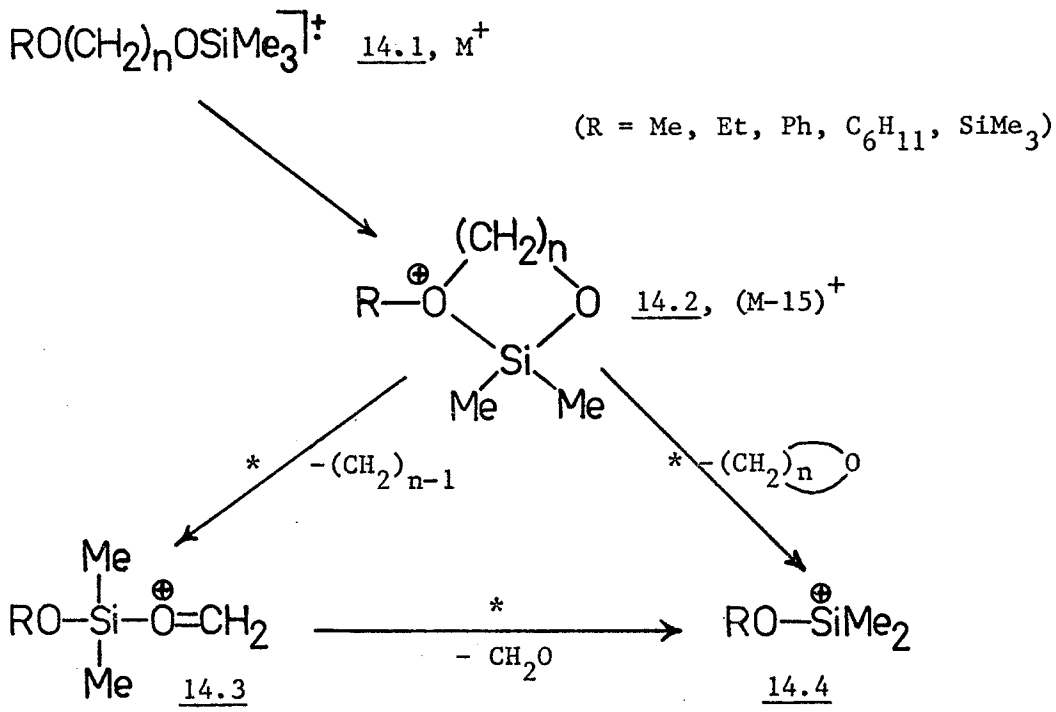
Examples of siliconium ion rearrangements are given in Schemes 13 (4 → 5), 14 (213), and 15 (214). Most of the rearrangements studied appear to be driven by a tendency to form stable Si-O (or Si-N) bonding. This may be aided by a lowering of transition-state and product-ion energies through $(p \rightarrow d)_{\pi}$-bonding. In addition, the siliconium ion rearrangements occur, for the most part, essentially independent of the separation between the interacting functions, as long as such interactions are sterically feasible. Therefore, these rearrangements have been proposed to occur as gas-phase internal ion-molecule reactions (215), with "cyclic silyloxonium ions" as intermediates (e.g., structures 13.4, 14.2, and 15.3). In support of this view, it has been shown that siliconium cations do react via an ion-molecule reaction with various heteroatom-containing molecules to give silyloxonium ions (216-220). This also supports the



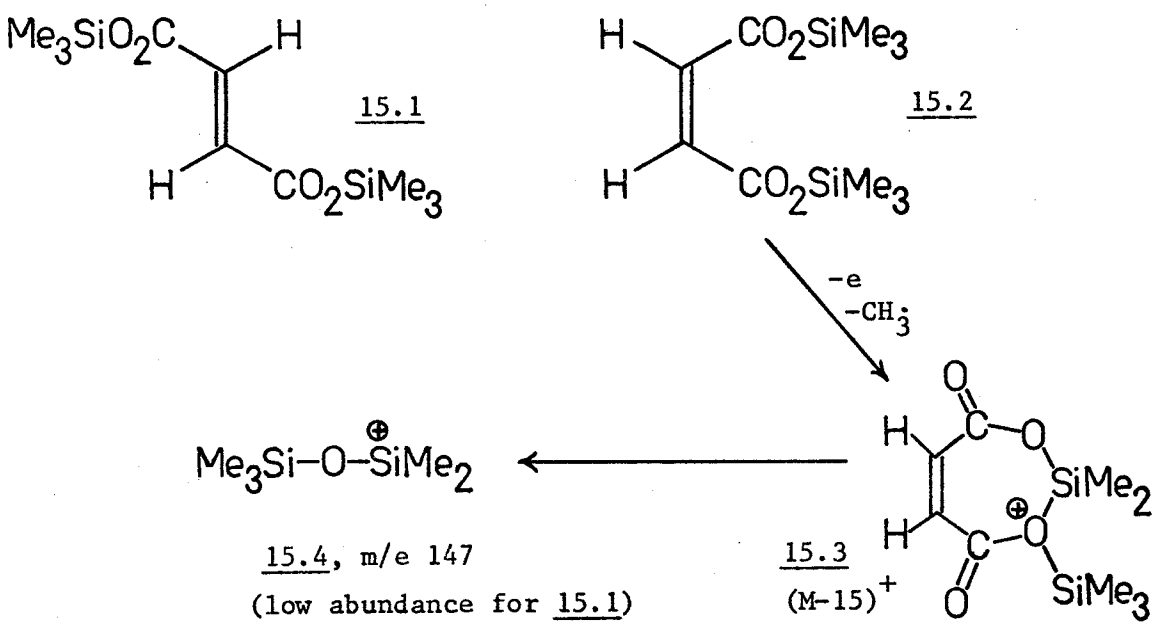
Scheme 12. Trimethylsilyl McLafferty rearrangement (211).



Scheme 13. Rearrangements of TMSi esters of ω-phenoxyalkanoic acids (212).



Scheme 14. Siliconium ion rearrangement of TMSi aliphatic glycol derivatives (213).

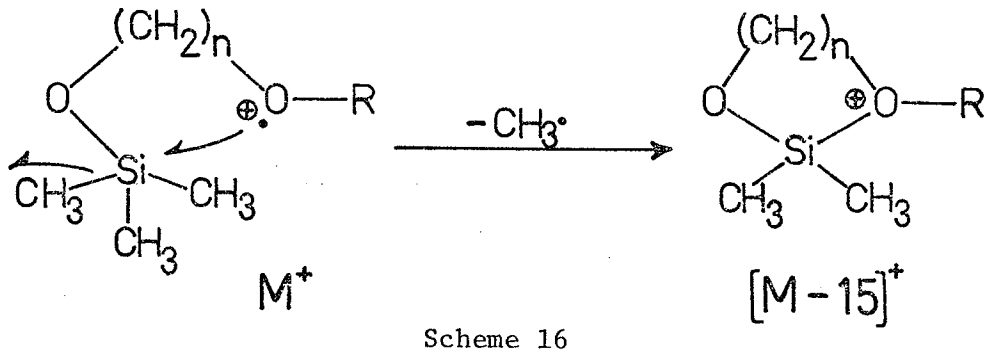


Scheme 15. Stereospecific formation of m/e 147 for the TMSi derivative of maleic acid (15.2) versus that of fumaric acid (15.1) (214).

view that the siliconium ion structure 10.2 is a more realistic representation than 10.3 with regard to its reactivity.

However, there is still some controversy over the existence of cyclic silyloxonium ions. Koppel *et al.*(221) have presented some appearance potential (AP) data for α,ω-bis-TMSi-ethers of the type $TMSiO(CH_2)_n$-OTMSi (n = 2 to 7). From the fact that the AP for $[M-15]^+$ is constant for all values of n (about 9.4 eV) they have concluded that stable $[M-15]^+$ ions do not possess a cyclic structure. Their conclusion is incorrect in two respects. First of all, a constant AP value could simply indicate initial formation of a linear $[M-15]^+$ ion; this does not disallow subsequent collision of the ends of the molecule to form a cyclic structure. Many such collisions should be possible within the lifetime of ions (approx. 10^{-6}s) in the ion source (222). Secondly, and most importantly, they have neglected to consider the AP of $[M-15]^+$ in systems where such interactions are not possible, namely mono-TMSi ethers. For such compounds, the ionization potential for M and the AP for $[M-15]^+$ are approximately 9.7 and 10.2 eV respectively (223). If Koppel's results (9.4 eV for M-15) are compared to these, it is apparent that some sort of "assistance" must be available for $[M-15]^+$ formation in bis-TMSi ethers. This could come from one oxygen inducing the expulsion of a methyl radical soon after electron impact and forming a cyclic silyloxonium ion structure, as shown in Scheme 16. This would be promoted by the formation of an additional Si-O bond (stronger than the broken Si-C bond) and the elimination of a radical to produce an even-electron species.

This concept could account for the wide variations in the M/(M-15) ratio observed in various compounds. In conformationally rigid systems, an adjacent electron dense function could very readily induce the



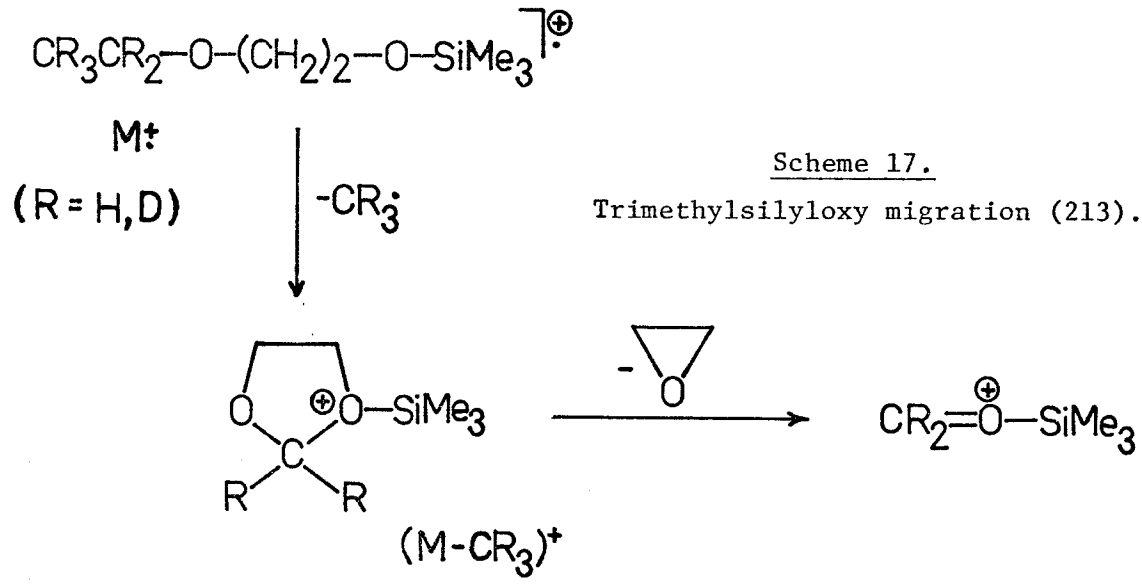
Scheme 16

formation of a cyclic $[M-15]^+$ structure which could be quite stable. (Of course, "internal ion-molecule" reactions of initially linear forms of $[M-15]^+$ must make a major contribution to cyclic silyloxonium ions and their rearrangement products in many systems where conformations are not rigid.) Some evidence is presented later in this thesis (Section C2) to support this argument.

Many papers have noted interactions between remote functional groups in other systems (222,224-231). It has been suggested that these kinds of fragmentations will provide the greatest amount of information by MS on the finer structural features such as stereochemistry (232,233).

Siliconium ion rearrangements may be the most powerful probe for such information. In support of this, several papers have demonstrated the strong stereochemical dependence of these rearrangements (214(Scheme 15), 234,235).

Other rearrangements observed for silyl derivatives include migration of trimethylsilyloxy (TMSiO) groups to remote carbonium centers (e.g., Scheme 17 (213)) and rearrangement of alkyl groups attached to silicon.



Scheme 17.
Trimethylsilyloxy migration (213).

d) SCTASi Derivatives

The results reported in this thesis demonstrate that the mass spectra of SCTASi ether derivatives are quite different from those of TMSi derivatives. The main reason is that fragmentations at b and c (Scheme 10) are greatly diminished in favor of siliconium ion formation via fragmentation a. This is rationalized by the favorable elimination of a stable, branched alkyl radical (R· = iPr· or tBu·) and the relief of steric crowding in the silyl group, in addition to those reasons stated previously for $[M-15]^+$ in TMSi ethers. Thus, the spectra have an abundant $[M-R]^+$ siliconium ion, and usually a very weak or undetectable molecular ion. $[M-R]^+$ is the precursor of most other fragment ions in the spectra, and rearrangements are almost exclusively of the siliconium ion type.

There are advantages for this behavior compared to that of TMSi derivatives. For the latter, the ratio of M^+ to $[M-15]^+$, and therefore the proportions of different types of fragmentations (i.e., α-cleavage, TMSi migration, siliconium ion interactions, or those due to charge localization

elsewhere in the molecule), can vary widely and unpredictably between various compounds. The resulting spectra may be quite complex due to several different processes operating simultaneously. Also, the delicate balance between formation of $[M-15]^+$ and alternative fragmentations of M^+ can be influenced seriously by instrumental conditions, especially temperature. The degree of fragmentation that occurs depends upon the internal energy of the molecule, which is derived from both electron-impact and contact with a hot ion source chamber and solid probe or GC inlet systems (236). Drastic differences in mass spectra have been reported for TMSi ethers of steroids (203). The diagnostically important fragment at m/e129 in TMSi-cholesterol (Scheme 11) is the base peak if a GC/MS system is used, but is reduced to 2% relative intensity if a crystalline sample is introduced directly by solid probe. Variations in the relative heights of M^+ and $[M-15]^+$ have also been observed in the literature (203), and by this author, to depend upon the inlet system and ion source temperatures. No such variations have been observed for SCTASi derivatives of either nucleosides or steroids.

The fact that stereochemically informative siliconium ion rearrangements are enhanced with SCTASi ethers, promises to make these derivatives of great value for stereochemical elucidation by MS. This is supported by results in later sections of this thesis.

The mass spectra of poly-TMSi and -SCTASi ether derivatives do have some similarities. For example, the elimination of silanol occurs for both TMSi and SCTASi ether derivatives. Other aspects are discussed in Sections B2 and C2.

e) Labelling

Specific labelling with deuterium (and sometimes oxygen-18) is an

important technique for elucidating mass spectral fragmentation pathways. McCloskey et al.(52) introduced the technique of TMSi-d_9 (dTMSi) labelling, which has since become valuable in deducing fragmentations and rearrangements of TMSi derivatives. For polysilylated compounds, mixed TMSi/dTMSi derivatives are useful. Initially, McCloskey et al.(52,237) employed randomly mixed derivatives prepared with an equimolar mixture of labelled and unlabelled silylating reagents. Mixed derivatives in which the dTMSi group occupies a specific position have also been prepared, by utilizing the higher lability of acidic as compared with ethereal TMSi groups in exchange reactions on a GC column (192,238,239). More recently, Vouros and Harvey (53) have developed a procedure for introducing dTMSi to specific positions in steroids by selective silylation. This is based on the different silylation rates of sterically hindered and unhindered hydroxyl groups.

The three SCTASi groups selected for this study, TBDMSi, TMIPSi and TMTBSi, together form a series which effectively labels the silyl substituents. In general, the different derivatives fragment similarly and fragment ion types have about the same relative intensities. The mass shifts that occur when changing a silyl goup (designated hereafter as RX_2Si, where R is the bulkiest alkyl substituent (iPr or tBu) and X_2 represents the remaining substituents (Me_2 or C_4H_8) (Table X)) are unique: TBDMSi $\xrightarrow{+26u}$ TMTBSi, which labels the X substituents; TBDMSi $\xrightarrow{+12u}$ TMIPSi, which labels both the R and X substituents. Other common mass shifts observed in SCTASi derivatives' spectra are given in Table X. The use of these groups can avoid the preparation of expensive deuterium-labelled silyl groups.

Of course, there are some minor variations in fragmentation

TABLE X. Nomenclature for SCTASi groups and common mass shifts that occur for fragment ions containing specific silyl substituents when the silyl group is changed from TBDMSi to TMTBSi or TMIPSi.

RX_2Si	R	X_2	mass (amu)
TBDMSi	tBu	Me_2	115
TMTBSi	tBu	C_4H_8	127
TMIPSi	iPr	C_4H_8	141

	Content of Fragment Ion[a]	Mass Shift (amu) from TBDMSi	
		TMTBSi	TMIPSi
b	no X or R	0	0
	RX_2Si	26	12
1	X_2Si(or TX_2Si)	26	26
	$2(RX_2Si)$	52	24
	$RX_2Si + X_2Si$	52	38
2	$TX_2Si + X_2Si$	52	52
	$3(RX_2Si)$	78	36
	$2(RX_2Si) + X_2Si$	78	50
3	$RX_2Si + TX_2Si + X_2Si$	78	64

[a] T = H or CH_3, after portion of R group has been eliminated.

[b] Maximum number of silyl groups in compound for which shifts are applicable.

between the three silyl derivatives. With TMIPSi, the iPr· radical is not lost as readily as tBu· for TBDMSi and TMTBSi, resulting in the retention of some "TMSi behavior"(e.g., M^+ is often observed for TMIPSi derivatives). Both TMTBSi and TMIPSi have some unique fragmentations involving rearrangement of the cyclo-tetramethylene ring, while TBDMSi tends to lose CH_3· to a slight extent, leading to a series of ions that parallel those formed from $[M-R]^+$. The ideal labelling system would be TBDMSi + TBDMSi-d_{15}. However, the latter group was not available for this study.

For derivatives with more than one SCTASi group, there is the possibility of more than one type of $[M-R]^+$ion. Each of these siliconium ions will "direct" their own series of fragmentations and rearrangements. The resulting composite spectrum can be quite complicated to interpret in detail unless mixed derivatives are used. Replacing one SCTASi group with another allows specific labelling of the substituents in that group. Such mixed SCTASi derivatives of nucleosides are relatively easy to prepare with the controllable selectivity of SCTASi reagents and the stability of the partial derivatives during manipulations. In some cases, however, a particular siliconium ion may direct a fragmentation, but not be part of the final product ion. Simple labelling of the SCTASi group in these cases does not reveal the mechanism. Partial SCTASi and mixed TMSi/SCTASi derivatives, as developed in this thesis, are valuable in this respect. These derivatives allow the "isolation" of one particular $[M-R]^+$ type and all the daughter ions that result from its fragmentation direction. The mixed TMSi/SCTASi derivatives are the most valuable for interpreting the spectra of fully-O-silylated compounds. The TMSi group behaves as a "passive" SCTASi group since elimination of CH_3· cannot compete with tBu· elimination. The partial SCTASi derivatives, on the

other hand, appear to undergo more extensive fragmentation. The labile hydrogen on an unprotected hydroxyl appears to enhance many fragmentations. However, a few fragmentations which depend upon the charge localizing ability of an intact silyloxy group can be suppressed.

3. Objectives

The objective of this chapter is to present the interpretation of the mass spectra of SCTASi derivatives of nucleosides. Most of the work reported deals with isomeric substituted derivatives of synthetic interest. Also of concern, however, are the fully-O-silylated derivatives which could be of considerable analytical importance, a topic that is discussed further in Chapter V. For these compounds, the isomeric substituted derivatives have proven invaluable for MS interpretation, as pointed out above.

There is little research reported in the literature that deals with the MS of isomeric substituted nucleosides, despite the interest in characterizing these types of compounds for synthetic and sequencing purposes.

Isomeric mononucleotides have been examined as TMSi derivatives (192). Isomerization occurs for the 2'- and 3'-phosphates during silylation and after electron-impact, leading to spectra which are indistinguishable from each other but clearly different from the 5'-phosphate isomer. Isomeric methyl substituted adenosines have been examined (240) and give rise to different mass spectra without further derivatization. Westmore et al.(241) were able to characterize isomeric substituted 2,2'-anhydronucleosides by Ac, TFA, or TMSi derivatization. In the same study, 2'- versus 3'-linked anhydronucleosides could be differentiated as TMSi derivatives (242). Also, the combination of isopropylidation or phenyl-

boronation with other blocking groups (TMSi, Ac, TFA) has been investigated as a method for nucleotide sequencing (190).

B. SCTASi Derivatives of Deoxynucleosides

1. Results

The mass spectral data for SCTASi derivatives of 2'-deoxynucleosides, and a discussion of the general significance of the mass spectra, are presented in this section. Following this, detailed interpretations of the principal fragment ions are given (Section B2).

a) Partial O-SCTASi Derivatives

This entire project began with the observation that isomeric partial-TBDMSi derivatives of deoxynucleosides had very characteristic mass spectra (65,87). Figures 31 and 32 illustrate this with the spectra for 5'-O-TBDMSi-thymidine (Iba) and 3'-O-TBDMSi-thymidine (Iab), respectively. In order to make a detailed study of these spectra, the entire series of deoxynucleosides (I-V) were examined as partial TBDMSi derivatives (Figures 33 to 40) to determine the influence of the base, and the partial TMTBSi and TMIPSi derivatives of I were studied to effectively label the silyl substituents (Figures 41 to 44). In addition, the labile hydrogens (OH and NH) in Iba and Iab were exchanged for deuterium to determine their fate.

Table XI summarizes the fragment ions that are most important for the characterization of partial SCTASi derivatives of deoxynucleosides. The interpretations are discussed in detail in section B2, and are based upon the results given in Tables XII and XIII.

Some problems were encountered with the partial derivatives of deoxycytidine (IIIba and IIIab) and deoxyguanosine (IVba and IVab), which

required rather high sample temperature to achieve volatilization. Slight thermal decomposition of these derivatives was indicated by a variation in the intensity of the $[B+H]^+$ ion during some runs. This is the molecular ion of the free base that is released during thermal decomposition, a process which has been observed for underivatized nucleosides (15). Also, for compound IIIba, peaks corresponding to those of the bis-silyl-derivative (IIIbb) appeared in some runs at higher temperatures. This indicated that some thermally-induced intermolecular exchange of silyl groups was occurring prior to vaporization. (A similar observation was made for partially acylated derivatives of anhydronucleosides (243).) These results suggest that further derivatization of the more labile nucleosides should be studied, especially since they may then be amenable to GC/MS analysis as well.

b) Mixed Derivatives

Further derivatization of partial O-SCTASi derivatives by TMSi, Ac and TFA reagents was studied in Chapter III for the purpose of improving chromatographic behavior. The mass spectral behavior of these "mixed derivatives" is also of interest for a number of reasons. Further derivatization is a prerequisite for a general GC/MS approach to the analysis of partial derivatives, particularly for the more labile deoxy-nucleosides (III and IV) and the ribonucleosides. If the spectra of isomers are still distinctly different after derivatization, identification would still be possible, in addition to allowing quantitative analysis by GC/MS-SIR techniques (even for those isomers that do not separate by GC). As discussed previously, mixed derivatives are also valuable for the interpretation of the spectra of fully-O-silylated derivatives.

i) Ac/SCTASi Derivatives

The mixed 3',5'-O-(Ac/SCTASi) derivatives have characteristic spectra, as illustrated in Figures 45 and 46 for the isomers Ibg and Igb. In the case of deoxyadenosine, N6-acetylation is possible, resulting in the isomers Vbgg and Vgbg. These also yield excellent spectra (Figures 47 and 48) with different fragmentation patterns and abundant $[M-R]^+$ ions. Although they are easy to prepare and have useful spectra, the acetyl derivatives are not as volatile as their TFA and TMSi analogs. This limits their value in GC/MS, particularly for the more labile nucleosides.

Assignments for major fragment ions are presented in Table XIV. Interpretations were aided with the dAc derivatives, Ibh and Ihb.

ii) TFA/SCTASi Derivatives

The mixed TFA/SCTASi derivatives not only give characteristic spectra but are separable by GC (Chapter III). Figures 49 to 52 illustrate the spectra of the 3',5'-O-(TFA/TBDMSi)-thymidine isomers, Ibi and Iib, and the 3',5'-O-(TFA/TBDMSi)-N6-TFA-deoxyadenosine isomers, Vbii and Vibi. Table XIV presents the interpretation of major fragment ions. The Ac and dAc derivatives were useful in supporting the proposed assignments.

iii) TMSi/SCTASi Derivatives

The spectra of these derivatives are also characteristically different for isomer pairs, as illustrated in Figures 53 to 56 for Ibe and Ieb (3',5'-O-(TMSi/TBDMSi)-thymidines) and Vbee and Vebe (3',5'-O-(TMSi/TBDMSi)-N6-TMSi-deoxyadenosines). Although isomers could not be separated by GC, these may be the best derivatives for a general GC/MS approach to the analysis of partial-O-SCTASi derivatives. They are easily prepared, have good volatility and thermal stability, are suitable for

the ribonucleoside series as well, and their very different spectra should allow GC/MS-SIR to be used for quantitative analysis. Extension of this work to the more thermally labile nucleosides may allow the GC/MS of substituted cytosine and guanine nucleosides.

Interpretation of the major fragment ions in mixed 3',5'-O-(TMSi/SCTASi)-deoxynucleosides is presented in Table XVd. The assignment of ion types was aided by the spectra of dTMSi/TBDMSi derivatives, and the 3',5'-O-(TMSi/TBDMSi)-deoxyadenosine isomers, Vbea and Veba. (The latter were prepared by selective silylation of hydroxyls only.) Fragmentation mechanisms are discussed in Section B2. The N6-TMSi deoxyadenosine derivatives have not yet been interpreted in detail, although some of the major fragment ions are assigned in Figures 55 and 56.

iv) Mixed SCTASi Derivatives

Three pairs of isomeric mixed SCTASi derivatives of thymidine were examined (Ibc/Icb, Ibd/Idb, and Icd/Idc) for three reasons: 1) to aid the interpretation of 3',5'-bis-O-SCTASi-deoxynucleoside spectra; 2) they are important synthetic intermediates; and 3) SCTASi derivatization could be a useful analytical approach for characterizing isomeric mono-O-SCTASi derivatives, especially since bis-O-SCTASi compounds are very stable towards hydrolytic conditions (compared to TFA and TMSi). The spectra of isomeric pairs are quite different as indicated in Figures 57 and 58 for the mixed TMIPSi/TBDMSi-thymidine derivatives, Ibd and Idb. Table XVc presents a detailed interpretation of major fragment ions, while the discussion in Section B2 deals with fragmentation mechanisms.

The above results indicate that SCTASi groups appear to direct the fragmentations of mixed derivatives in such a way as to give excellent information on isomeric substitution in the sugar ring. Therefore, SCTASi

derivatization may be useful for the characterization of other types of isomeric partial derivatives, such as acyl or alkyl substituted nucleosides, nucleoside monophosphates, or even dinucleotides. The investigation of mixed derivatives presented in this thesis should be of value in future studies.

c) 3',5'bis-O-SCTASi Derivatives

The interpretation of the spectra of these derivatives is important not only due to their potential analytical value (Chapter V), but also because they are important synthetic intermediates that need to be characterized. In addition, the interpretations give additional support to those for partial and mixed derivatives. Tables XVa and XVb present detailed assignments of ion types for the various derivatives studied, and the discussion in Section B2 deals with fragmentation mechanisms. Figures 59 to 65 present the spectra of 3',5'bis-O-TBDMSi, -TMTBSi, and -TMIPSi derivatives of thymidine (Ibb, Icc, Idd) and the 3',5'bis-O-TBDMSi derivatives of deoxyuridine (IIbb), deoxyadenosine (Vbba), deoxycytidine (IIIbb), and deoxyguanosine (IVbb). Higher sample temperatures for IIIbb and IVbb presented some inconvenience, although no signs of thermal decomposition were evident. However, since they were not amenable to GC analysis, further derivatization of the base functions should be studied.

It is interesting to compare the spectra of the corresponding TMSi derivatives to see if SCTASi derivatives offer more structural information. The spectra of 3',5'bis-O-TMSi ethers of thymidine (Iee) and deoxyuridine (IIee) are displayed in Figures 66 and 67. Comparing these to the spectra in Figures 59 and 60, it is apparent that the TBDMSi derivatives are more sensitive to changes in the nucleoside's base. The TMSi derivatives'

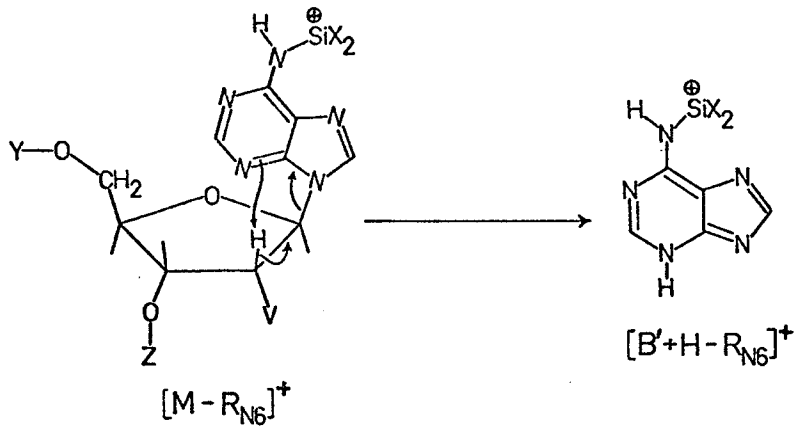
spectra are dominated by sugar fragments. Although the fragmentations of TMSi derivatives are sensitive to isomeric substitution in the sugar, SCTASi derivatives are also sensitive, perhaps to an even greater degree, as illustrated by the mixed derivatives' spectra.

Other notable features of analytical value for SCTASi derivatives include an abundant $[M-R]^+$ for the determination of molecular weight (vs. weak M^+ or $[M-15]^+$ ions for TMSi derivatives) and excellent hydrolytic stability that allows easy isolation for MS analysis. The complete and detailed interpretation of ion compositions and fragmentation mechanisms presented in Section B2 should allow many future applications of these derivatives (e.g., biochemical studies such as determining the position of isotope incorporation into nucleoside structures).

d) N,O-persilylated Derivatives

Since the fully N,O-SCTASi derivatized deoxynucleosides have potential analytical value (see Chapter V), a preliminary investigation was made into their mass spectral behavior. The spectrum of 3',5'bis-O-TBDMSi-N6-TBDMSi-deoxyadenosine (Vbbb) is given in Figure 68. It is quite different from that of the corresponding TMSi derivative (Veee), given in Figure 69. The TBDMSi derivative has a simple spectrum with most of the total ionization current carried by two ions: m/e536 ($[M-R]^+$) and m/e192. The latter is assigned as $[B'+H-R_{N6}]^+$, where B' is the base unit with a TBDMSi group at N6. The composition of this ion is established by examining the spectrum of the mixed derivative 3',5'bis-O-TBDMSi-N6-TMTBSi-deoxyadenosine (Vbbc), in Figure 70, in which the same ion type has shifted to m/e218. The most probable mechanism of formation is that given in Scheme 18.

Since trimethylsilylation appears to be a useful approach for



Scheme 18.

analyzing partial SCTASi derivatives, it is important to consider the mass spectrometry of N-TMSi derivatives of the bis-O-SCTASi compounds which usually occur with the former in silylation mixtures. The spectrum of 3',5'bis-O-TBDMSi-N6-TMSi-deoxyadenosine (Vbbe) is given in Figure 71. A more detailed interpretation of the spectrum than that indicated in Figure 71 requires labelling experiments. However, it is apparent that this type of derivative allows better characterization of the sugar moiety than do the fully N,O-SCTASi derivatized compounds. This type of work should be extended to deoxycytidine and deoxyguanosine.

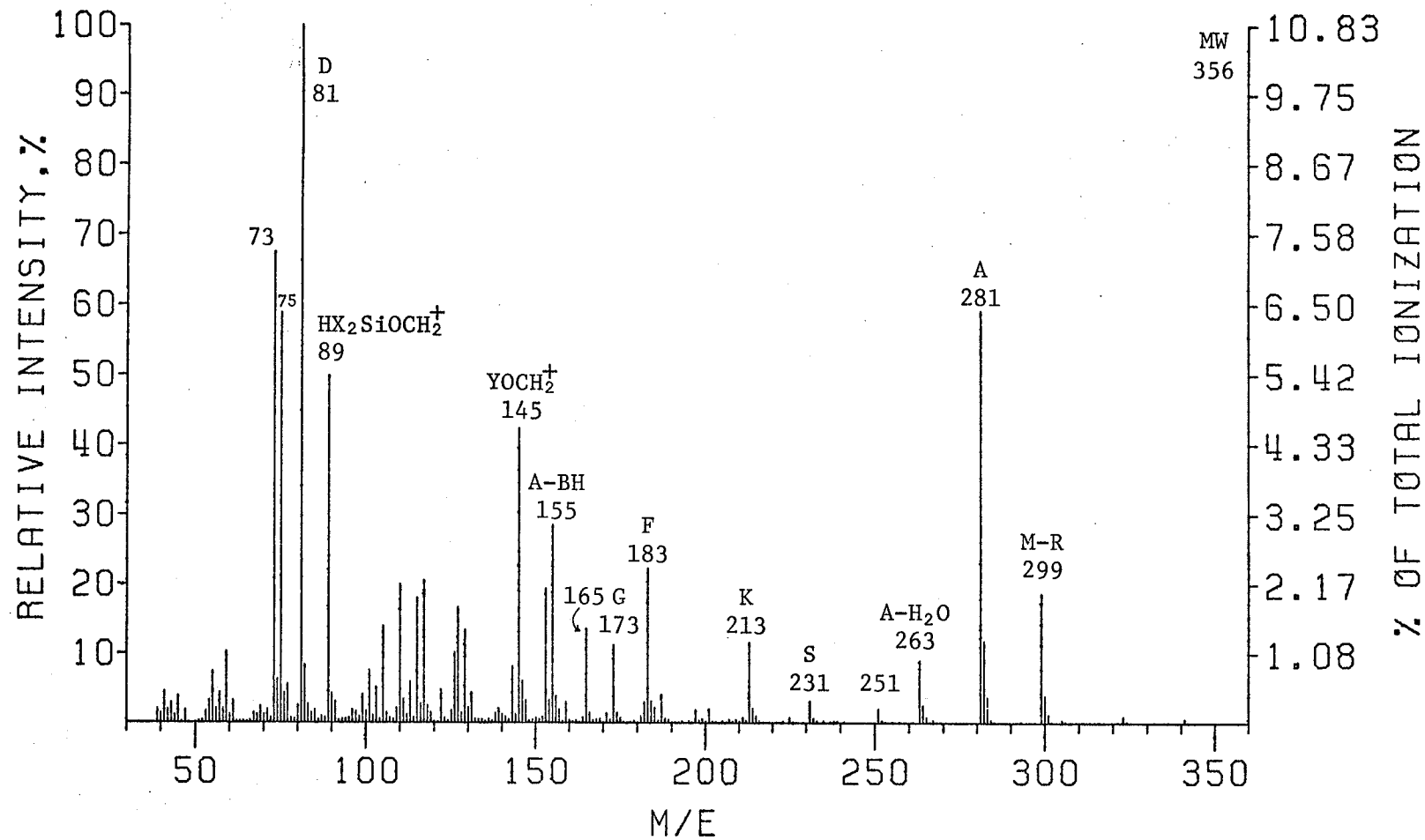


Figure 31. Mass spectrum of 5'-O-TBDMSi-thymidine (Iba) recorded on Hitachi MS at 50 eV.

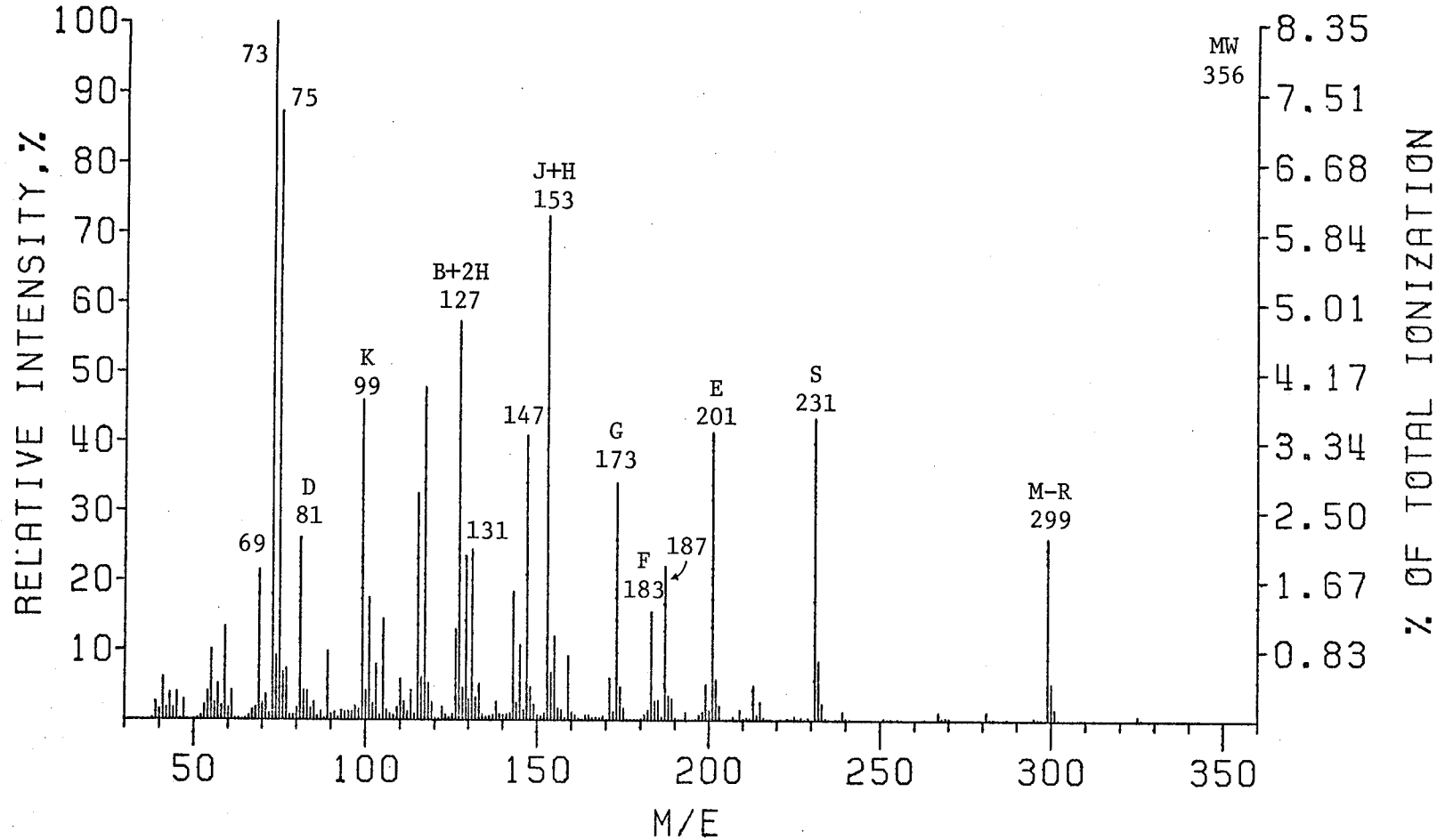


Figure 32. Mass spectrum of 3'-O-TBDMSi-thymidine (Iab) recorded on Hitachi MS at 50 eV.

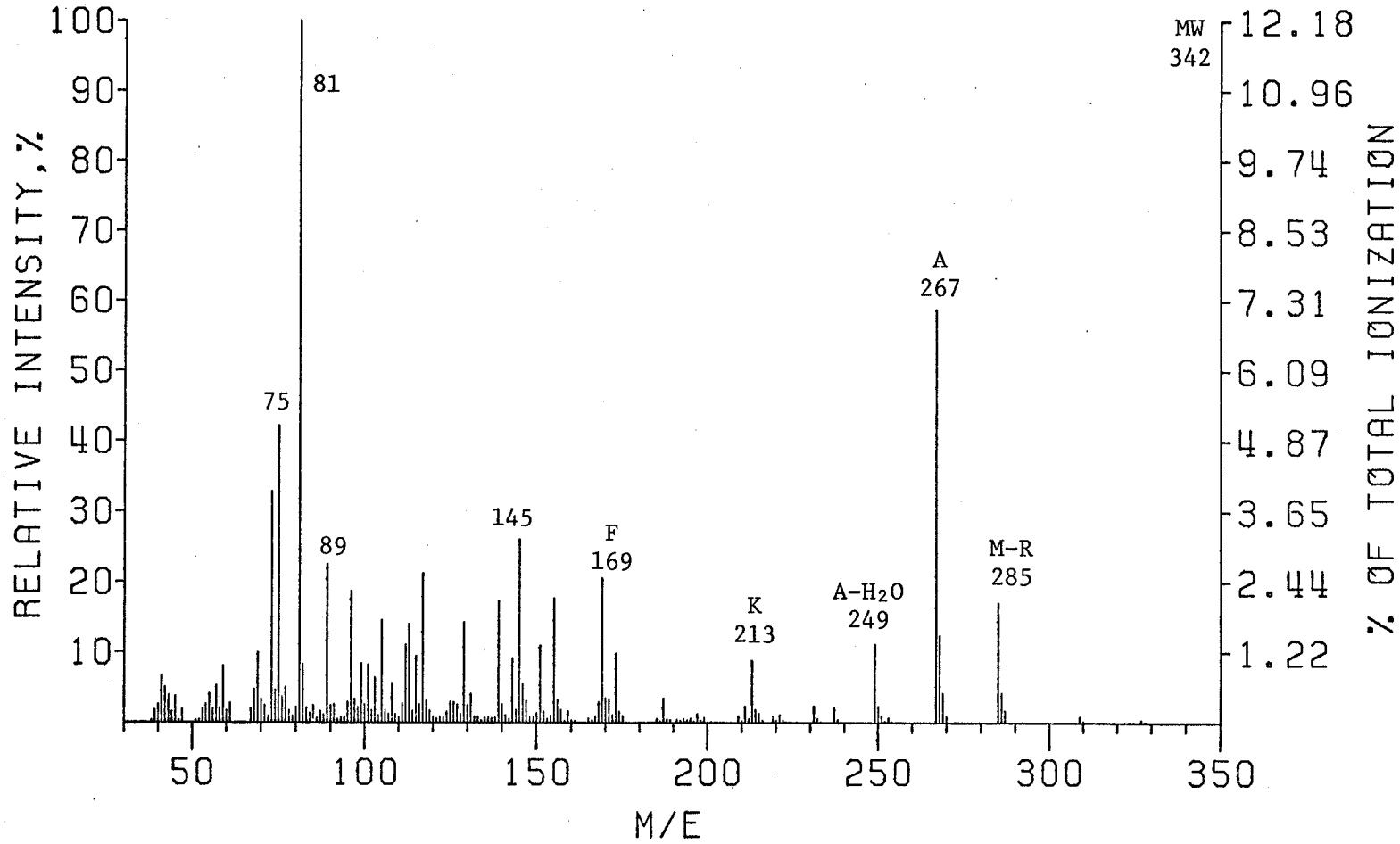


Figure 33. Mass spectrum of 5'-O-TBDMSi-deoxyuridine (IIba) recorded on Finnigan MS at 70 eV.

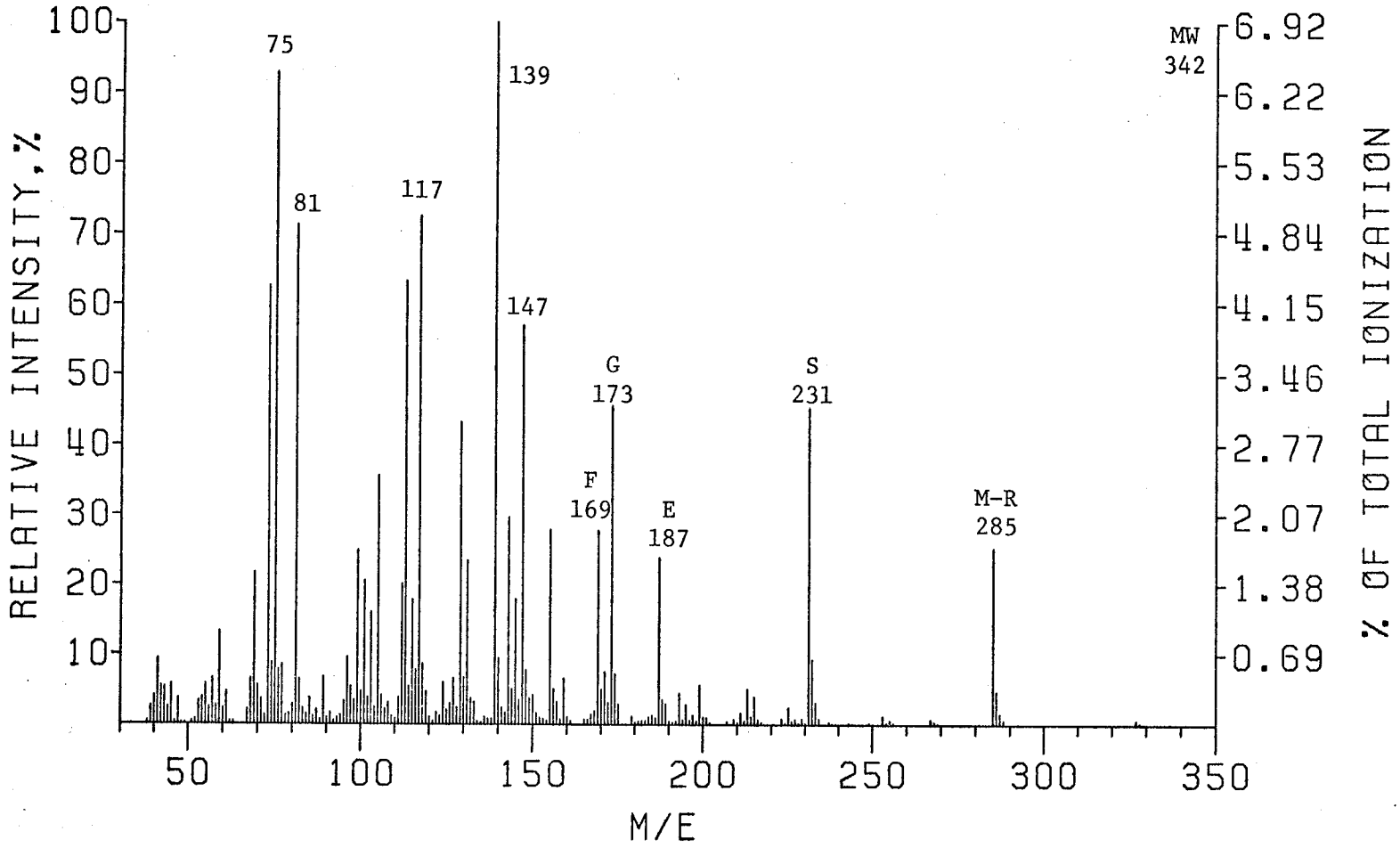


Figure 34. Mass spectrum of 3'-O-TBDMSi-deoxyuridine (IIab) recorded on Finnigan MS at 70 eV.

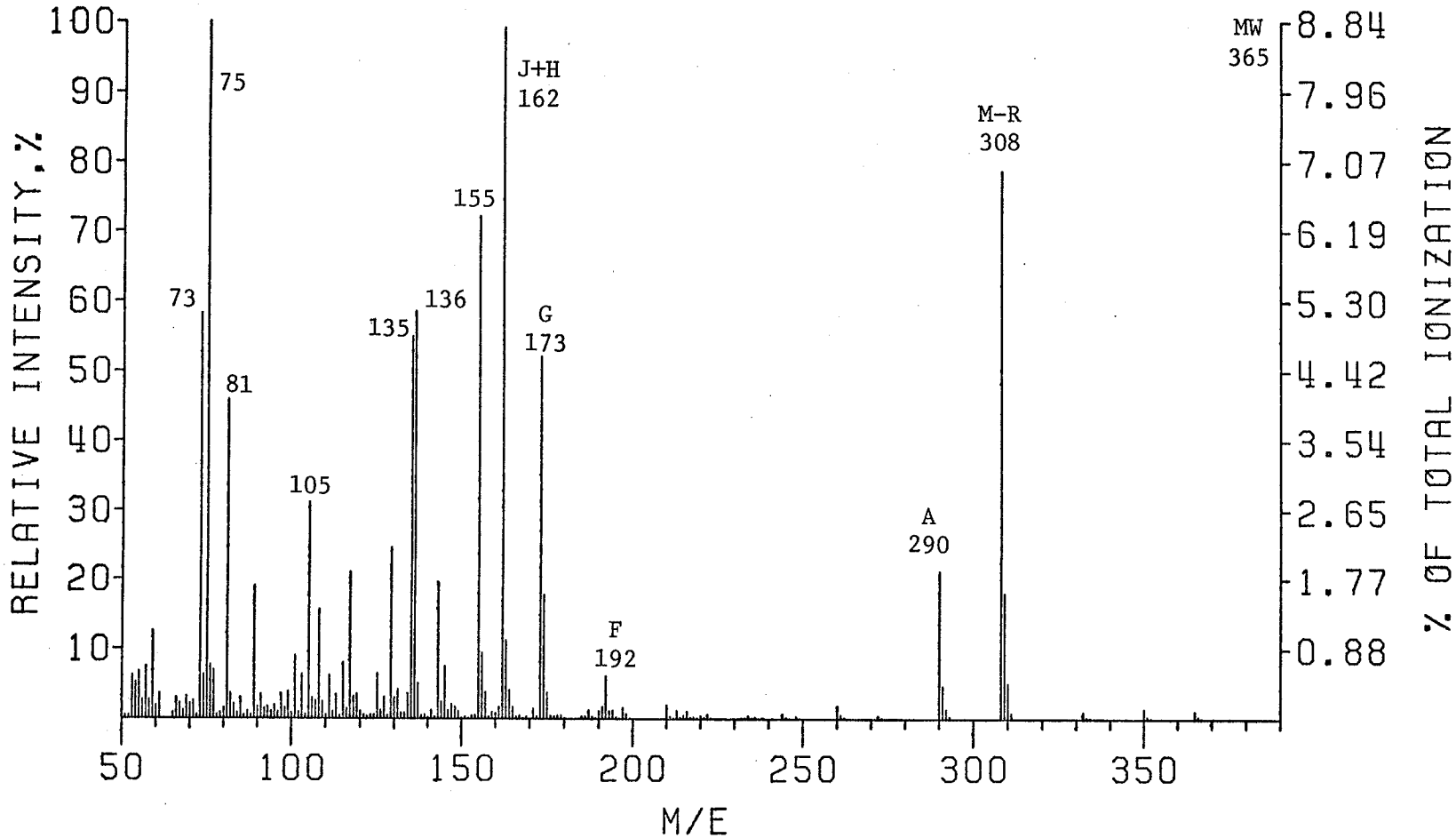


Figure 35. Mass spectrum of 5'-O-TBDMSi-deoxyadenosine (Vbaa) recorded on Hitachi MS at 50 eV.

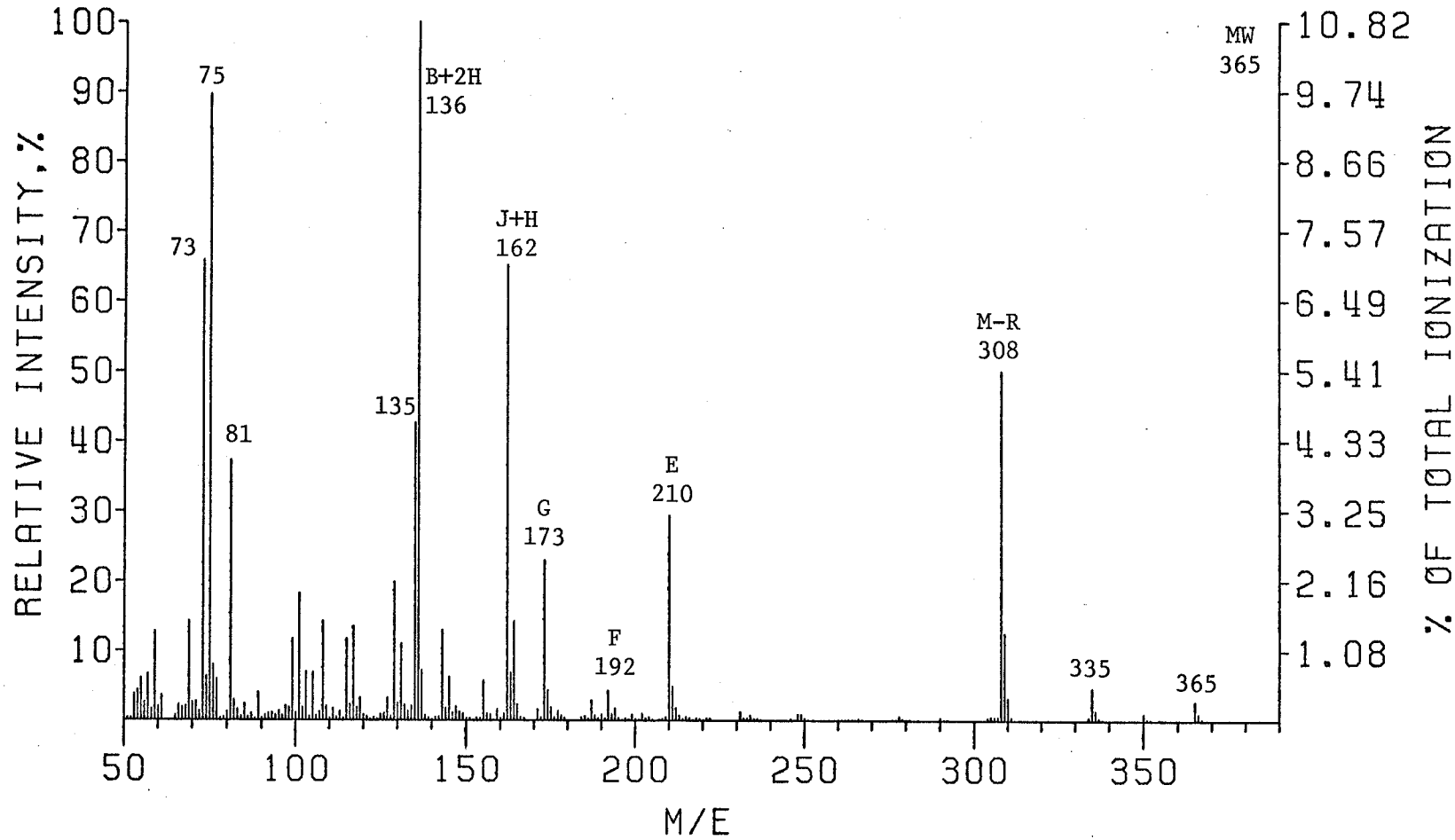


Figure 36. Mass spectrum of 3'-O-TBDMSi-deoxyadenosine (Vaba) recorded on Hitachi MS at 50 eV.

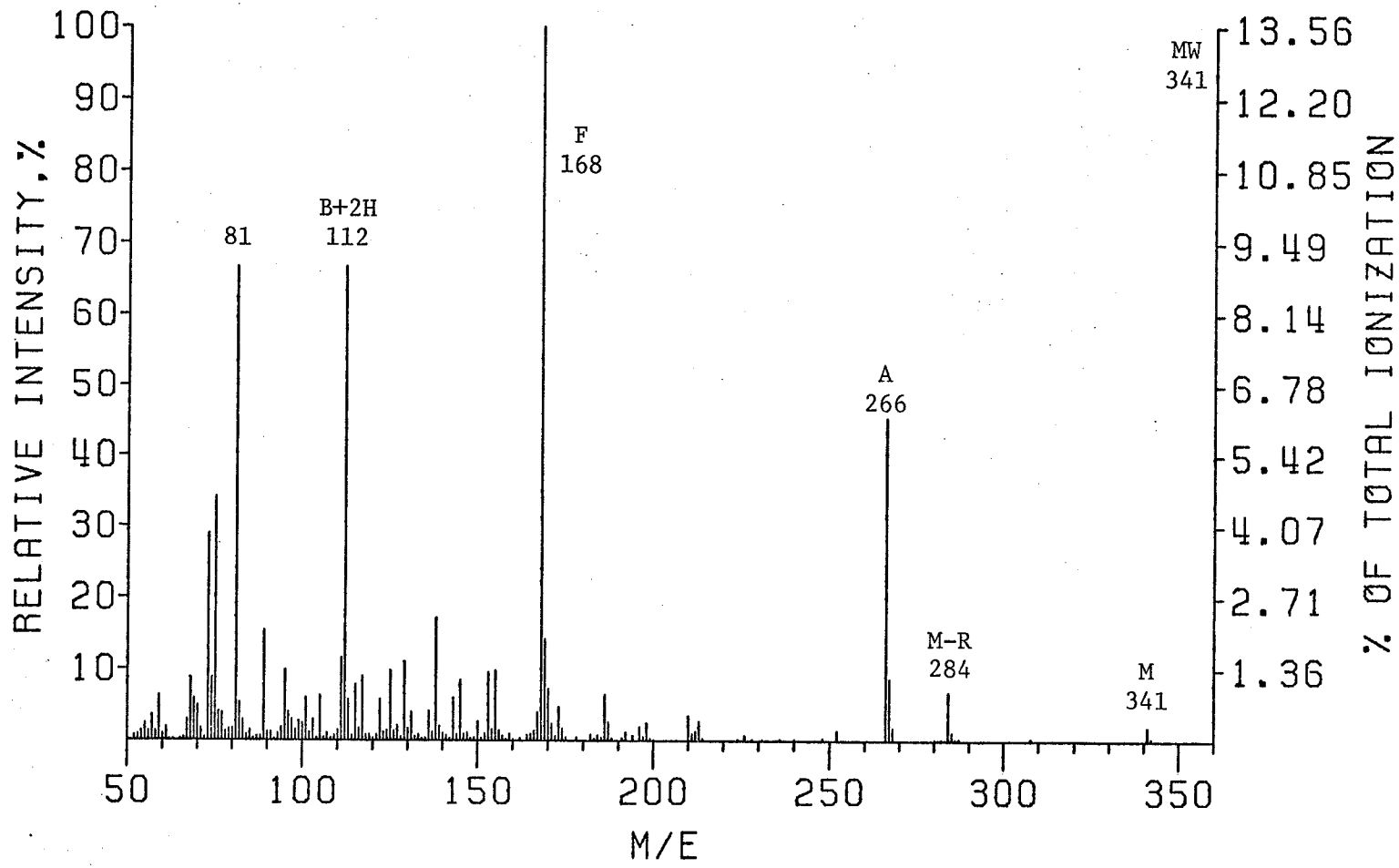


Figure 37. Mass spectrum of 5'-O-TBDMSi-deoxycytidine (IIIba) recorded on Finnigan MS at 70 eV.

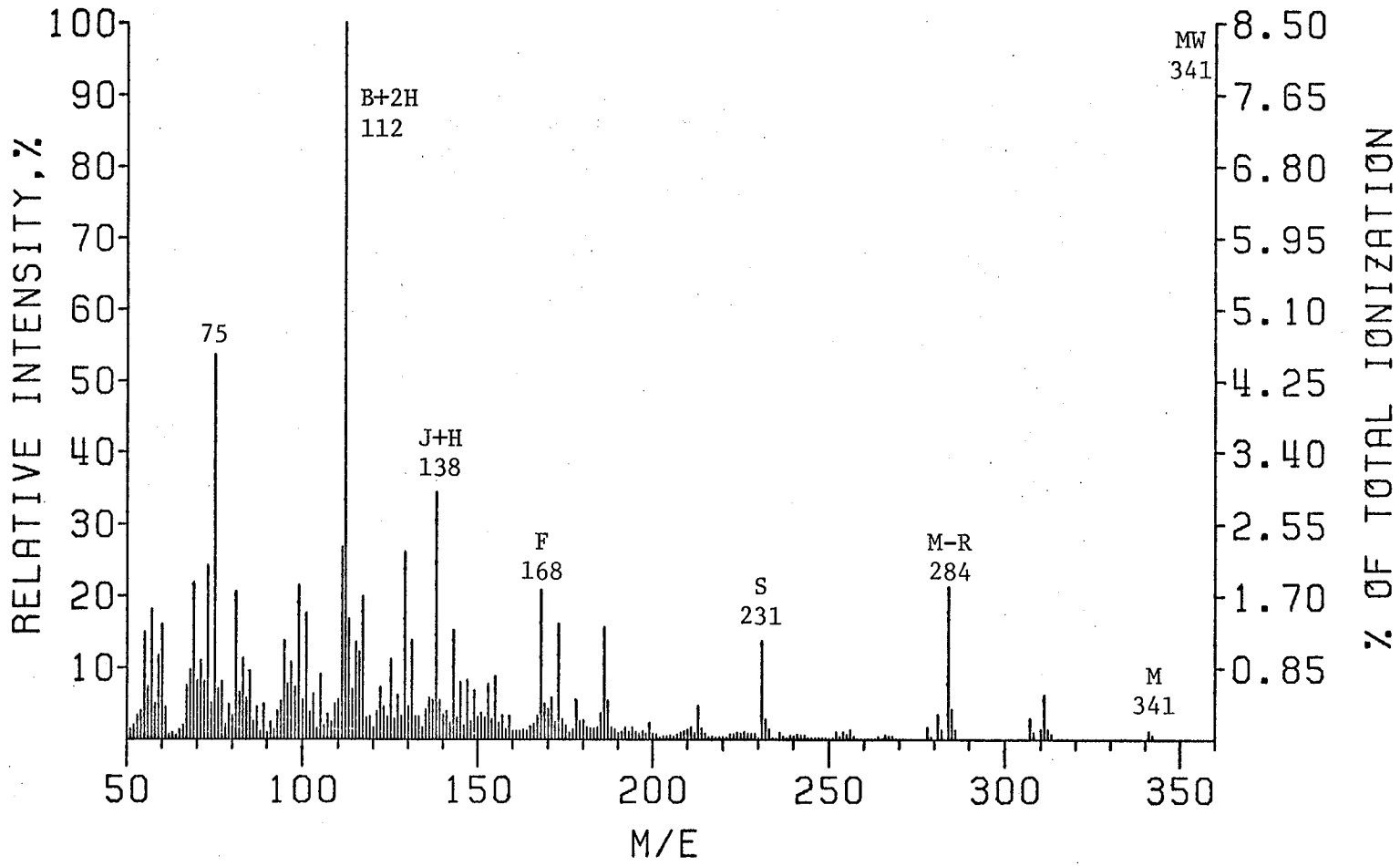


Figure 38. Mass spectrum of 3'-O-TBDMSi-deoxycytidine (IIIab) recorded on Finnigan MS at 70 eV.

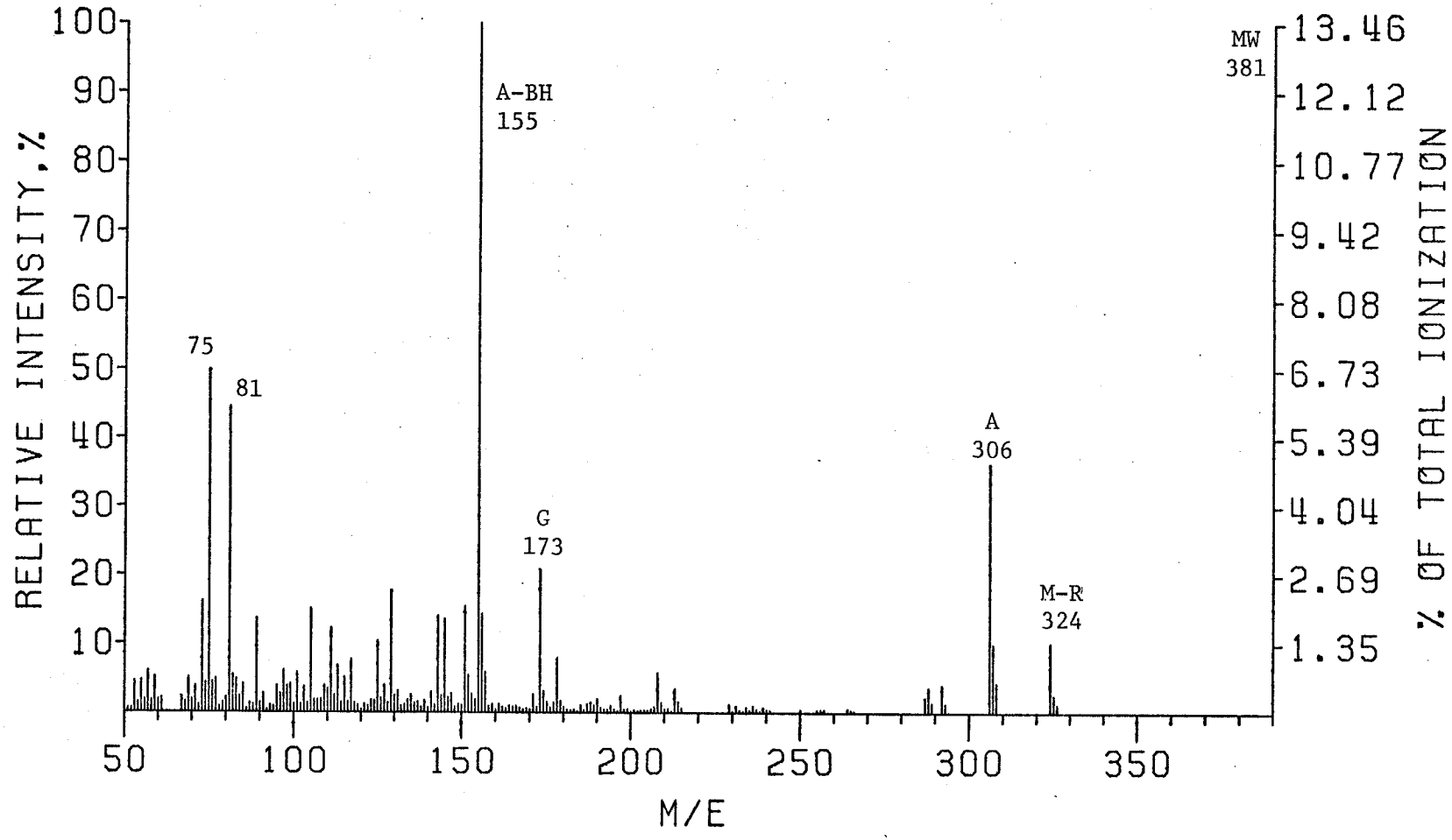


Figure 39. Mass spectrum of 5'-O-TBDMSi-deoxyguanosine (IVba) recorded on Finnigan MS at 70 eV.

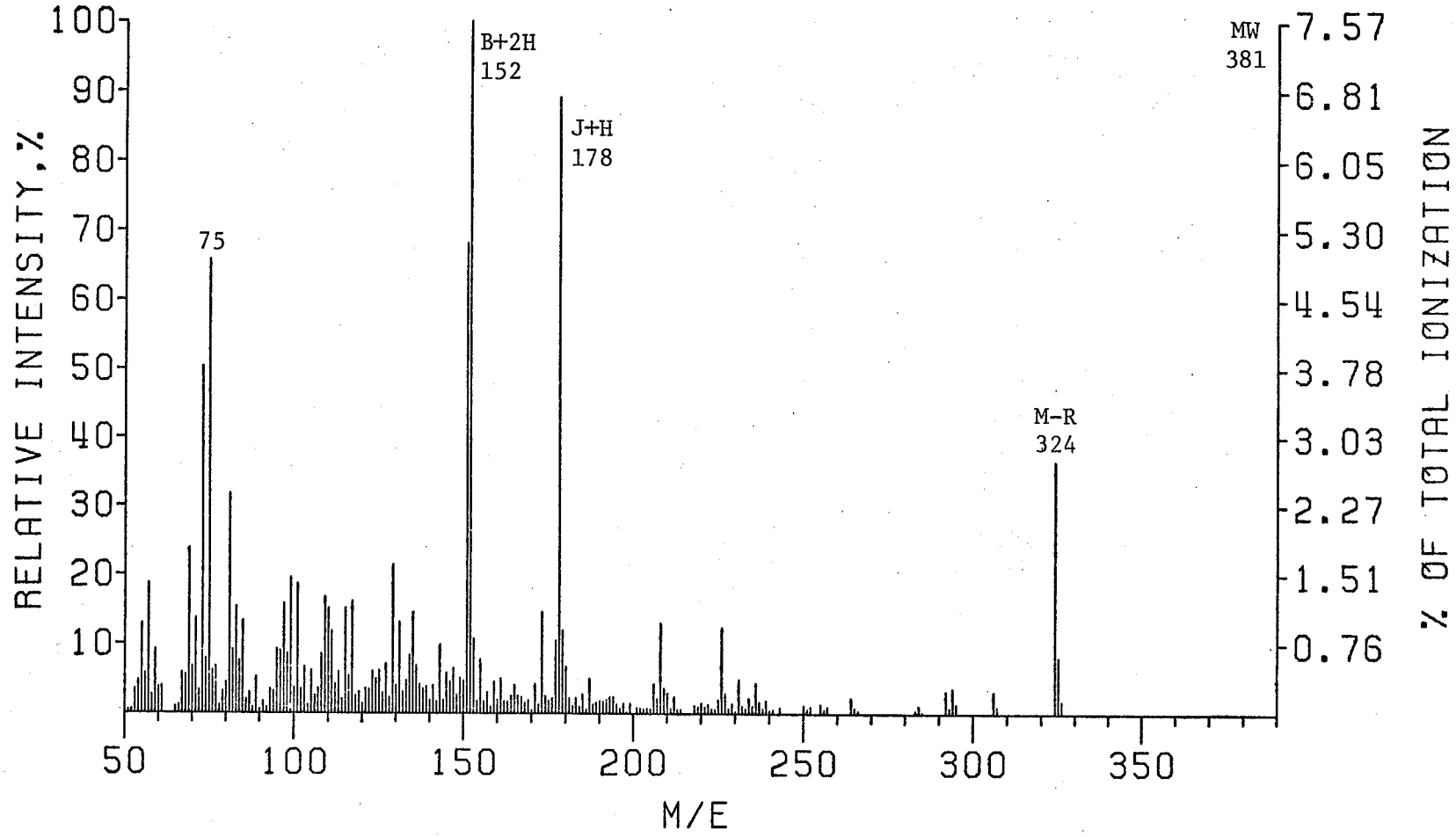


Figure 40. Mass spectrum of 3'-O-TBDMSi-deoxyguanosine (IVab) recorded on Finnigan MS at 70 eV.

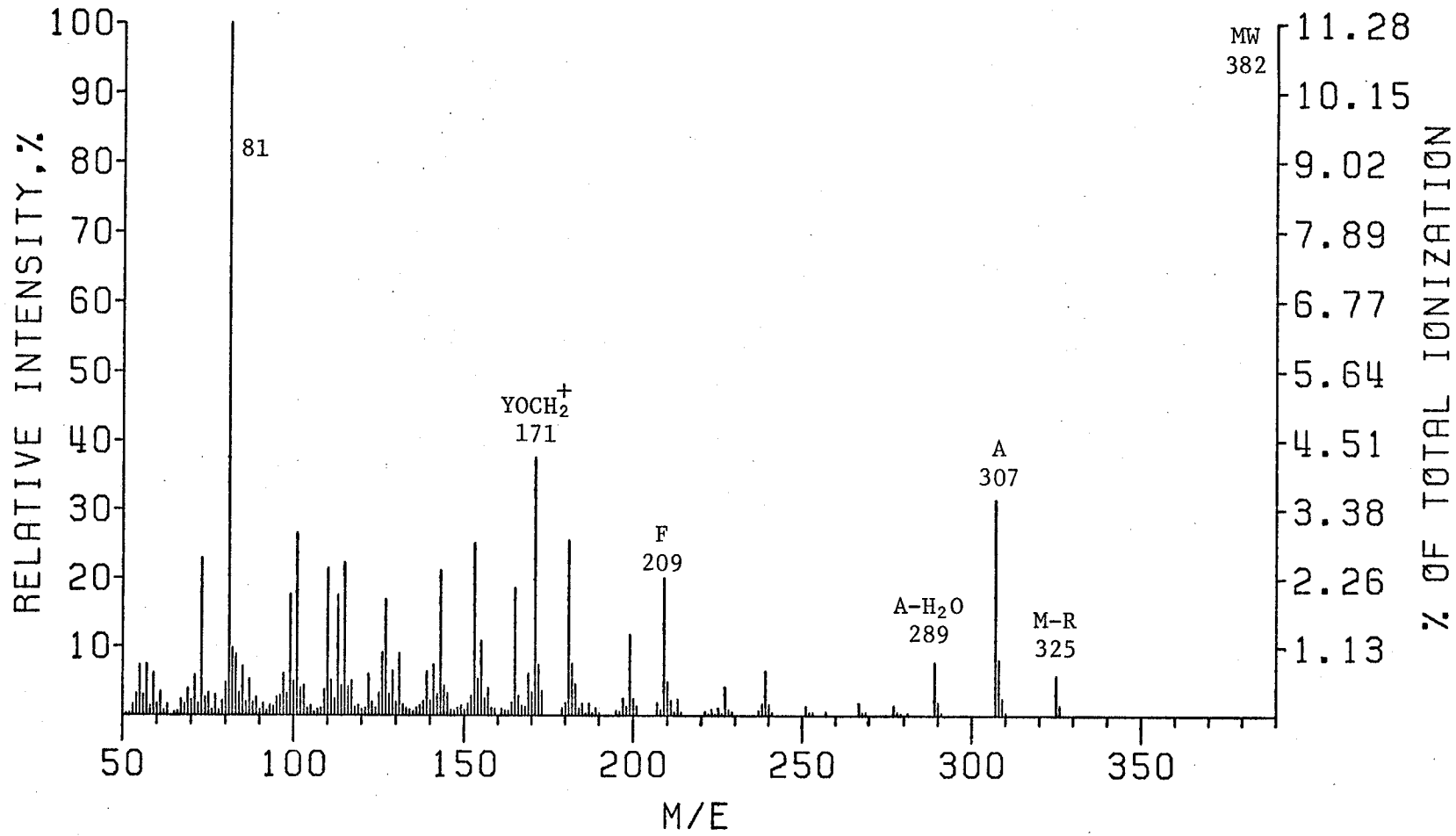


Figure 41. Mass spectrum of 5'-O-TMTBSi-thymidine (Ica) recorded on Finnigan MS at 70 eV.

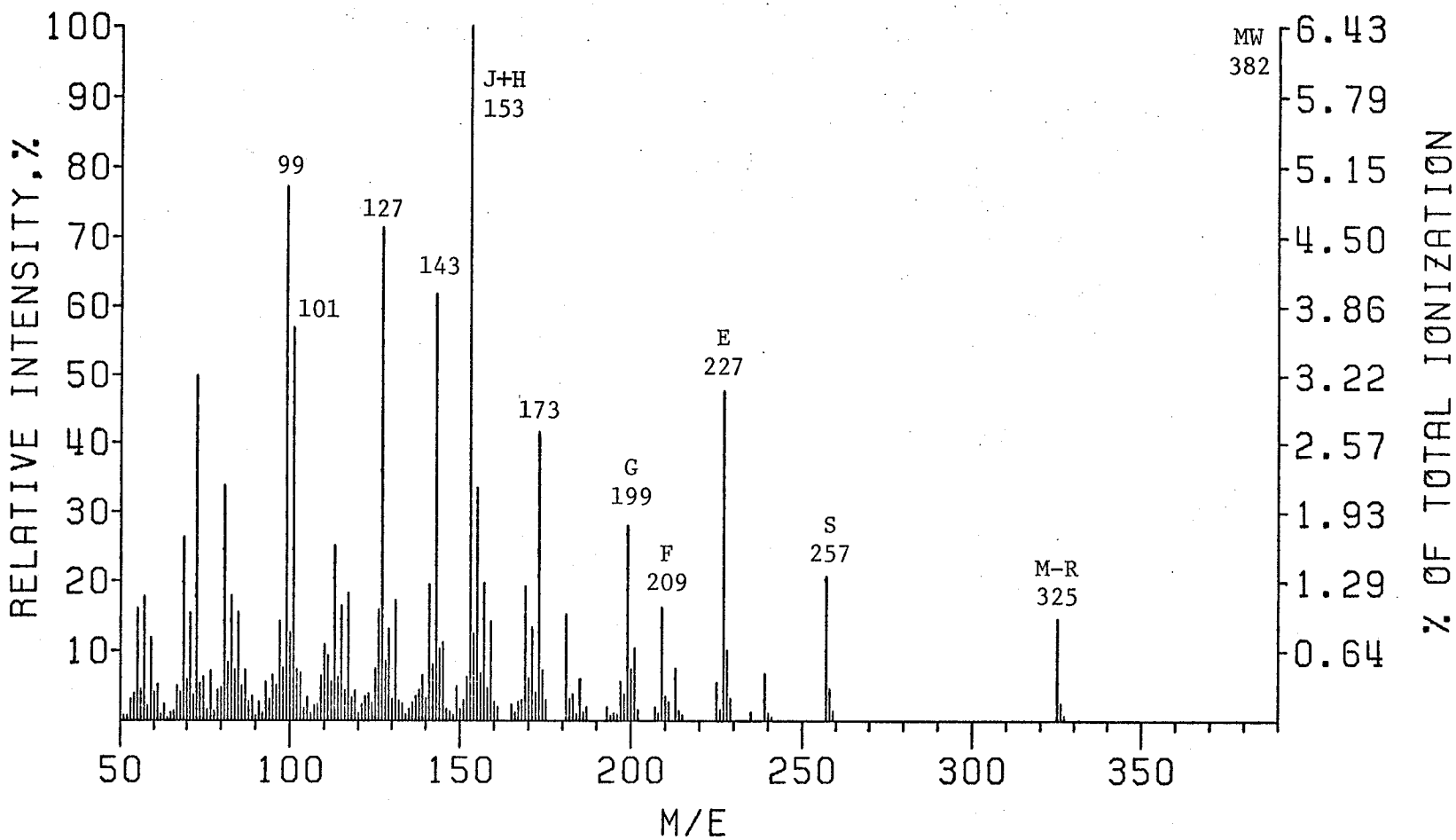


Figure 42. Mass spectrum of 3'-O-TMTBSi-thymidine (Iac) recorded on Finnigan MS at 70 eV.

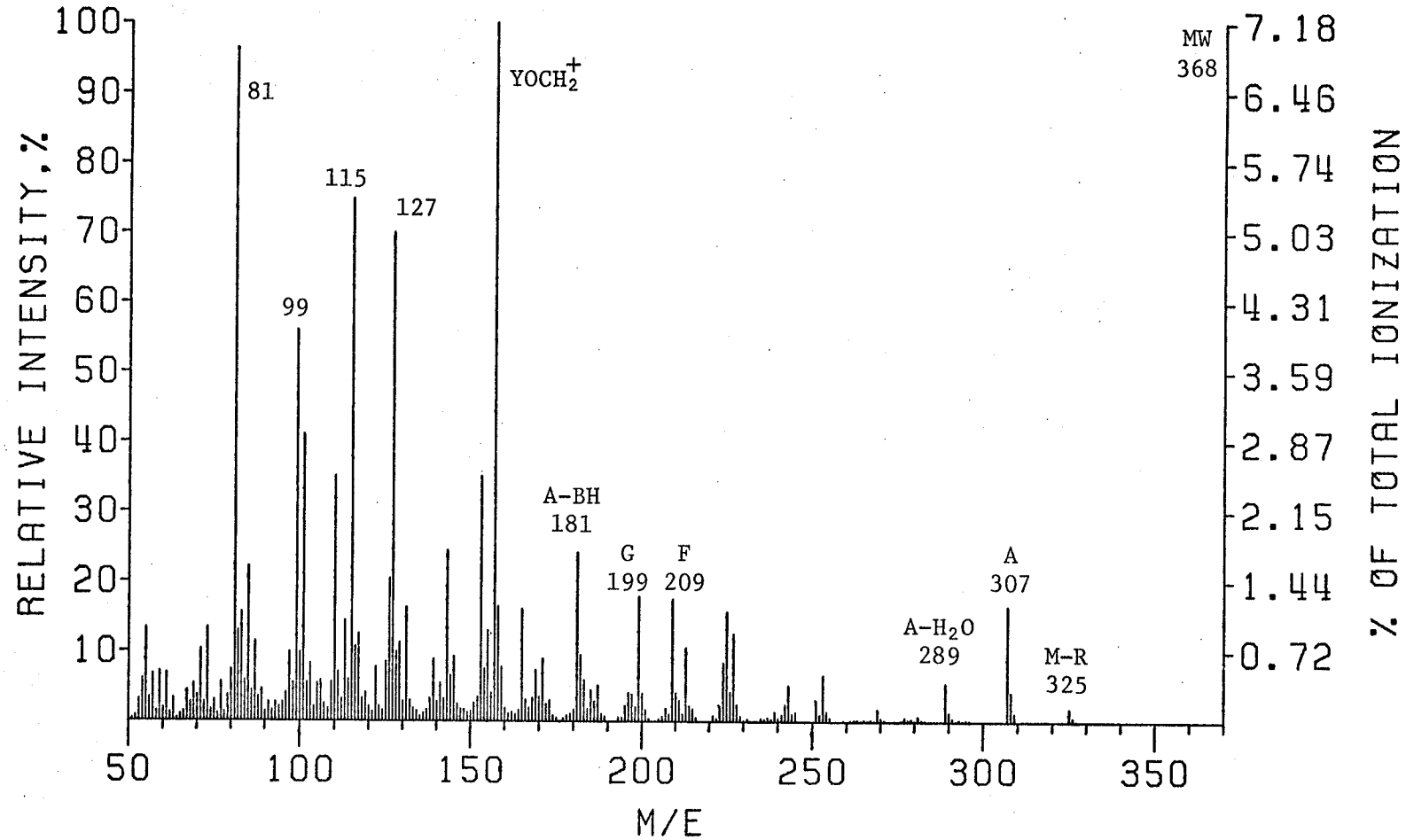


Figure 43. Mass spectrum of 5'-O-TMIPSi-thymidine (Ida) recorded on Finnigan MS at 70 eV.

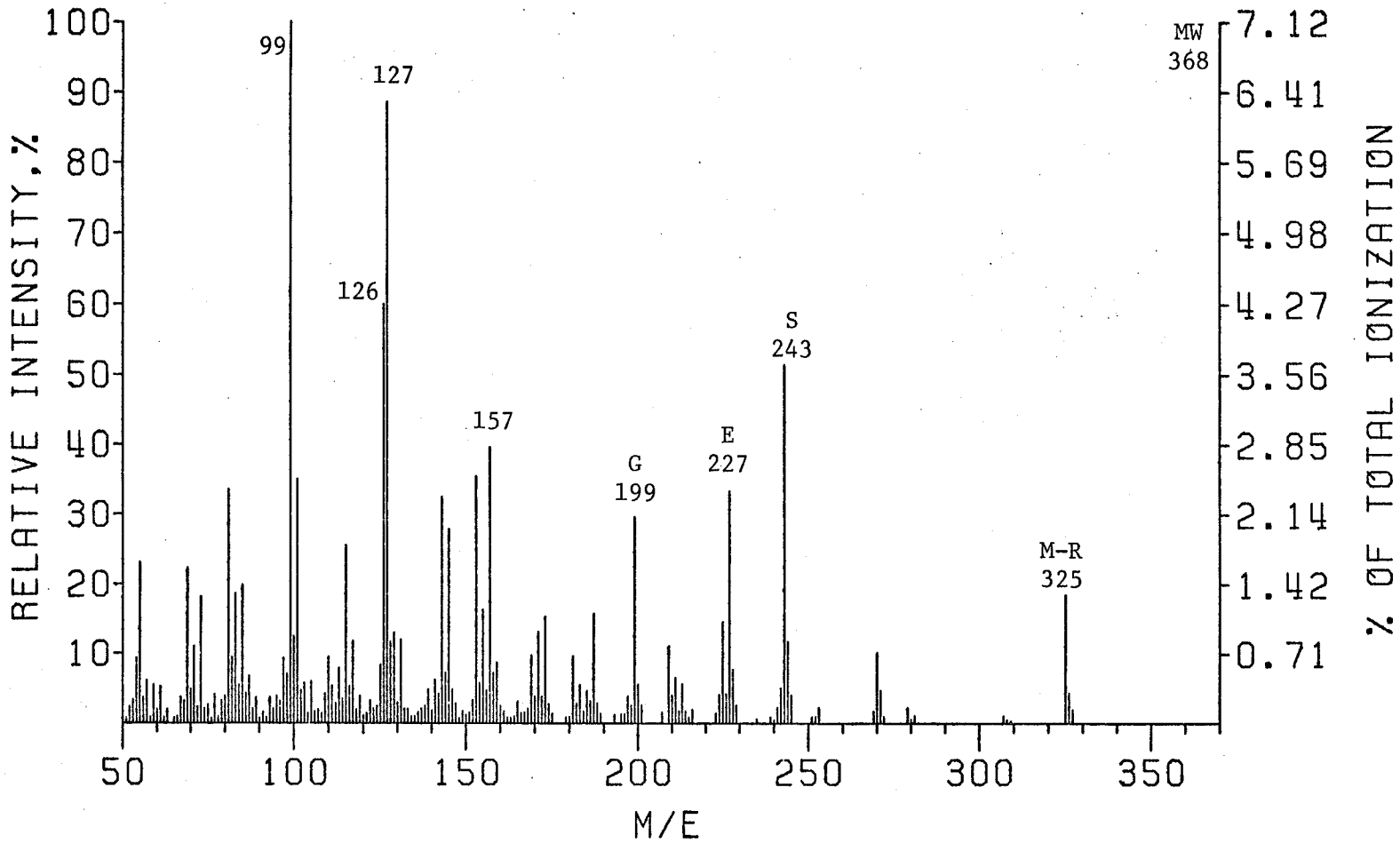


Figure 44. Mass spectrum of 3'-O-TMIPSi-thymidine (Iad) recorded on Finnigan MS at 70 eV.

TABLE XI. Diagnostically important fragment ions for partial-O-SCTASi derivatives of deoxynucleosides.

Characteristic for 5'-isomers			Characteristic for 3'-isomers		
Assignment[d]	Comment	m/e-Iba	Assignment[d]	Comment	m/e-Iab
[M-R-H_2O] or A	b	281	S	b	231
[A-H_2O]	a	263	[B+X_2Si+H_2O] or E	b	201
[S-H_2O]	b	213	[RX_2Si+72]	b	187
[B+40]	b	165	[J+H]	b	153
[M-R-H_2O-BH] or [A-BH]	b	155	[M-R-J]	b	147
			[X_2Si+73]	b	131
RX_2SiOCH_2	b	145[c]	[B+2H]	b	127[c]
HX_2SiOCH_2	b	89	[S-RX_2SiOH]	b	99[c]
C_5H_5O or D	b	81	[S-RX_2SiOH-CH_2O]	b	69

Present in Both Isomers	
Assignment[d]	m/e-Iba or Iab
[M-R]	299
[B+X_2Si] or F	183
[M-R-BH] or G	173
X_2SiOH	75
CH_3X_2Si	73

[a] Unique to this isomer.

[b] Much more abundant for this isomer.

[c] Overlap with other ion types or isotope of ion type of lower mass interferes in some cases.

[d] Positive ions; assignments are discussed in Section B2.

TABLE XIIa. Mass spectral data for 5'- and 3'-O-TBDMSi-thymidine derivatives (Iba and Iab).

Line #	Assignment[e]	Elemental Composition C-H-Si-N-O	Exact Mass Calc.	Observed: Iba	Observed: Iab	m/e	Rel.Int.%[f] Iba	Rel.Int.%[f] Iab	d-shift Iba	d-shift Iab
1.	M	[16-28-1-2-5]	-	-	-	356	-	-	(2)	(2)
2.	$M-CH_3$	15-25-1-2-5	341.153	341.155	-	341	0.4	0.03	2	-
3.	$M-CH_2OH(31)$	15-25-1-2-4	325.158	-	325.158	325	-	0.6	-	1
4.	$M-CH_3-H_2O$	15-23-1-2-4	323.143	323.143	-	323	0.9	-		-
5.	M-R	12-19-1-2-5	299.106	299.108	299.107	299	19.	26.	2	2
6.	$M-C_2H_5O_2(61)$	14-23-1-2-3	295.148	-	295.149	295	-	0.4	-	
7.	$[M-R-H_2O]$ or A	12-17-1-2-4	281.096	281.096	281.096	281	59.	1.0	0(80%), 1(20%)	1
8.	$M-R-CH_2O$	11-17-1-2-4	269.096	-	269.097	269	0.2	0.4	-	2
9.	$J+RX_2Si$	13-23-1-2-2	267.153	-	267.153	267	0.4	1.1	-	1
10.	$[M-R-2H_2O]$ or $[A-H_2O]$	12-15-1-2-3	263.085	263.087	-	263	9.1	0.1	0	-
11.	$M-R-H_2O-CH_2O$	11-15-1-2-3	251.085	251.086	-	251	2.0	0.3	1	-
12.	$M-R-C_2H_4O_2(60)$	10-15-1-2-3	239.085	-	239.085	239	0.3	1.3	-	1
13.	S	11-23-1-0-3	231.142	231.143	231.143	231	3.2	43.	1	1
14.	$M-CH_3-BH$	10-19-1-0-3	215.110	-	215.110	215	1.0[d]	2.7	-	0(70%), 1(30%)
15.	$[S-H_2O]$ or K (for 5' isomer)	11-21-1-0-2	213.131	213.131[a]	213.131	213	12.	4.8	0(95%), 1(5%)	0(90%), 1(10%)
16.		8-13-1-2-3	213.070	213.070[c]	-					

continued...

TABLE XIIa. continued

Line #	Assignment[e]	C-H-Si-N-O	Exact Mass Calc.	Observed: Iba	Observed: Iab	m/e	Rel.Int.%[f] Iba	Rel.Int.%[f] Iab	d-shift Iba	d-shift Iab
17.		11-19-1-0-2	211.115	211.116	-	211	0.7	0.3		-
18.		9-13-1-2-2	209.075	209.076	209.075	209	0.6	1.5	0	0
19.	$[B+X_2Si+H_2O]$ or E	7-13-1-2-3	201.070	201.070	201.071	201	2.0	41.	1(25%), 2(75%)	1(85%), 2(15%)
20.		10-19-1-0-2	199.115	-	199.117	199	0.5	5.1	-	0
21.	$M-CH_3-BH-H_2O$	10-17-1-0-2	197.100	197.102	-	197	1.9	0.8	0	-
22.	$M-CH_2OH-RX_2SiOH$	9- 9-0-2-3	193.061	-	193.063	193	0.3	1.1	-	1
23.	$RX_2Si+C_3H_4O_2(72)$	9-19-1-0-2	187.115	187.117	187.117	187	4.2	22.	1	0(70%), 1(30%)
24.	$B+C_3H_6O(58)$	8-11-0-2-3	183.077	183.077[c]	-	183	22.	16.	0(40%), 1(60%)	0(30%), 1(70%)
25.	$[B+X_2Si]$ or F	7-11-1-2-2	183.059	183.059[a]	183.060					
26.	[M-R-BH] or G	7-13-1-0-3	173.063	173.064	173.064	173	11.	34.	1	0(90%), 1(10%)
27.	$RX_2Si+C_3H_4O(56)$	9-19-1-0-1	171.121	-	171.122[a]	171	1.3	5.9		0(70%), 1(30%)
28.	$RXSi+C_3H_3O_2(71)$	8-15-1-0-3	171.084	171.085[c]	171.086[b]					
29.	$X_2Si+C_5H_5O_3(113)$	7-11-1-0-3	171.048	171.049[a]	171.049[b]					
30.	$B+C_3H_4(40)$	8- 9-0-2-2	165.066	165.069	165.067	165	14.	0.7	1	1
31.		8-19-1-0-1	159.121	159.122[a]	159.122[a]	159	3.0	9.2	0(20%), 1(80%)	0(50%), 1(50%)
32.	$RXSi+C_2H_3O_2(59)$	7-15-1-0-2	159.084	159.085[c]	159.085[b]					

continued...

TABLE XIIa. continued

Line #	Assignment[e]	C-H-Si-N-O	Exact Mass Calc.	Observed: Iba	Observed: Iab	m/e	Rel.Int.%[f] Iba	Rel.Int.%[f] Iab	d-shift Iba	d-shift Iab
33.	[M-R-H_2O-BH] or [A-BH]	7-11-1-0-2	155.053	155.054	155.054	155	29.	12.	0	0
34.	J+H	7- 9-0-2-2	153.066	153.068	153.067	153	19.	74.	2	2
35.	[$X_2Si+C_3H_5O_3$(89)] or [M-R-J]	5-11-1-0-3	147.048	147.049	147.048	147	3.3[d]	41.	-	1
36.	RX_2SiOCH_2	7-17-1-0-1	145.105	145.107[a]	145.106[b]	145	42.	11.	0	0
37.	$HRSi+C_2H_3O_2$(59)	6-13-1-0-2	145.069	145.070[b]	145.069[a]					
38.	[M-R-BH-CH_2O] or [G-CH_2O]	6-11-1-0-2	143.053	143.054	143.053	143	8.1	18.	1	0
39.		6-17-1-0-1	133.105	-	133.108	133	0.6[d]	5.1	-	1
40.	$X_2Si+C_3H_5O_2$(73)	5-11-1-0-2	131.053	131.055	131.054	131	4.4	25.	0(80%), 1(20%)	0(80%), 1(20%)
41.	$R(CH_2)SiOCH_2$	6-13-1-0-1	129.074	129.073[b]	-	129	13.	24.	0	0
42.	$X_2Si+C_3H_3O_2$(71)	5- 9-1-0-2	129.037	129.036[b]	129.038					
43.	B+2H	5- 7-0-2-2	127.051	127.050	127.051	127	17.	57.	1(75%), 2(25%)	1(60%), 2(40%)
44.	B+H	5- 6-0-2-2	126.043	126.043	126.043	126	10.	13.	1	1
45.	(B+C_3H_4)-HNCO	7- 8-0-1-1	122.061	122.061	122.061	122	4.8	1.9	0	0
46.	RXSiOH	5-13-1-0-1	117.074	117.074[c]	117.074[c]	117	21.	48.	0(25%), 1(75%)	0(20%), 1(80%)
47.	[$X_2Si+C_2H_3O_2$(59)] or [M-R-J-CH_2O]	4- 9-1-0-2	117.037	117.037[a]	117.037[a]					

continued...

TABLE XIIa. continued

Line #	Assignment[e]	C-H-Si-N-O	Exact Mass Calc.	Observed: Iba	Observed: Iab	m/e	Rel.Int.%[f] Iba	Rel.Int.%[f] Iab	d-shift Iba	d-shift Iab
48.	RX_2Si	6-15-1-0-0	115.094	115.095[b]	115.095[b]					
49.		5-11-1-0-1	115.058	115.059[c]	115.058[b]	115	18.	33.	0(95%), 1(5%)	0
50.		4- 7-1-0-2	115.022	115.022[b]	115.021[b]					
51.		5- 9-1-0-1	113.042	113.043	113.043	113	5.9	4.2	0	0
52.	(J+H)-HNCO	6- 8-0-1-1	110.061	110.060[a]	110.060[a]	110	20.	5.9	1	1
53.		5- 4-0-1-2	110.024	110.024[b]	110.024[b]					
54.	$X_2Si+CH_3O_2$(47)	3- 9-1-0-2	105.037	105.038	105.038	105	14.	15.	1	0(70%), 1(30%)
55.	$CH_3X_2SiOCH_2$	4-11-1-0-1	103.058	103.059[c]	103.059[c]	103	5.1	8.0	0(70%), 1(30%)	0(60%), 1(40%)
56.	$HXSi+C_2H_3O_2$(59)	3- 7-1-0-2	103.022	103.022[a]	103.022[a]					
57.	$X_2Si+C_2H_3O$(43)	4- 9-1-0-1	101.042	101.043	101.043	101	7.6	18.	0(90%), 1(10%)	0
58.	$R(CH_2)Si$	5-11-1-0-0	99.063	99.064[b]	99.064[b]					
59.	[S-RX_2SiOH] or K (for 3' isomer)	5- 7-0-0-2	99.045	99.046[b]	99.046[b]	99	4.2	46.	0(90%), 1(10%)	0(50%), 0(50%)
60.	X_2Si+C_2HO(41)	4- 7-1-0-1	99.027	99.028[b]	99.028[b]					
61.	$H(X_2SiOCH_2)$	3- 9-1-0-1	89.042	89.042	89.042	89	50.	9.7	0	0(90%), 1(10%)
62.	C_5H_5O or D	5- 5-0-0-1	81.034	81.034	81.034	81	100.	26.	0	0

continued...

TABLE XIIa. continued

Line #	Assignment[e]	C-H-Si-N-O	Exact Mass Calc.	Observed: Iba	Observed: Iab	m/e	Rel.Int.%[f] Iba	Rel.Int.%[f] Iab	d-shift Iba	d-shift Iab
63.	X_2SiOH	2- 7-1-0-1	75.027	75.027	75.027	75	59.	87.	0(30%), 1(70%)	0(50%), 0(50%)
64.	CH_3X_2Si	3- 9-1-0-0	73.047	73.049[a]	73.049[a]	73	67.	100.	0(95%), 1(5%)	0(80%), 1(20%)
65.	$C_3H_5O_2$	3- 5-0-0-2	73.029	73.031[b]	73.031[b]					
66.	$S\text{-}RX_2SiOH\text{-}CH_2O$	-	-	-	-	69	2.4	22.		0(20%), 1(80%)
67.	HX_2Si	-	-	-	-	59	10.	13.	0	0

Base Peak as $\%\Sigma_{30}$ = 10.83 8.35

[a-c] Relative contribution to integer m/e intensity: a = major, b = medium, c = minor.

[d] Major isotopic contribution interferes.

[e] Positive ions; sign left out for simplification.

[f] Intensity data from spectra run on Hitachi instrument at 50 eV.

TABLE XIIb. Correlation of m/e values of principal fragment ions of mono-O-SCTASi derivatives of 2'-deoxynucleosides. Unless indicated by a footnote, ion abundances follow same trend as for 3'- and 5'-O-TBDMSi-thymidine isomers (see Table XIIa for RI% data).

Line #	Assignment	Deriv.→ Compd.→	TBDMSi I	TMTBSi I	TMIPSi I	TBDMSi II	TBDMSi V	TBDMSi III	TBDMSi IV
1.	M		356[c]	382[c]	368[c]	342[c]	365	341	381[c]
2.	$M-CH_3$		341	-	-	327	350	326	366
5.	M-R		299	325	325	285	308	284	324
7.	A		281	307	307	267	290	266	306
9.	$J+RX_2Si$		267	293	279	253	276	252	292
10.	$A-H_2O$		263	289	289	249	272[w]	248	288
11.	$M-R-H_2O-CH_2O$		251	277	277	237	260	236	276
13.	S		231	257	243	231	231[w]	231	231[w]
15.	$S-H_2O$		213	239	225	213	213[w]	213	213
19.	E		201	227	227	187[a]	210	186	226
23.	$RX_2Si+C_3H_4O_2(72)$		187	213	199[a]	187[a]	187[w]	187[b]	187[w]
25.	F		183	209	209	169	192	168	208
26.	G		173	199	199[a]	173	173	173	173
30.	$B+C_3H_4(40)$		165	165	165	151	174[b]	150[w]	190[w]
33.	A-BH		155	181	181	155	155	155	155
34.	J+H		153	153	153	139	162	138	178
35.	M-R-J		147	173	173	147	147[w]	147[w]	147[w]
36.	RX_2SiOCH_2		145	171	157[a]	145	145[w]	145	145
38.	$G-CH_2O$		143	169	169	143	143	143	143
40.	$X_2Si+C_3H_5O_2(73)$		131	157	157[a]	131	131	131	131
42.	$X_2Si+C_3H_3O_2(71)$		129	155	155	129	129	129	129
43.	B+2H		127	127[a]	127[a]	113	136	112	152
44.	B+H		126	126	126	112	135	111	151
47.	$M-R-J-CH_2O$		117	143	143	117	117	117	117
48.	RX_2Si		115[a]	141	127[a]	115[a]	115[a]	115[a]	115[a]
52.	(J+H)-HNCO		110	110	110	96	-	95	-
54.	$X_2Si+CH_3O_2(47)$		105	131	131	105	105	105	105

continued...

TABLE XIIb. continued

Line #	Assignment	Deriv.→ Compd.→	TBDMSi I	TMTBSi I	TMIPSi I	TBDMSi II	TBDMSi V	TBDMSi III	TBDMSi IV
57.	$X_2Si+C_2H_3O(43)$		101	127[a]	127[a]	101	101	101	101
59.	$S-RX_2SiOH$		99[a]	99[a]	99[a]	99[a]	99[a]	99[a]	99[a]
61.	$H(X_2SiOCH_2)$		89	115	115	89	89	89	89
62.	D		81	81	81	81	81	81	81
63.	X_2SiOH		75	101	101	75	75	75	75
64.	CH_3X_2Si		73	99[a]	99[a]	73	73	73	73
66.	$S-RX_2SiOH-CH_2O$		69	69	69	69	69	69	69
67.	HX_2Si		59	85	85	59	59	59	59

[a] Overlapping ion types present.

[b] Isotopic contribution predominant.

[c] Ion type absent or not detectable for either isomer.

[w] Weak compared to intensity of TBDMSi-thymidine.

TABLE XIIIa. Metastable transitions in the mass spectra of 3'-O-TBDMSi derivatives of 2'-deoxynucleosides.[a]

Transition	Compound: Iab	Compound: Vaba
[M-R] → [J+H]	299 → 153 (78.3, 78.5m)	
[M-R] → [B+X_2Si+H_2O] or E	299 → 201 (135.1, 135.2m)	308 → 210 (143.2, 143.5m)
E → [B+X_2Si] or F	201 → 183 (166.6, 166.7w)	210 → 192 (175.5, 175.7m)
G → [X_2Si+$C_3H_3O_2$(71)]	173 → 129 (96.2, 96.3w)	173 → 129 (96.2, 96.3w)
S → [RX_2Si+$C_3H_4O_2$(72)]	231 → 187 (151.4, 151.5m)	
S → [S-RX_2SiOH] or K	231 → 99 (42.4, 42.4m)	
K → [K-CH_2O]	99 → 69 (48.1, 48.2m)	99 → 69 (48.1, 48.2m)
[M-R-J] → [M-R-J-CH_2O]	147 → 117 (93.1,93m)	
[RX_2Si+$C_3H_4O_2$(72)] → [X_2Si+$C_3H_5O_2$(73)]	187 → 131 (91.8, 91.8m)	
RX_2Si → CH_3X_2Si	115 → 73 (46.3, 46.4m)	115 → 73 (46.3, 46.4m)
[M-R] → [B+2H]		308 → 136 (60.1, 60.2s)
[B+H] → [(B+H)-HCN]		135 → 108 (86.4, 86.5w)

[a] Data given are: $m_p \rightarrow m_d$, (m*(calc), m*(obs), intensity)
vs = very strong, s = strong, m = moderate, w = weak, vw = very weak

TABLE XIIIb. Metastable transitions in the mass spectra of 5'-O-TBDMSi derivatives of 2'-deoxynucleosides.[a]

Transition	Compound: Iba	Vba[a]
[M-R] → [M-R-H_2O] or A	299 → 281 (264.1, 264s)	308 → 290 (273.1, 273vs)
A → [A-H_2O]	281 → 263 (246.2, 246s)	
A → [A-BH]		290 → 155 (82.8, 83w)
A → [C_5H_5O] or D	281 → 81 (23.3, 23.3s)	
[M-R] → [M-R-BH] or G		308 → 173 (97.2, 97.2s)
G → [G-CH_2O]	173 → 143 (118.2, 118.4w)	173 → 143 (118.2, 118.1w)
S → [S-H_2O]	231 → 213 (196.4, 196.5m)	
RX_2SiOCH_2 → HX_2SiOCH_2	145 → 89 (54.6, 54.7s)	145 → 89 (54.6, 54.8w)
RX_2Si → CH_3X_2Si	115 → 73 (46.3, 46.4m)	115 → 73 (46.3, 46.4m)
[X_2Si+CH_3O_2(47)] → X_2SiOH		105 → 75 (53.6, 53.7w)
[M-R] → [B+2H]		308 → 136 (60.1, 60.3m)
[J+H] → [(J+H)-HNCO]	153 → 110 (79.1, 79.2m)	(NA)
[B+H] → [(B+H)-HCN]	(NA)	135 → 108 (86.4, 86.5m)

[a] see footnote a in Table XIIIa

NA = not applicable

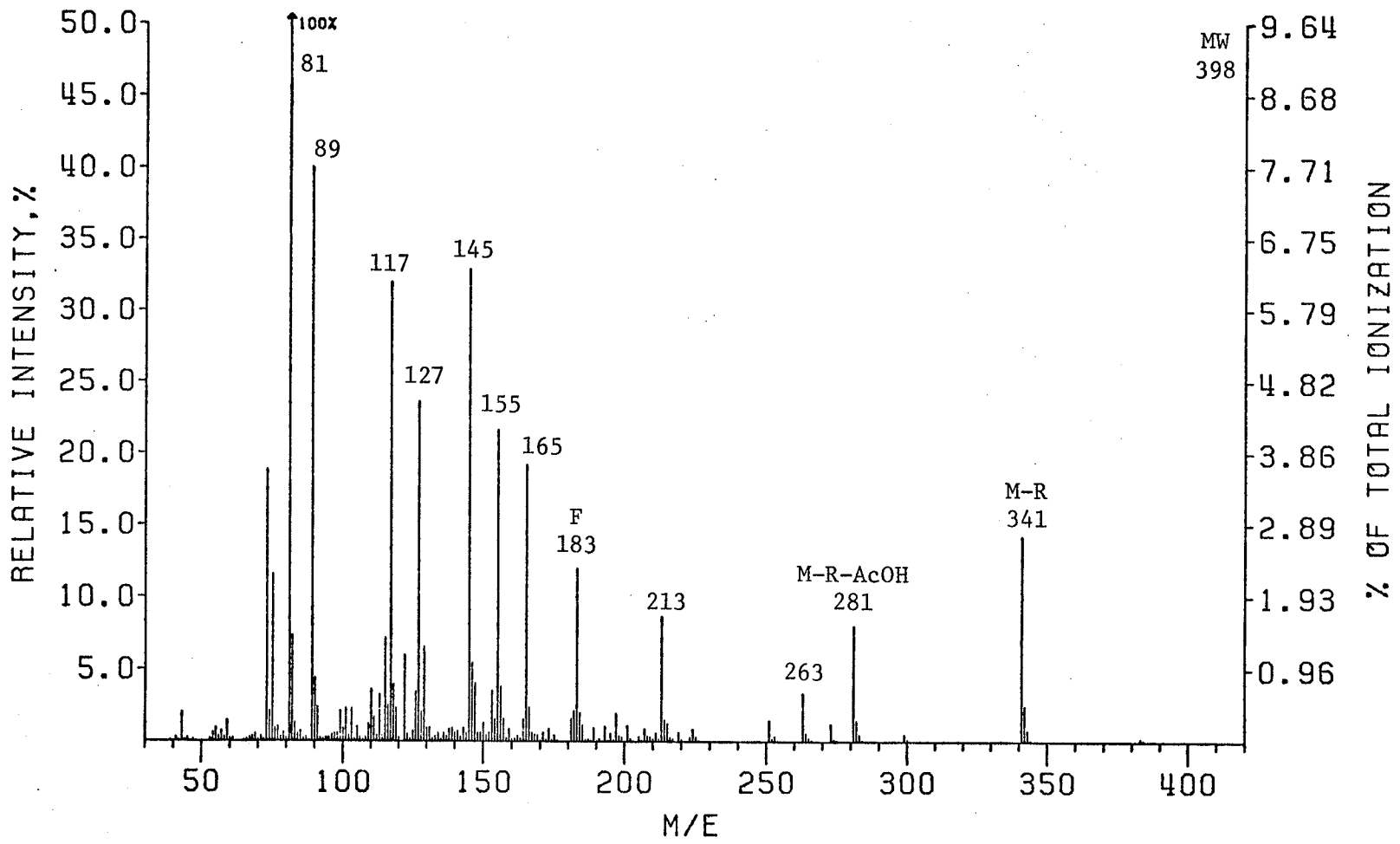


Figure 45. Mass spectrum of 3'-O-Ac-5'-O-TBDMSi-thymidine (Ibg) recorded on Finnigan MS at 70 eV.

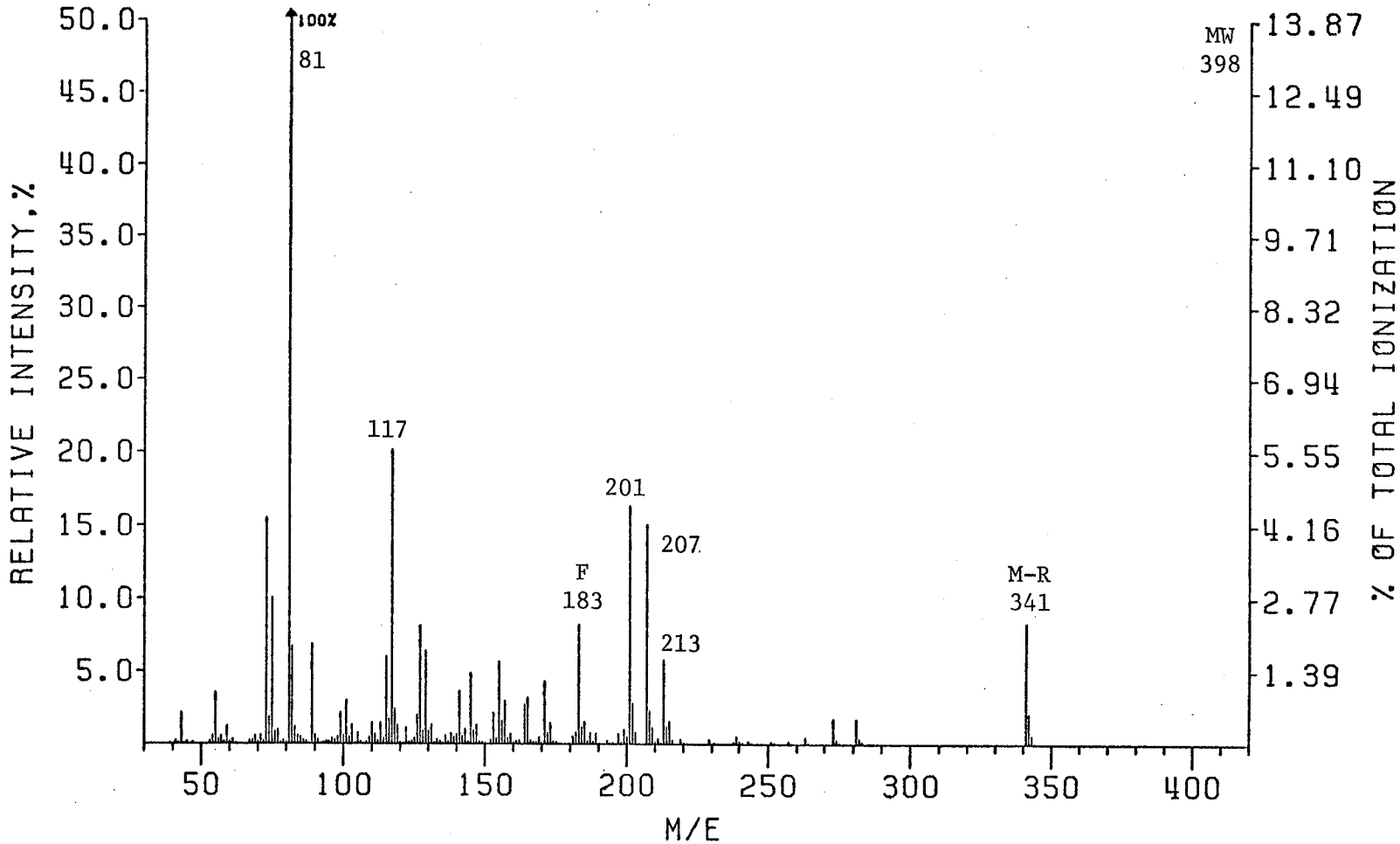


Figure 46. Mass spectrum of 3'-O-TBDMSi-5'-O-Ac-thymidine (Igb) recorded on Finnigan MS at 70 eV.

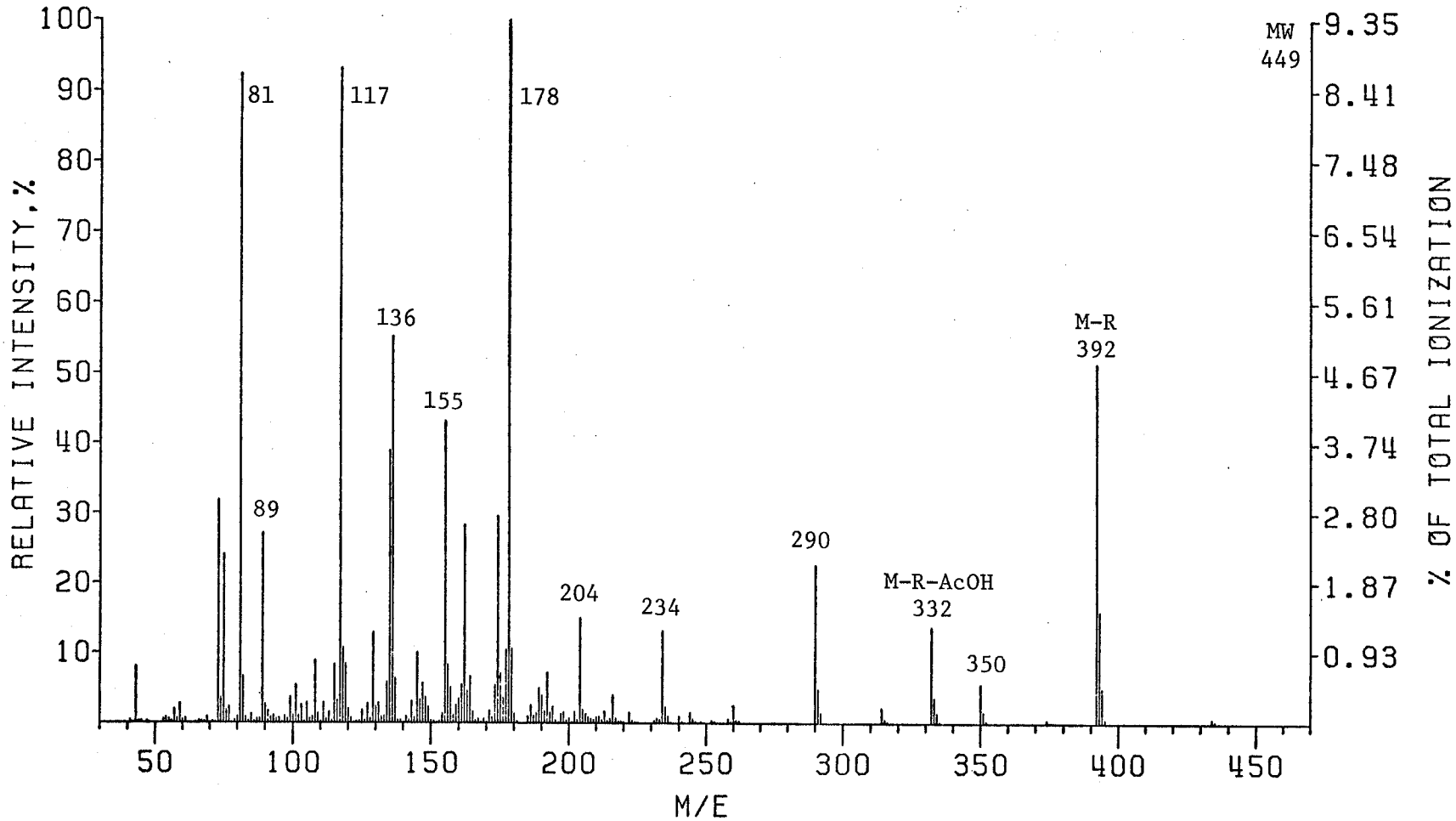


Figure 47. Mass spectrum of 3'-O-Ac-5'-O-TBDMSi-N6-Ac-deoxyadenosine (Vbgg) recorded on Finnigan MS at 70 eV.

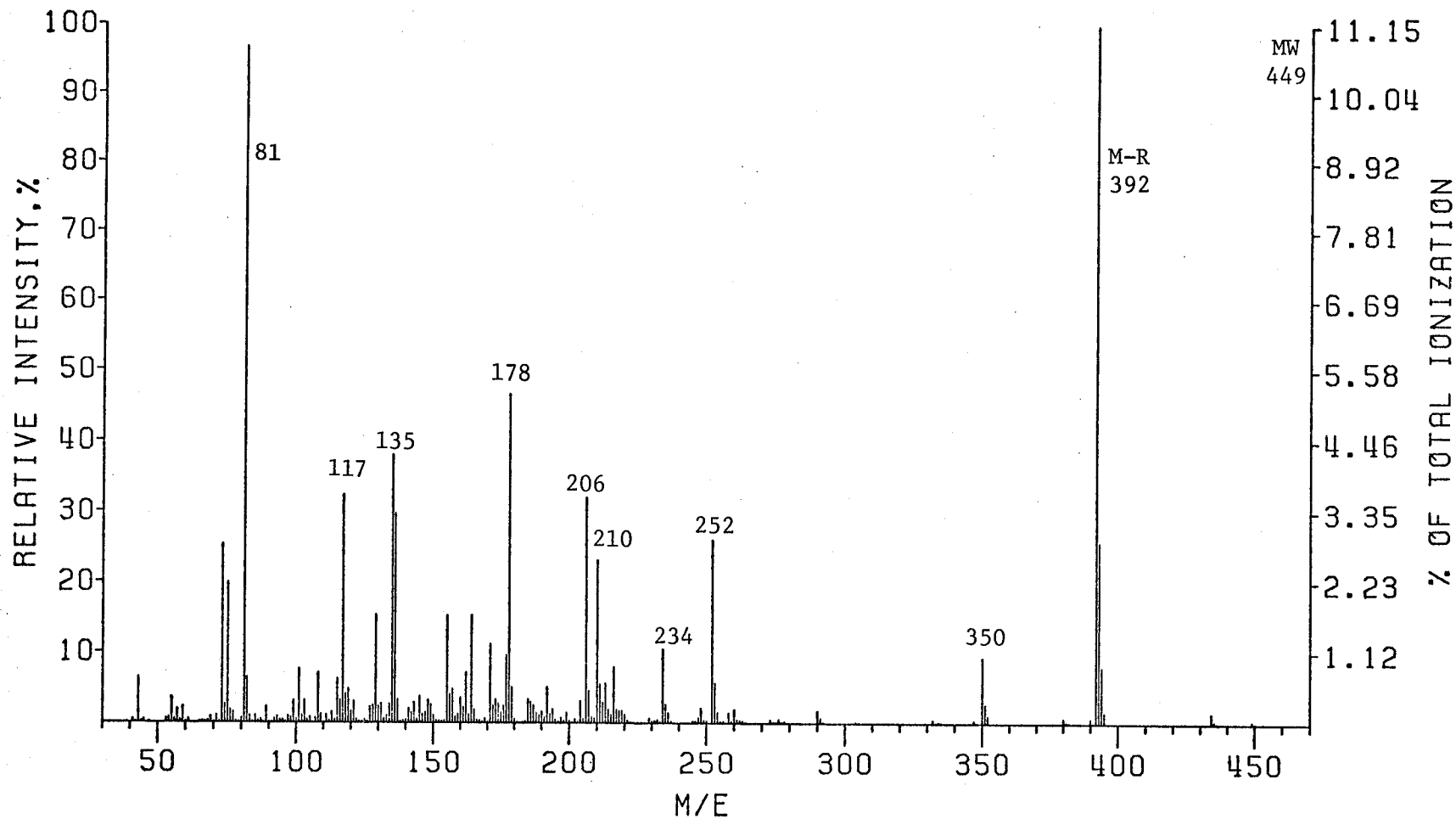


Figure 48. Mass spectrum of 3'-O-TBDMSi-5'-O-Ac-N6-Ac-deoxyadenosine (Vgbg) recorded on Finnigan MS at 70 eV.

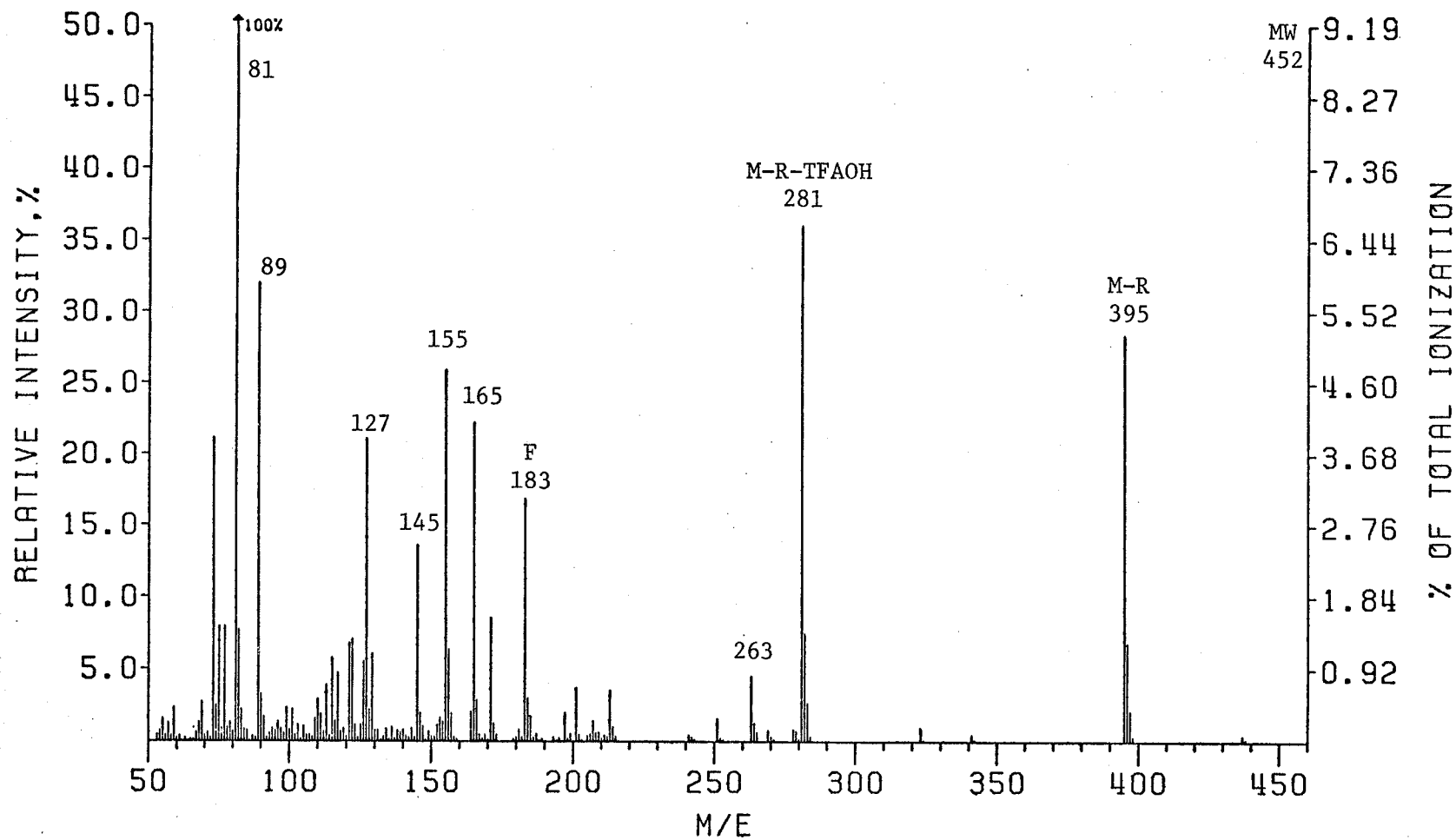


Figure 49. Mass spectrum of 3'-O-TFA-5'-O-TBDMSi-thymidine (Ibi) recorded on Finnigan MS at 70 eV.

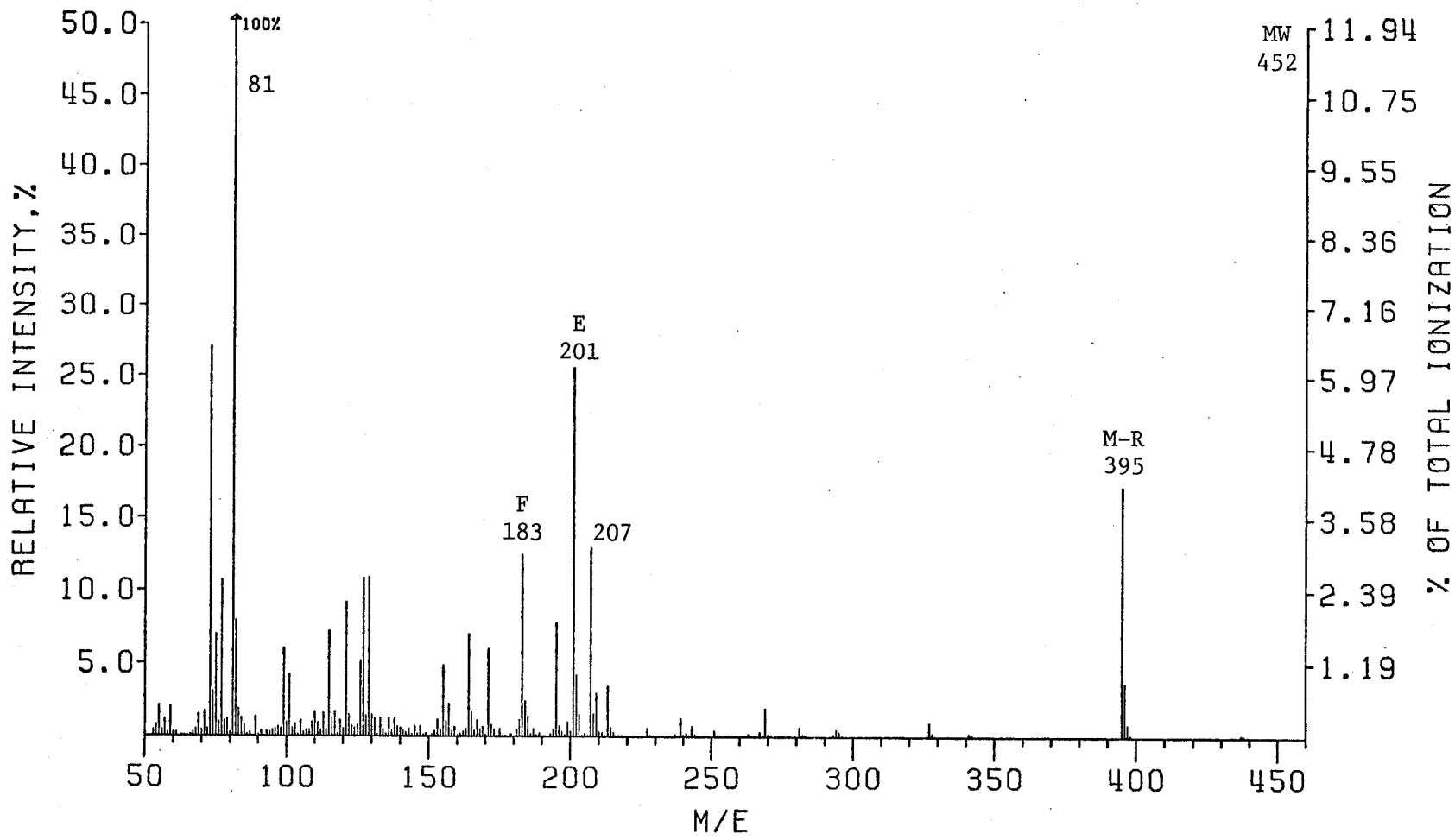


Figure 50. Mass spectrum of 3'-O-TBDMSi-5'-O-TFA-thymidine (Iib) recorded on Finnigan MS at 70 eV.

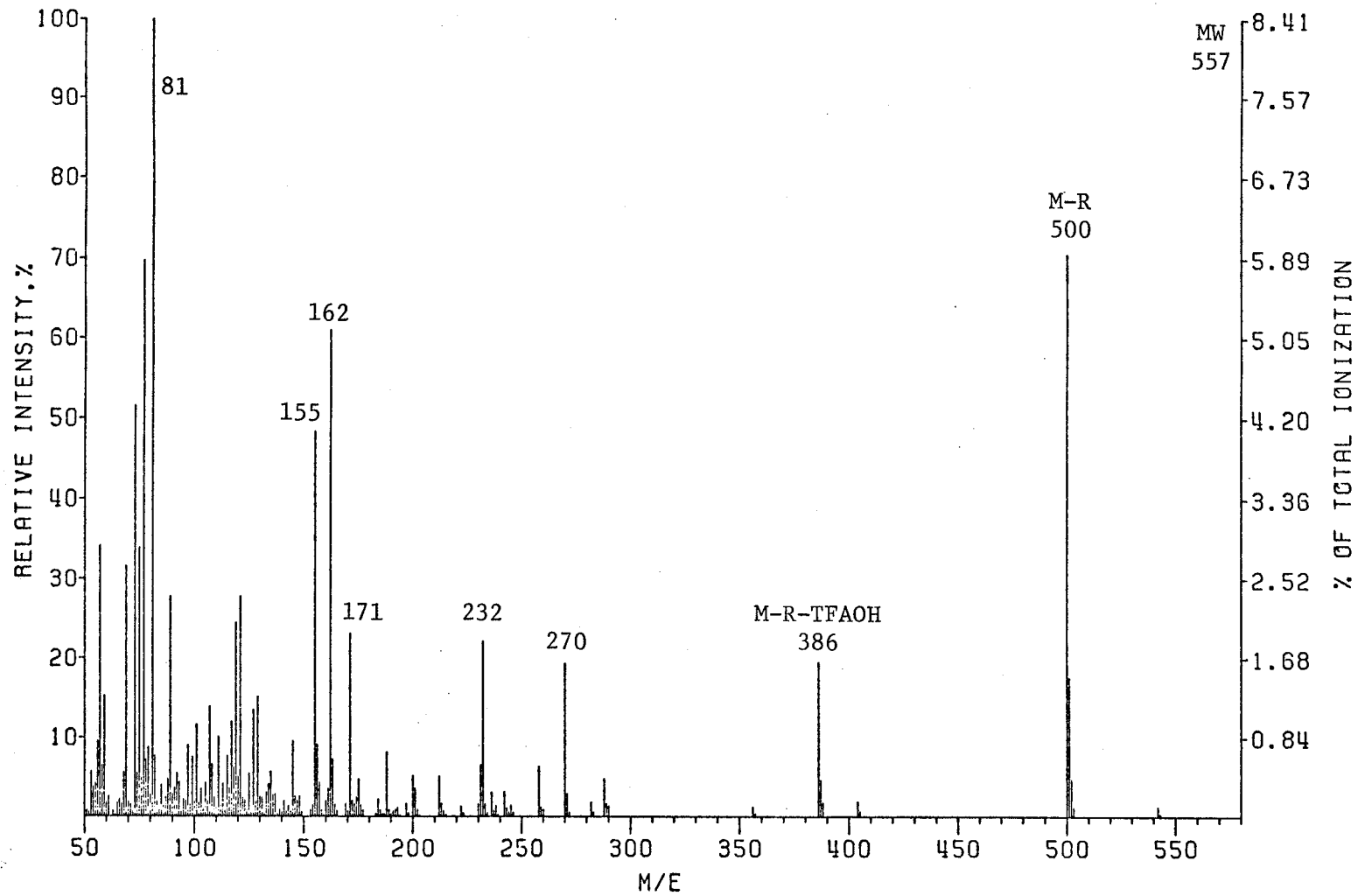


Figure 51. Mass spectrum of 3'-O-TFA-5'-TBDMSi-N6-TFA-deoxyadenosine (Vbii) recorded on Finnigan MS at 70 eV.

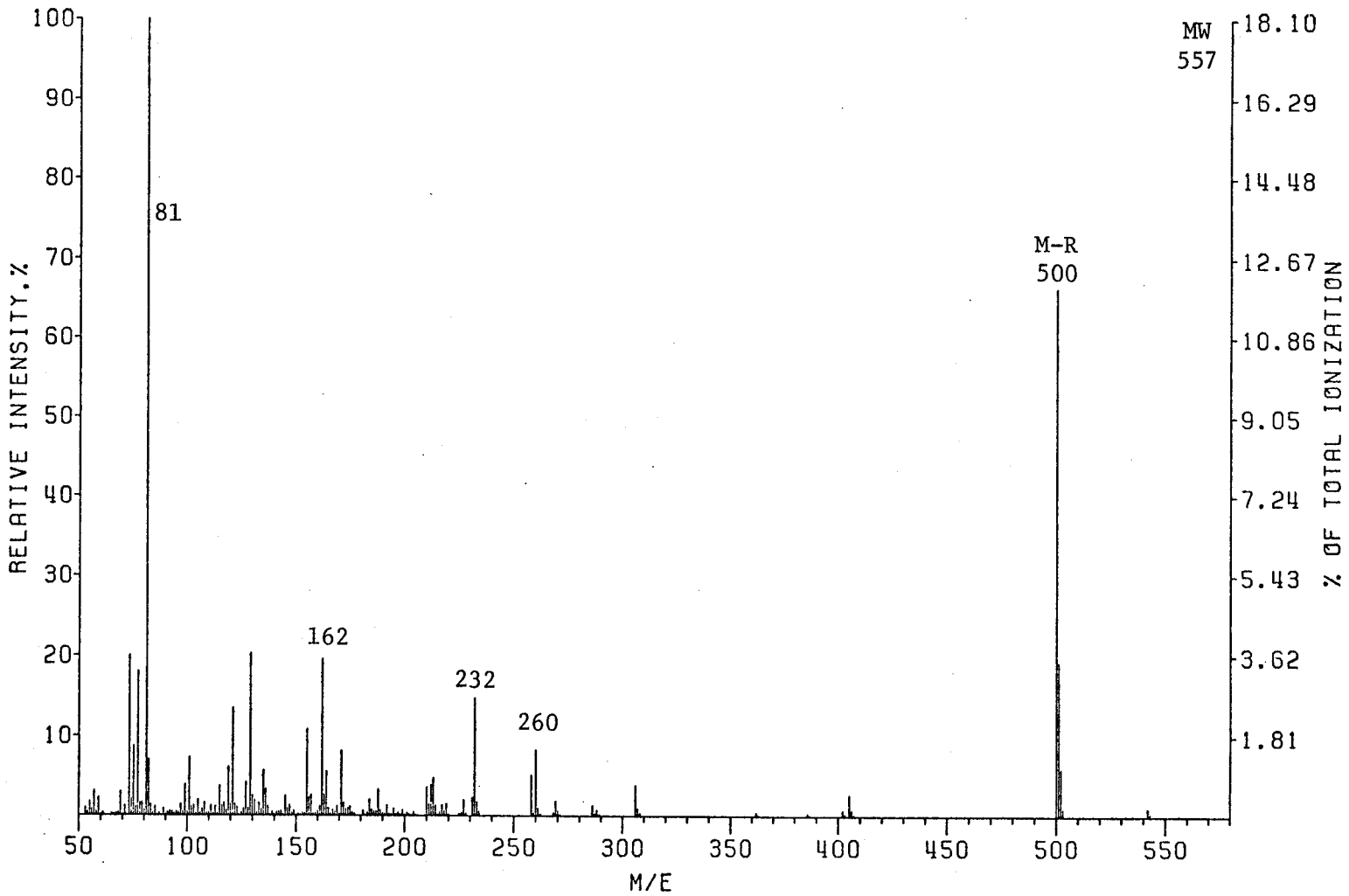


Figure 52. Mass spectrum of 3'-O-TBDMSi-5'-O-TFA-N6-TFA-deoxyadenosine (Vibi) recorded on Finnigan MS at 70 eV.

TABLE XIV. Correlation of m/e values for important fragment ions in the mass spectra of mixed acyl/SCTASi derivatives of thymidine (I) and deoxyadenosine (V).

Acyl Derivative:		Ac				dAc	TFA			
3' or 5'-O-Silyl Group:		TBDMSi	TMTBSi	TMIPSi	TBDMSi	TBDMSi	TBDMSi	TMTBSi	TMIPSi	TBDMSi
Assignment[a]	Comment[b] Compd.#	Ibg Igb	Icg Igc	Idg Igd	Vbgg Vgbg	Ibh Ihb	Ibi Iib	Ici Iic	Idi Iid	Vbii Vibi
M		398	424	410	449	401	452	478	464	557
M-R		341	367	367	392	344	395	421	421	500
M-R-CL_2CO	Ac,v	299	325	325	350	300	-	-	-	-
(M-R-CL_3CO_2H) or A	5'	281	307	307	332	281	281	307	307	386
A-CL_2CO	5',Ac,v	NA	NA	NA	290	NA	NA	NA	NA	-
A-H_2O	5'	263	289	289	314	263	263	289	289	368
S-CL_3CO_2H		213	239	225	213	213	213	239[c]	225	213
B+C_5H_6O	3',i	207	207	207	216[c]	207	207	207	207	270[c]
B'+X_2Si+H_2O	3',v	NA	NA	NA	252	NA	NA	NA	NA	306
B+X_2Si+H_2O	3'	201	227	227[c]	210	201	201	227	227	210
B'+X_2Si	v	NA	NA	NA	234	NA	NA	NA	NA	288
B+X_2Si		183	209	209	192	183	183	209	209	192
B'+CH_2O	3',v	NA	NA	NA	206	NA	NA	NA	NA	260
B'+C_3H_4	v	NA	NA	NA	216[c]	NA	NA	NA	NA	270[c]
B+C_3H_4	5'	165	165	165	174	165	165	165	165	174
A-BH	5'	155	181	181	155	155	155	181	181	155

continued...

TABLE XIV. continued

Assignment[a]	Comment	Acyl Derivative: 3' or 5'-O-Silyl Group: Compd.#:	Ac TBDMSi Ibg Igb	Ac TMTBSi Icg Igc	Ac TMIPSi Idg Igd	Ac TBDMSi Vbgg Vgbg	dAc TBDMSi Ibh Ihb	TFA TBDMSi Ibi Iib	TFA TMTBSi Ici Iic	TFA TMIPSi Idi Iid	TFA TBDMSi Vbii Vibi
$B'+C_2H_4$	v,d		NA	NA	NA	204	NA	NA	NA	NA	258
$B+C_2H_4$	v,d		153	153	153	162	154[d]	153	153	153	162
RX_2SiOCH_2	5'		145	171[c]	157	145	145	145	171[c]	157	145
B'+2H	v,d		NA	NA	NA	178	NA	NA	NA	NA	232
B+2H			127	127	127[c]	136	127, 128[d]	127	127	127[c]	136
$X_2Si+CO_2CL_3$			117	143	143	117[c]	120	171[c]	197	197	171
HX_2SiOCH_2	5'		89	115	115	89	89	89	115	115	89
C_5H_5O			81	81	81	81	81	81	81	81	81

[a] L = H, D, or F for Ac, dAc, and TFA derivatives respectively; B' = B(base) + acyl group at N6.

[b] Fragment ion is prominent only in the spectra of: 5' = 5'-O-SCTASi derivatives; 3' = 3'-O-SCTASi derivatives; Ac = Ac derivatives; i = derivatives of thymidine (I); v = derivatives of deoxyadenosine (V). Otherwise, ions are abundant in all derivatives. See representative spectra in Figures 45 to 52.

[c] Overlap with other ion type.

[d] A d-shift of 1 for Ibh and Ihb indicates that for Ac derivatives, one H originates from acetyl group.

NA = not applicable

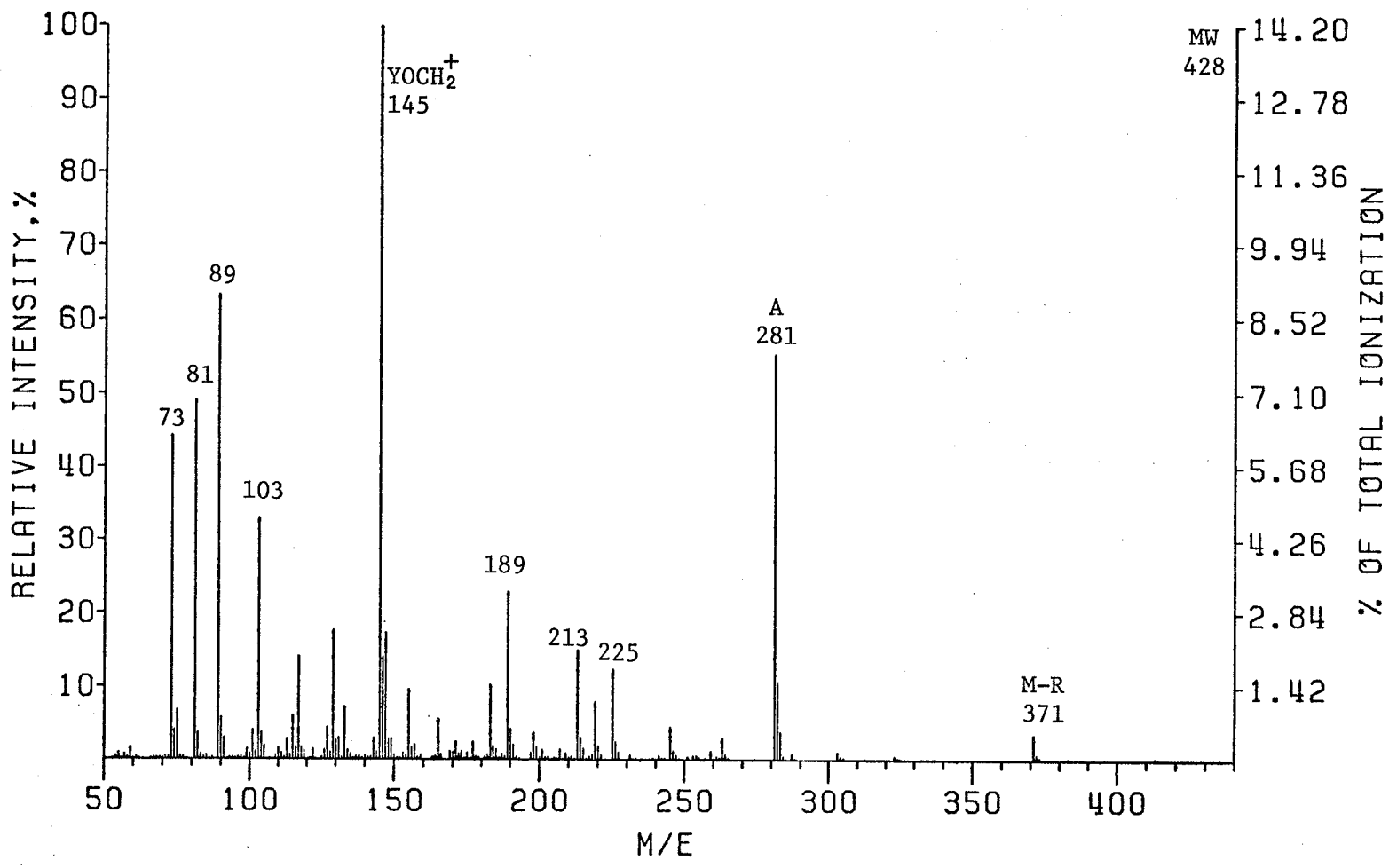


Figure 53. Mass spectrum of 3'-O-TMSi-5'-O-TBDMSi-thymidine (Ibe) recorded on Finnigan MS at 70 eV.

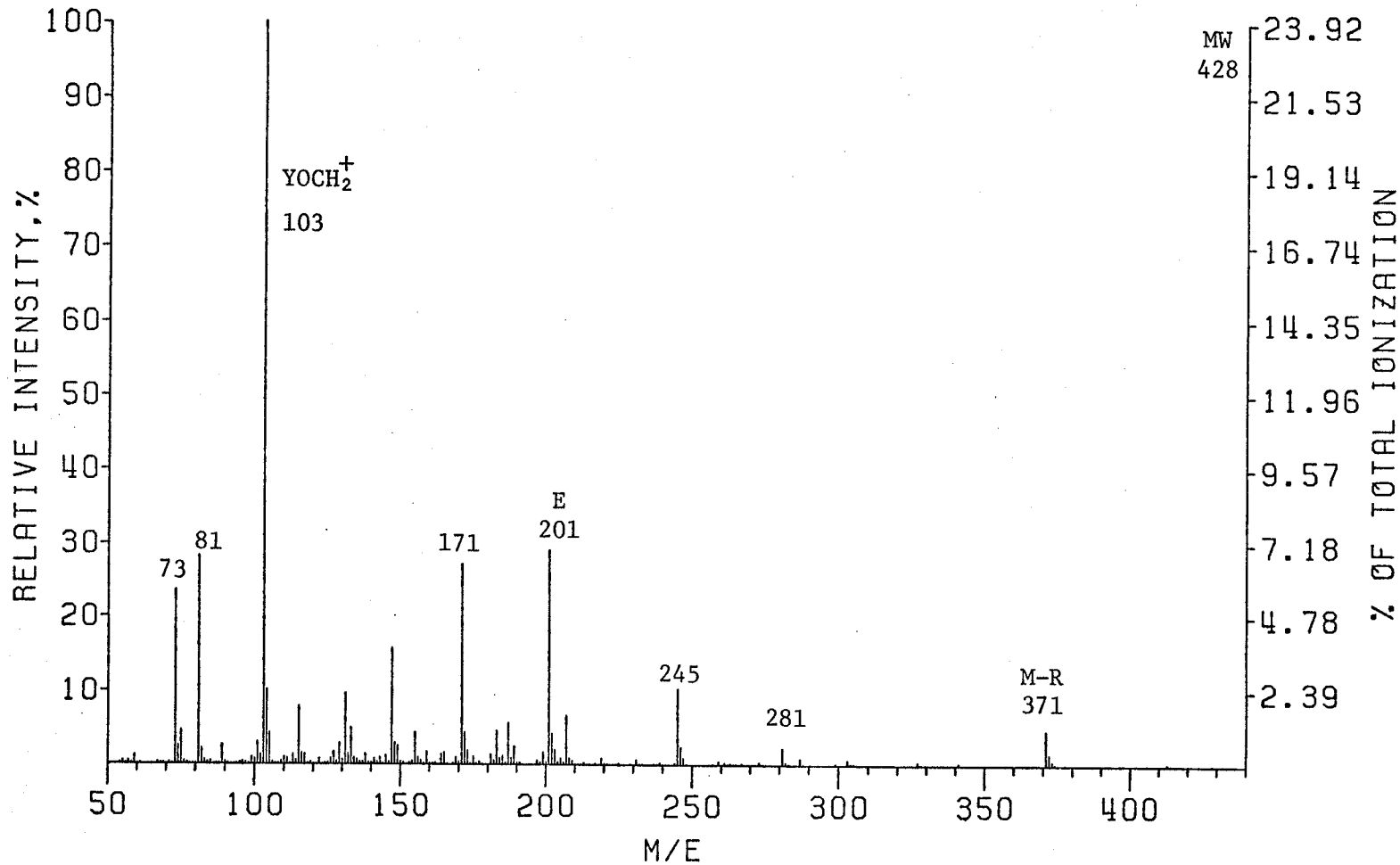


Figure 54. Mass spectrum of 3'-O-TBDMSi-5'-O-TMSi-thymidine (Ieb) recorded on Finnigan MS at 70 eV.

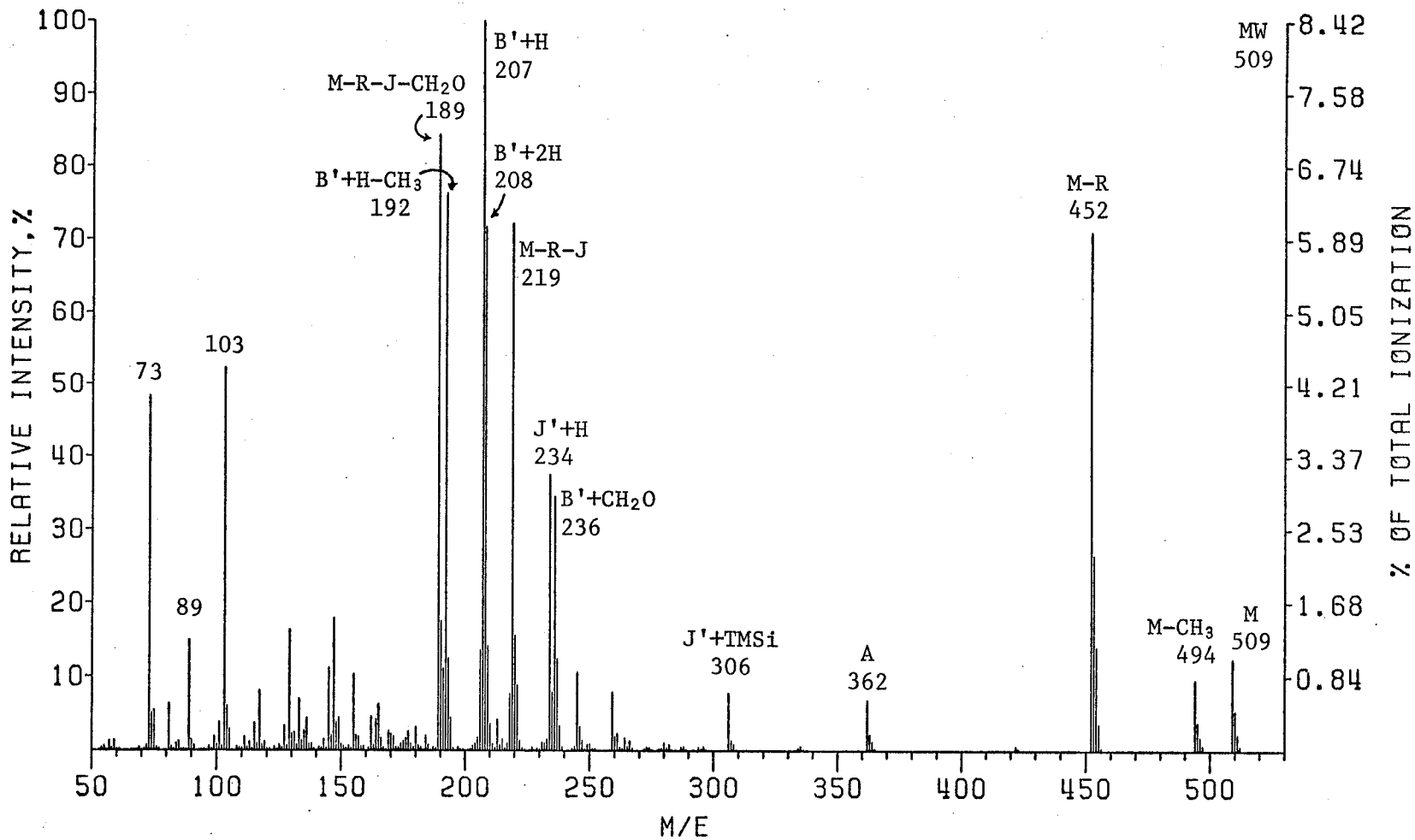


Figure 55. Mass spectrum of 3'-O-TMSi-5'-O-TBDMSi-N6-TMSi-deoxyadenosine (Vbee) recorded on Finnigan MS at 70 eV.

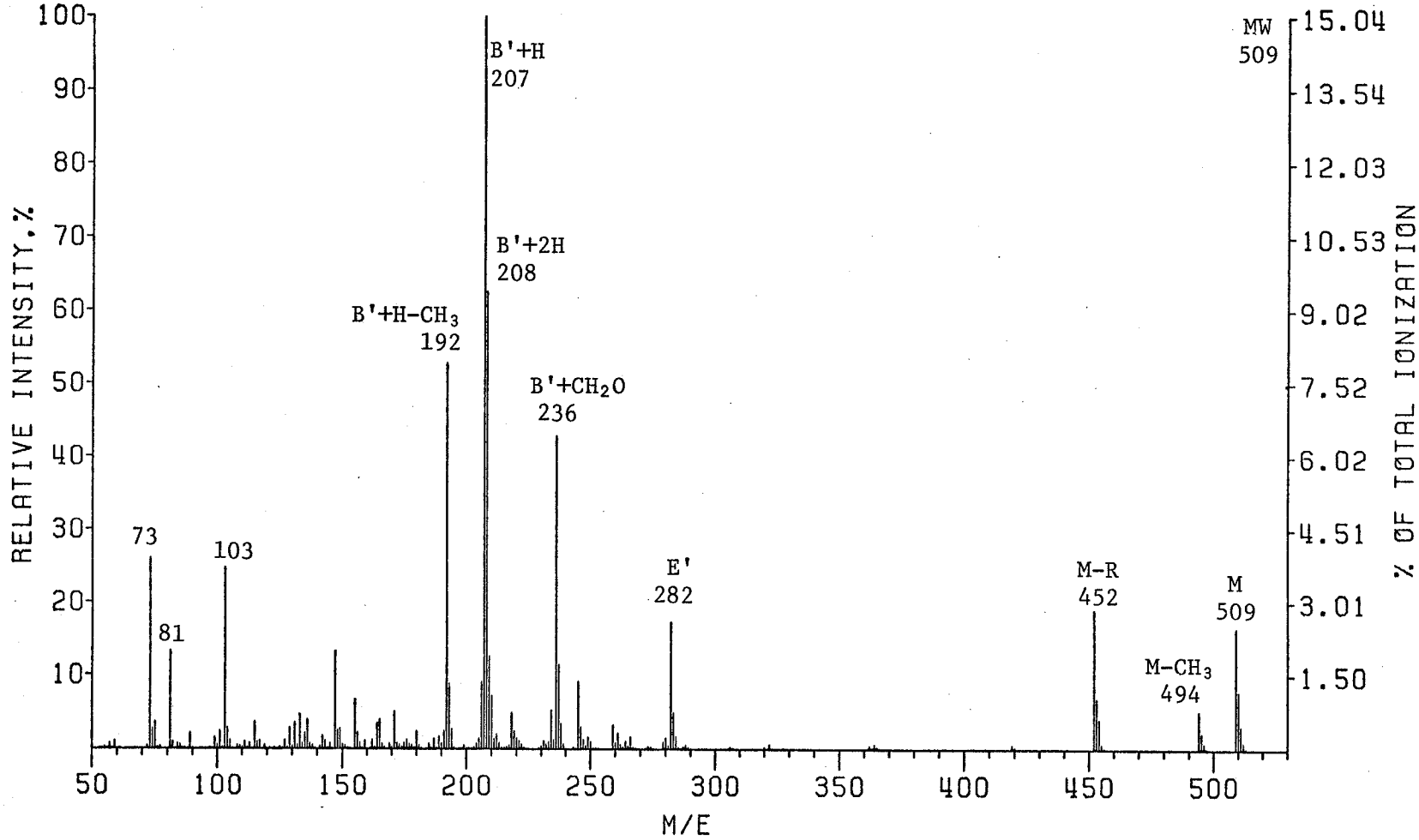


Figure 56. Mass spectrum of 3'-O-TBDMSi-5'-O-TMSi-N6-TMSi-deoxyadenosine (Vebe) recorded on Finnigan MS at 70 eV.

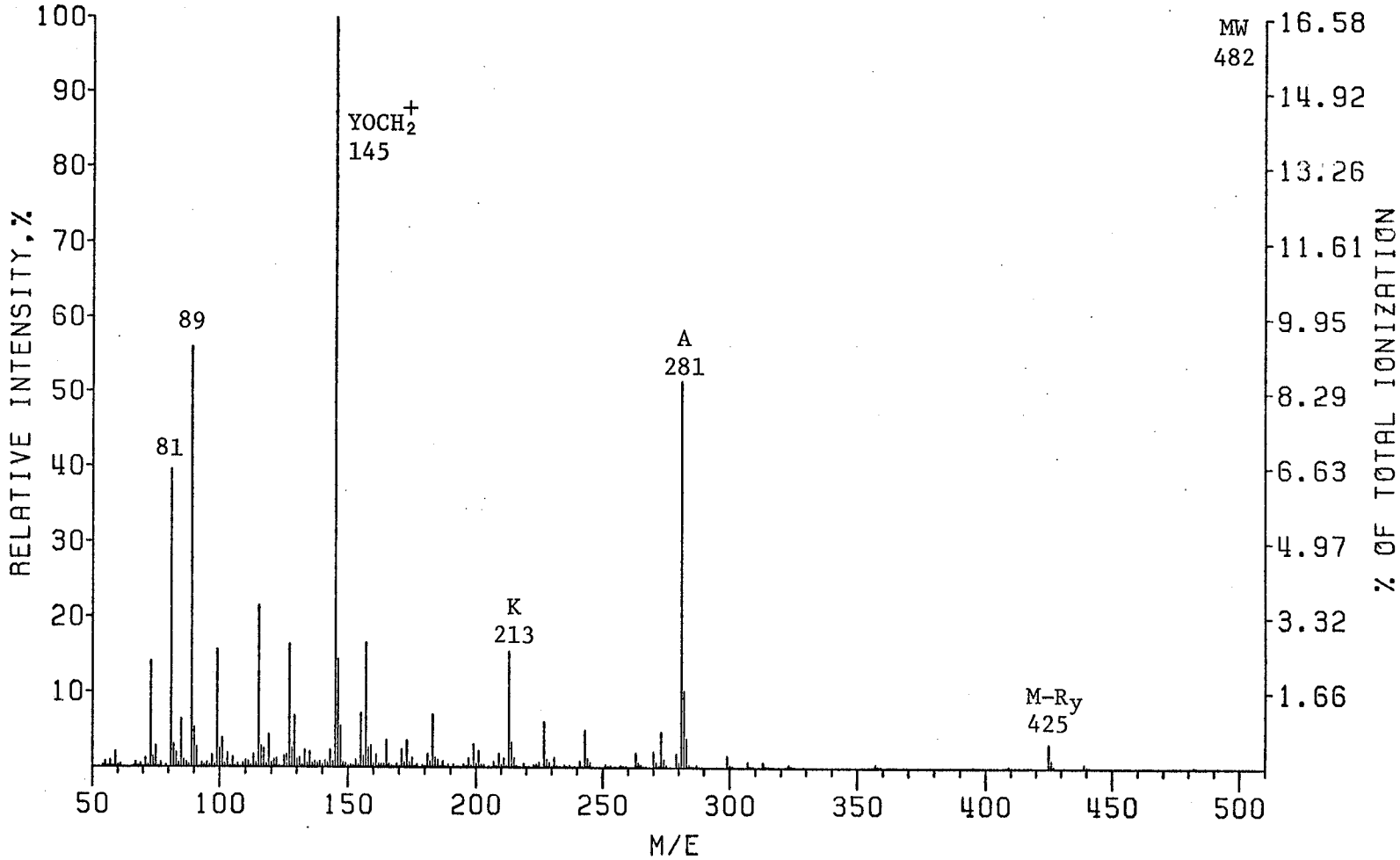


Figure 57. Mass spectrum of 3'-O-TMIPSi-5'-O-TBDMSi-thymidine (Ibd) recorded on Finnigan MS at 70 eV.

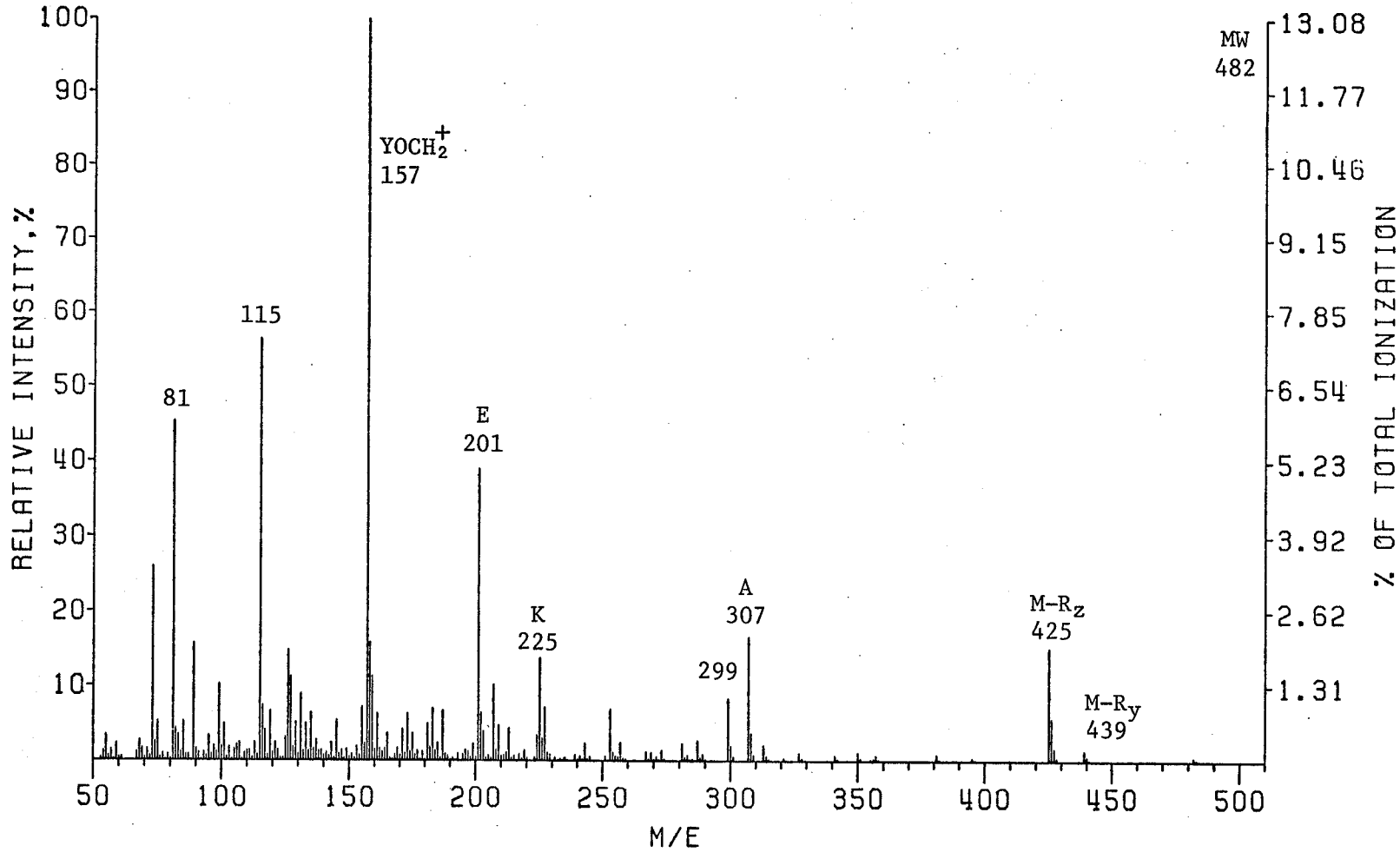


Figure 58. Mass spectrum of 3'-O-TBDMSi-5'-O-TMIPSi-thymidine (Idb) recorded on Finnigan MS at 70 eV.

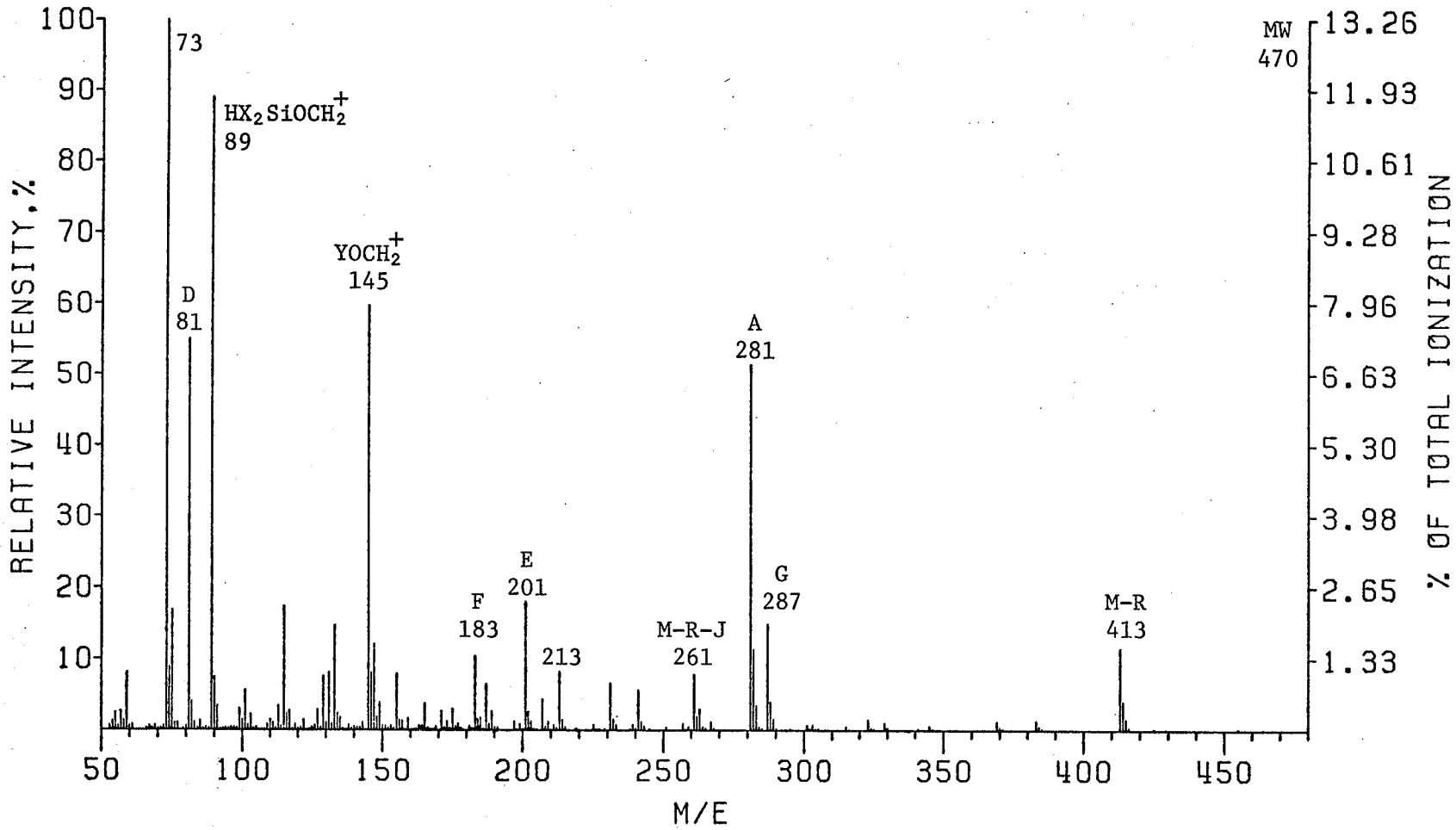


Figure 59. Mass spectrum of 3',5'bis-O-TBDMSi-thymidine (Ibb) recorded on Hitachi MS at 50 eV.

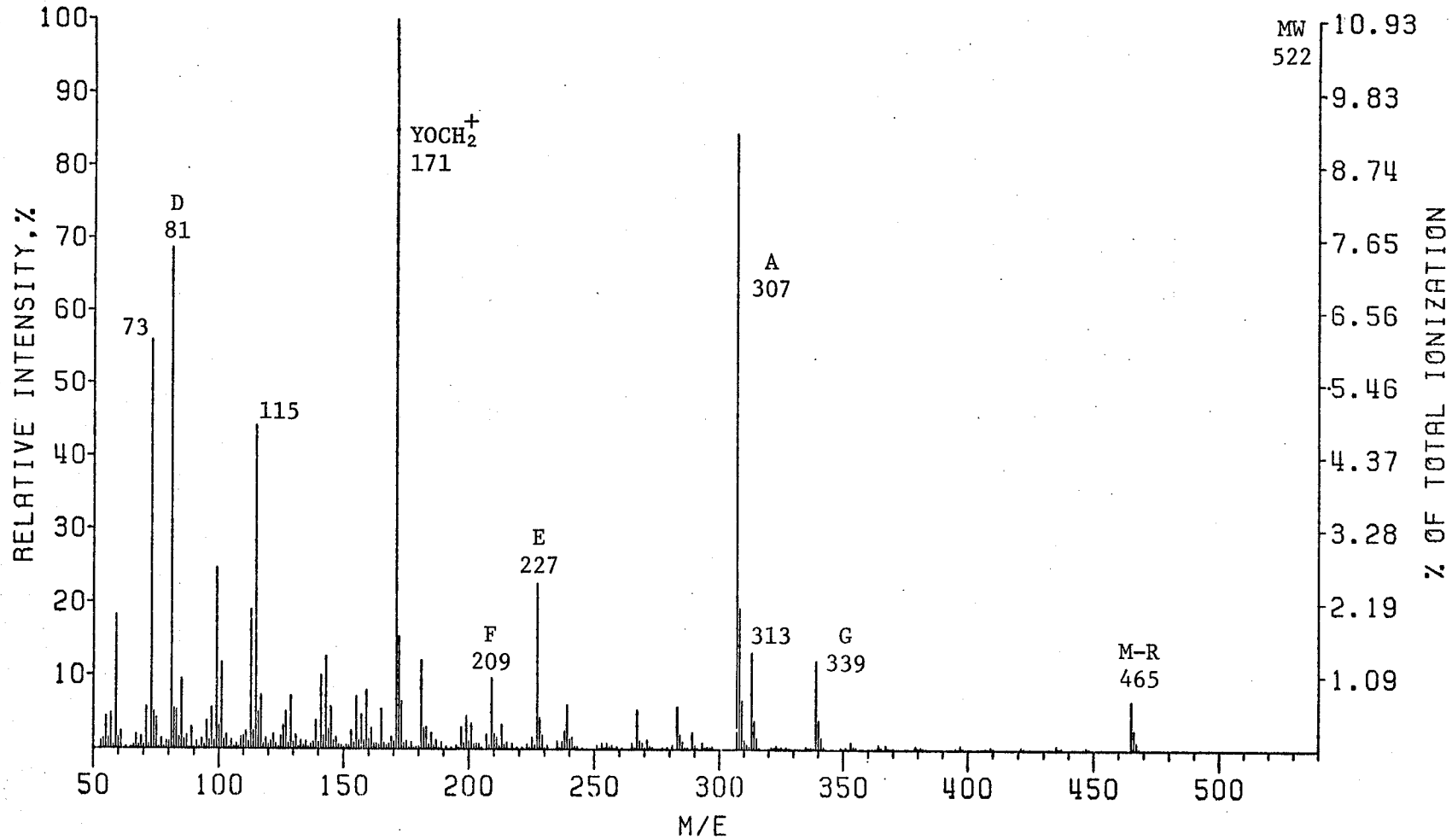


Figure 60. Mass spectrum of 3',5'bis-O-TMTBSi-thymidine (Icc) recorded on Hitachi MS at 50 eV.

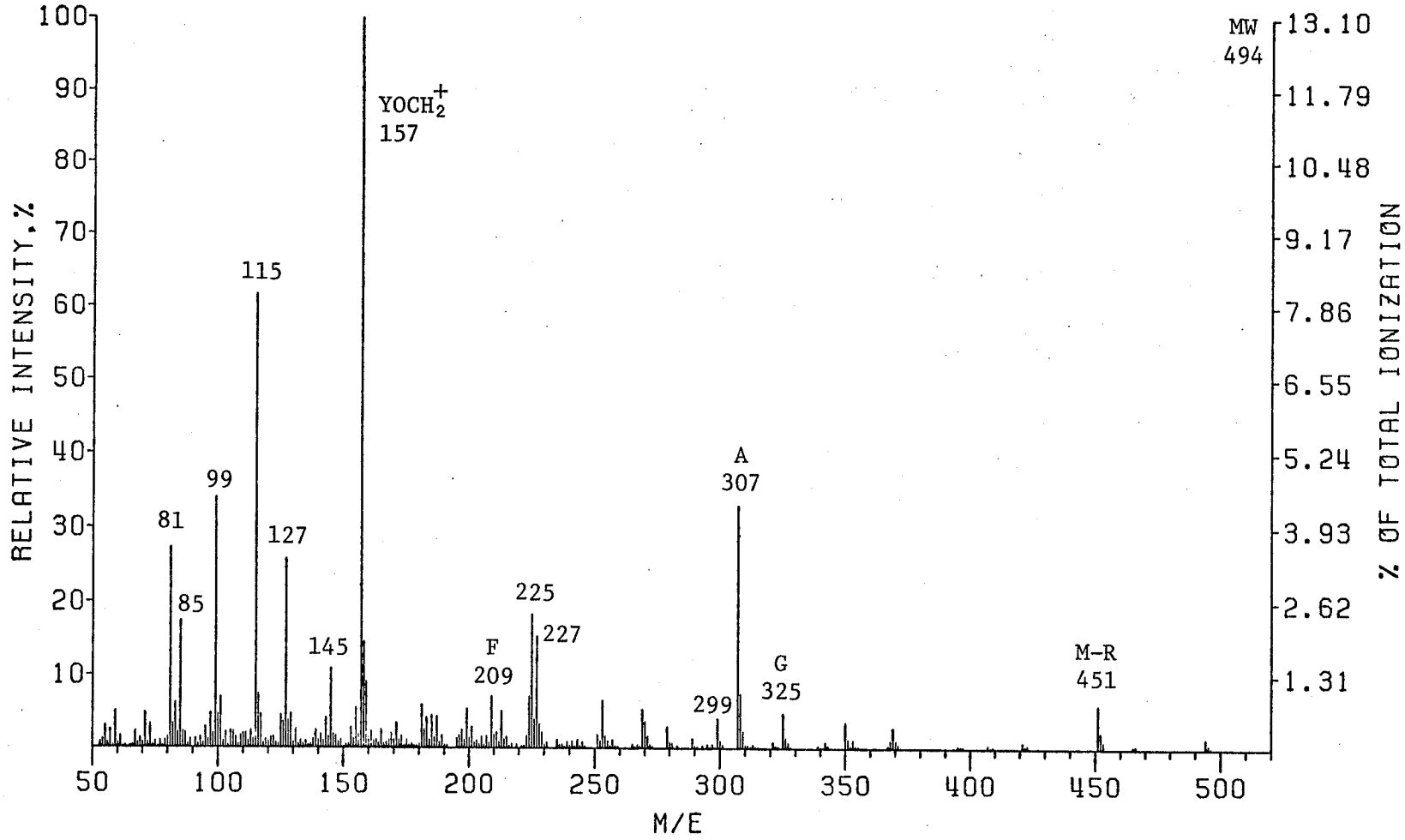


Figure 61. Mass spectrum of 3',5'bis-O-TMIPSi-thymidine (Idd) recorded on Hitachi MS at 50 eV.

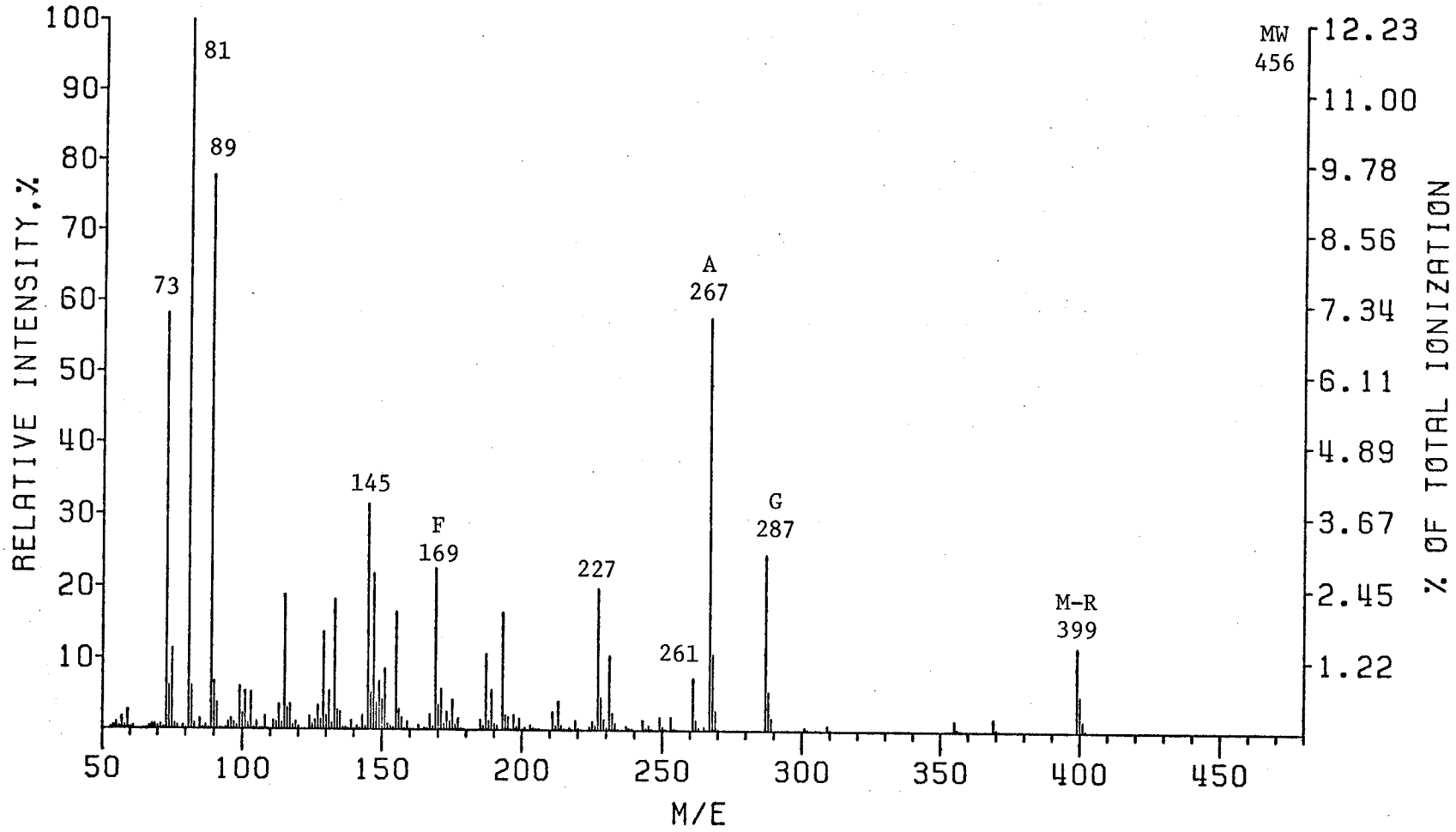


Figure 62. Mass spectrum of 3',5'bis-O-TBDMSi-deoxyuridine (IIbb) recorded on Finnigan MS at 70 eV.

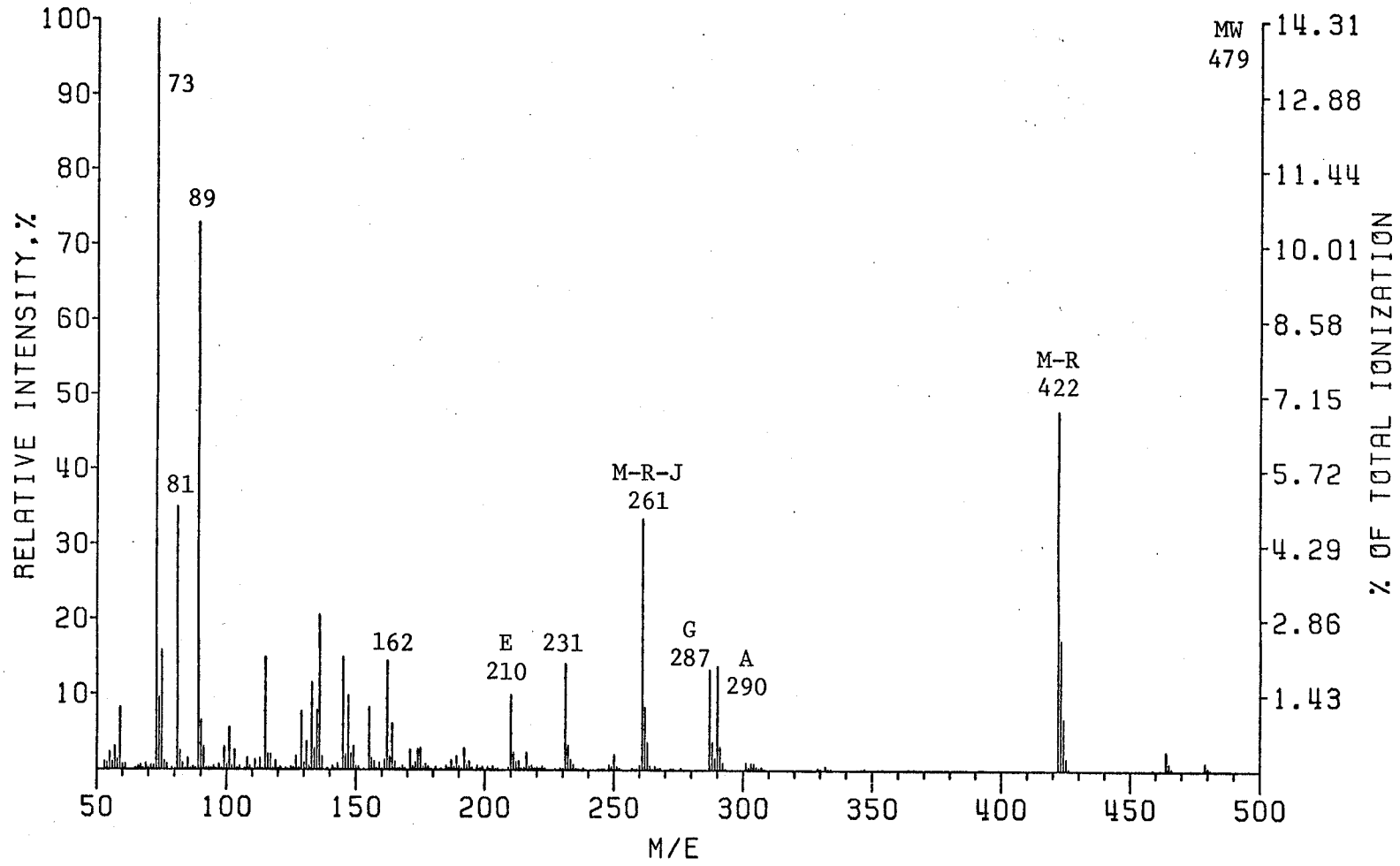


Figure 63. Mass spectrum of 3',5'bis-O-TBDMSi-deoxyadenosine (Vbba) recorded on Hitachi MS at 50 eV.

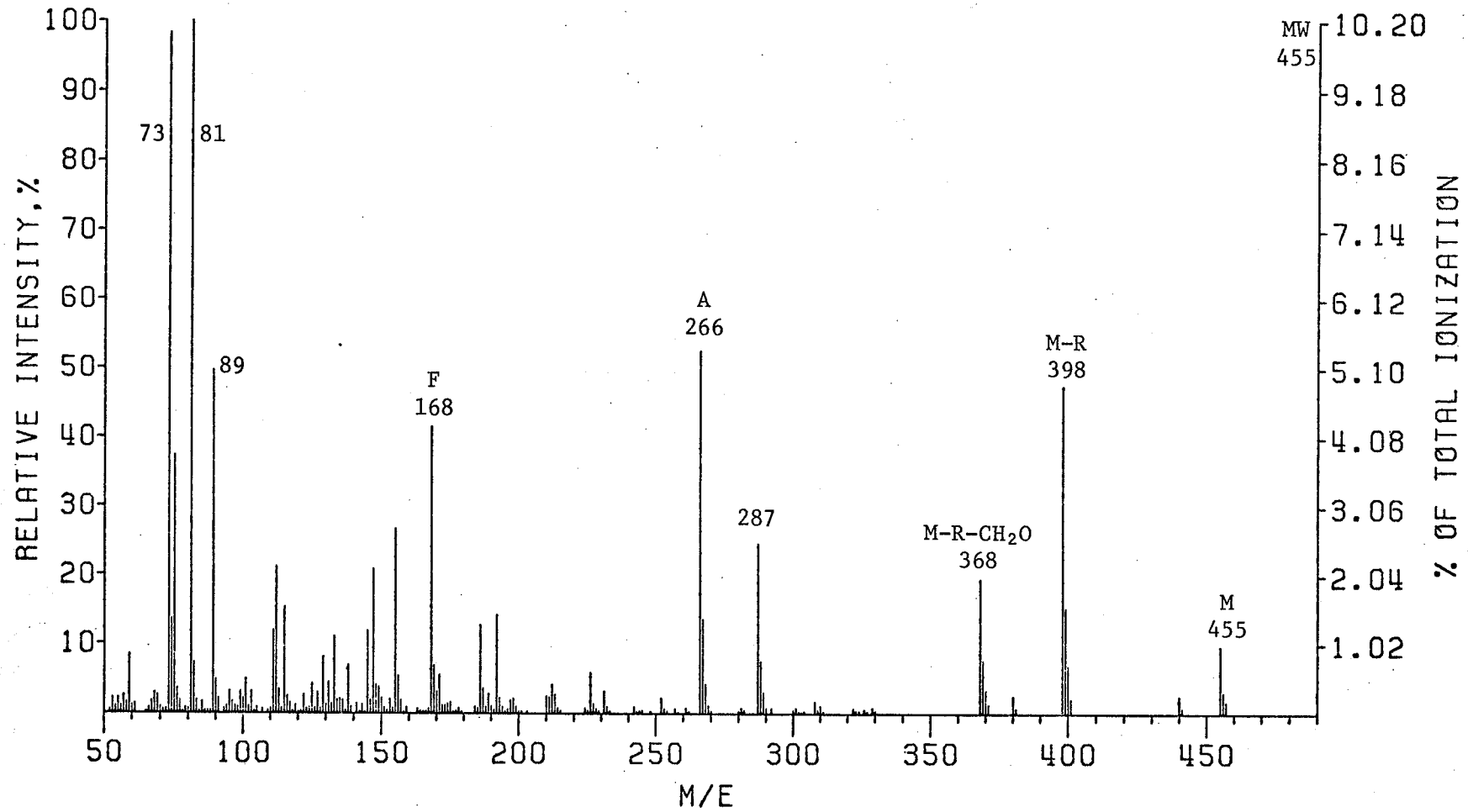


Figure 64. Mass spectrum of 3',5'bis-O-TBDMSi-deoxycytidine (IIIbb) recorded on Hitachi MS at 50 eV.

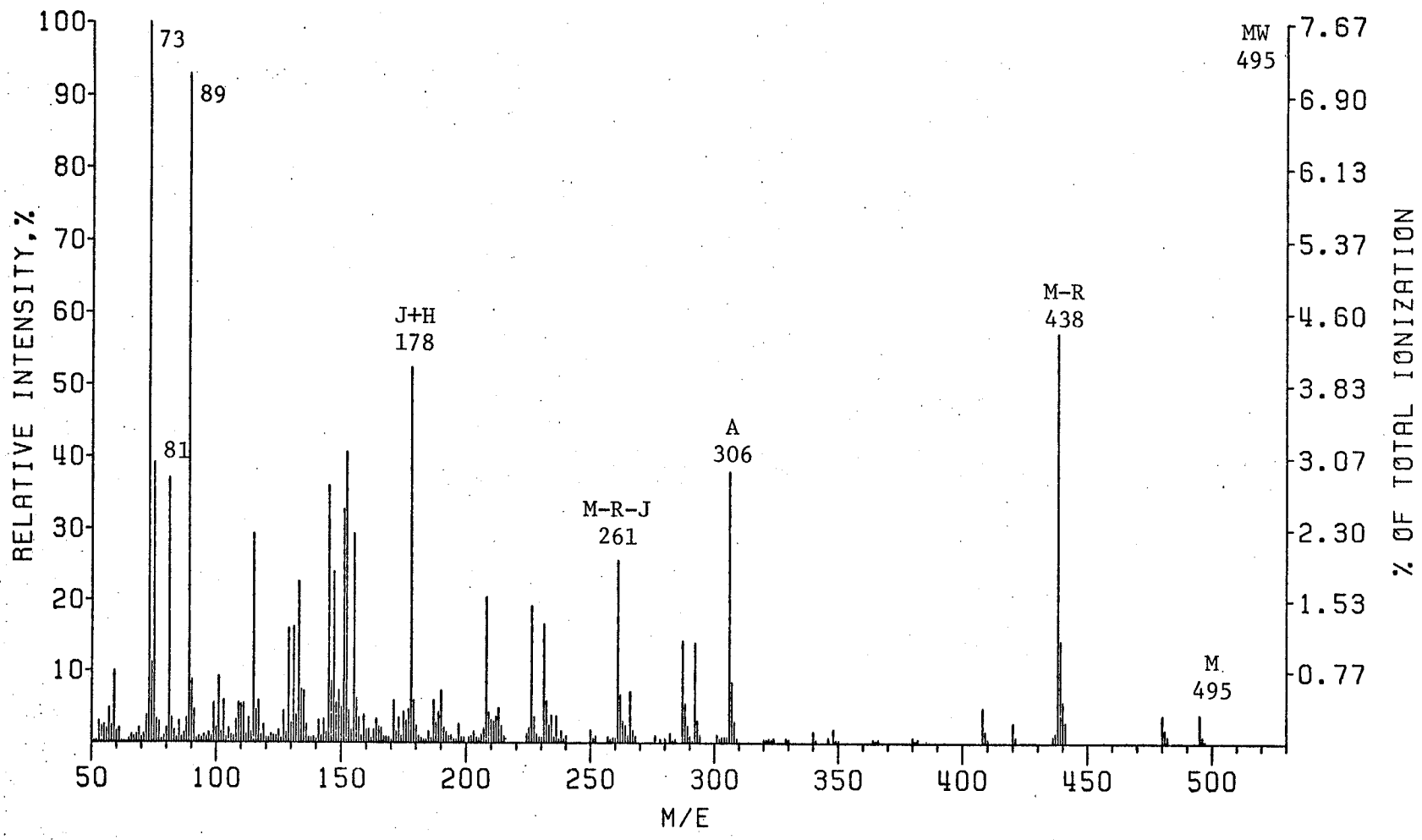


Figure 65. Mass spectrum of 3',5'bis-O-TBDMSi-deoxyguanosine (IVbb) recorded on Finnigan MS at 70 eV.

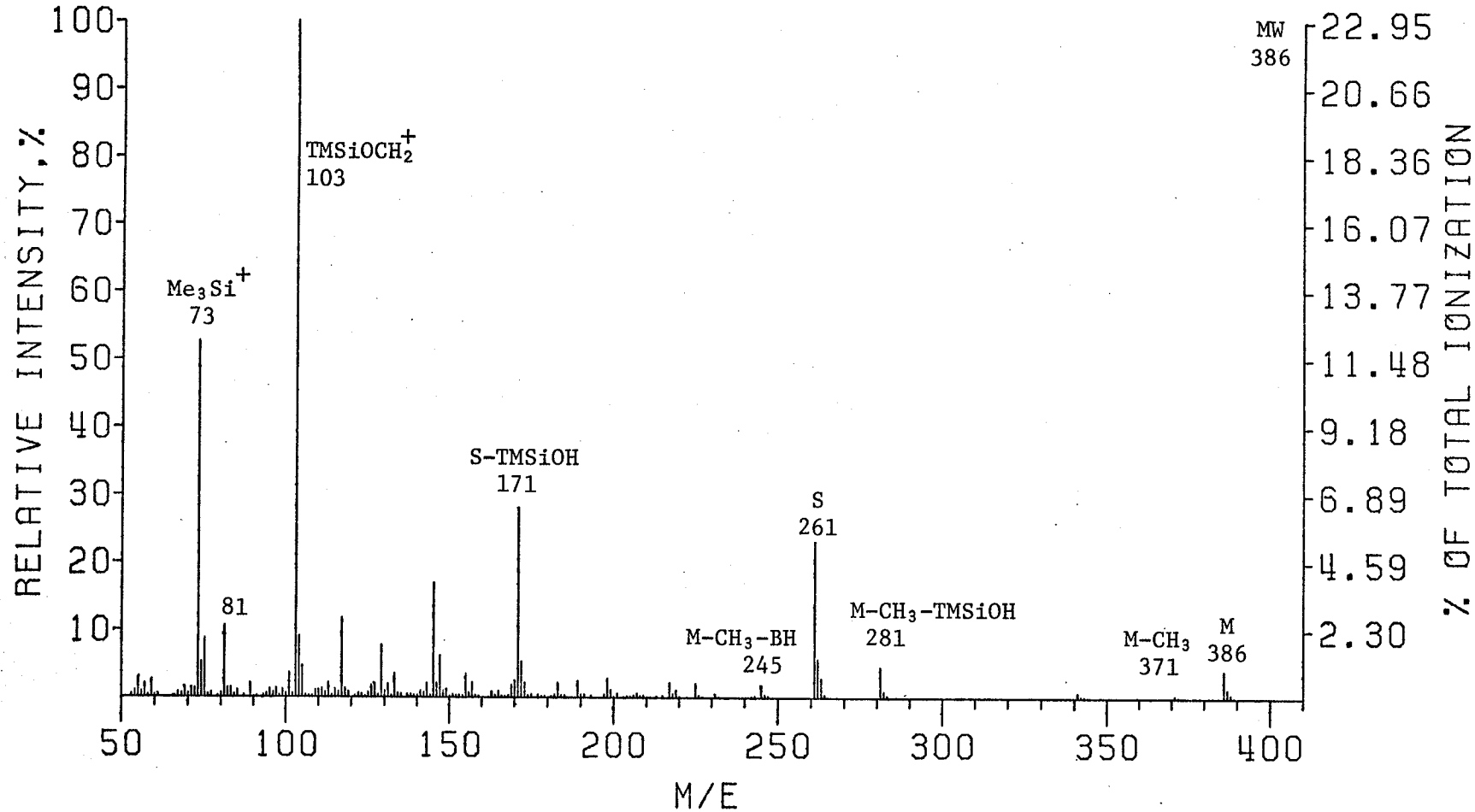


Figure 66. Mass spectrum of 3',5'bis-O-TMSi-thymidine (Iee) recorded on Hitachi MS at 50 eV.

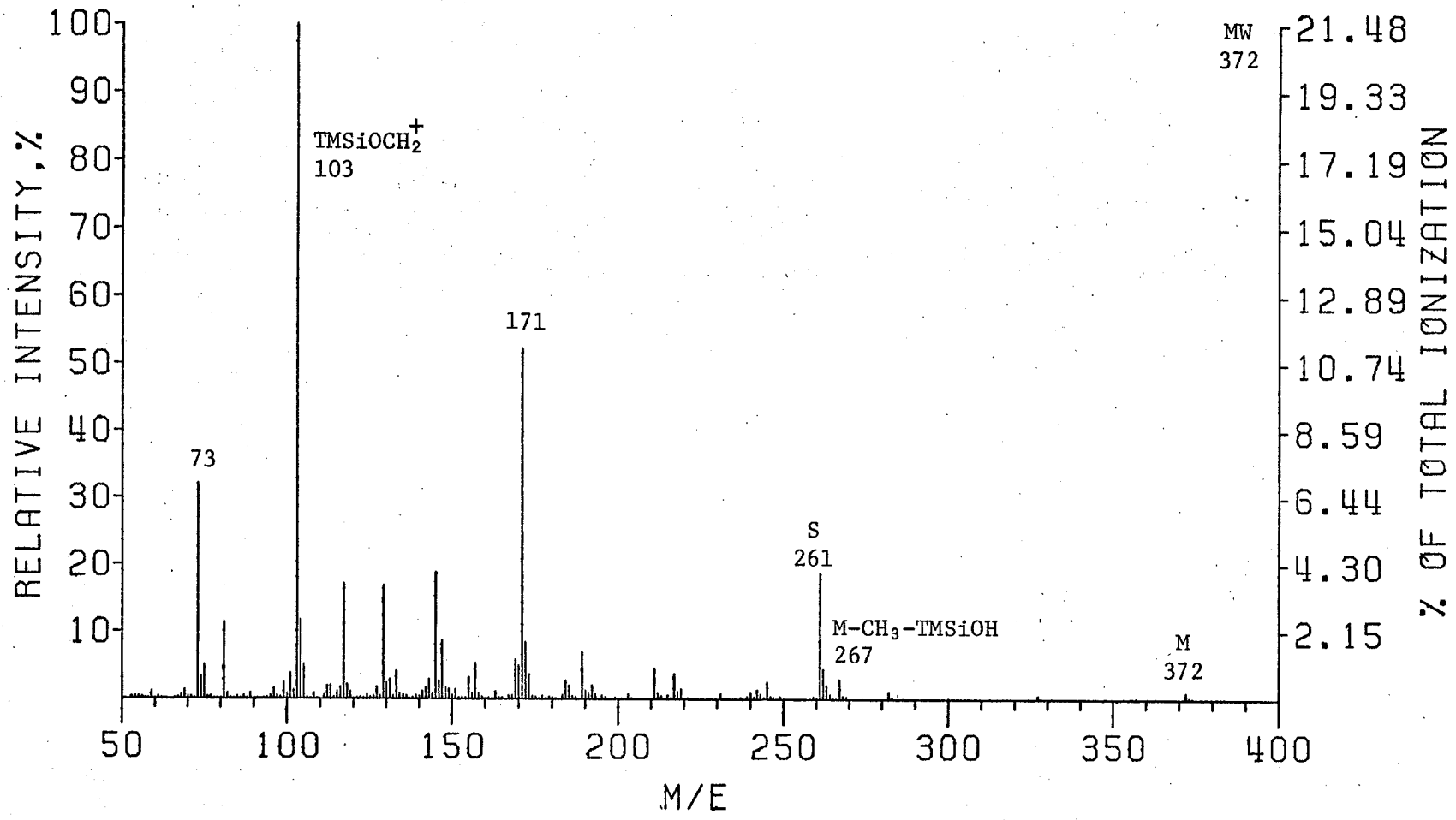


Figure 67. Mass spectrum of 3',5'bis-O-TMSi-deoxyuridine (IIee) recorded on Finnigan MS at 70 eV.

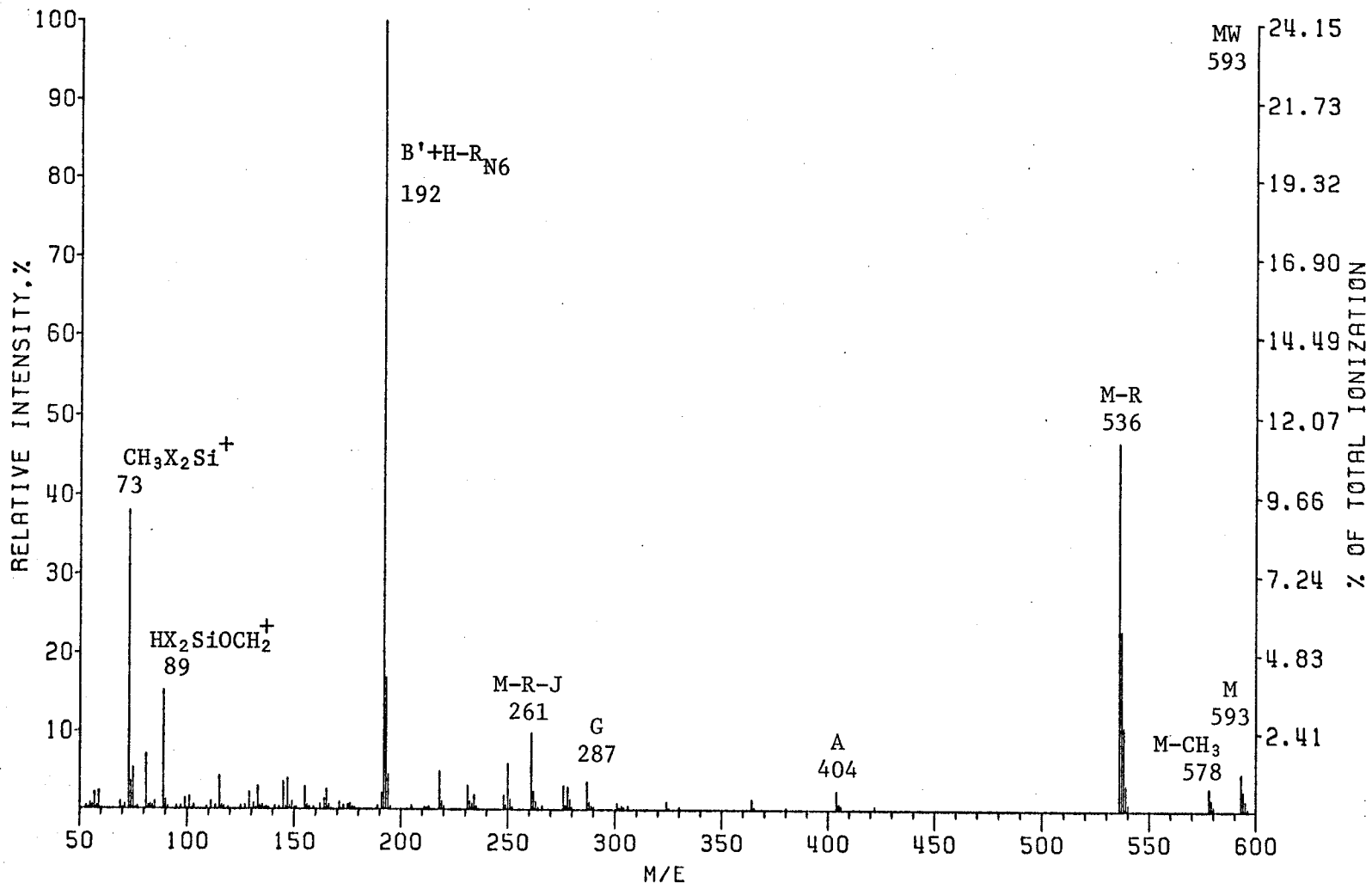


Figure 68. Mass spectrum of 3',5'bis-O-TBDMSi-N6-TBDMSi-deoxyadenosine (Vbbb) recorded on Hitachi MS at 50 eV.

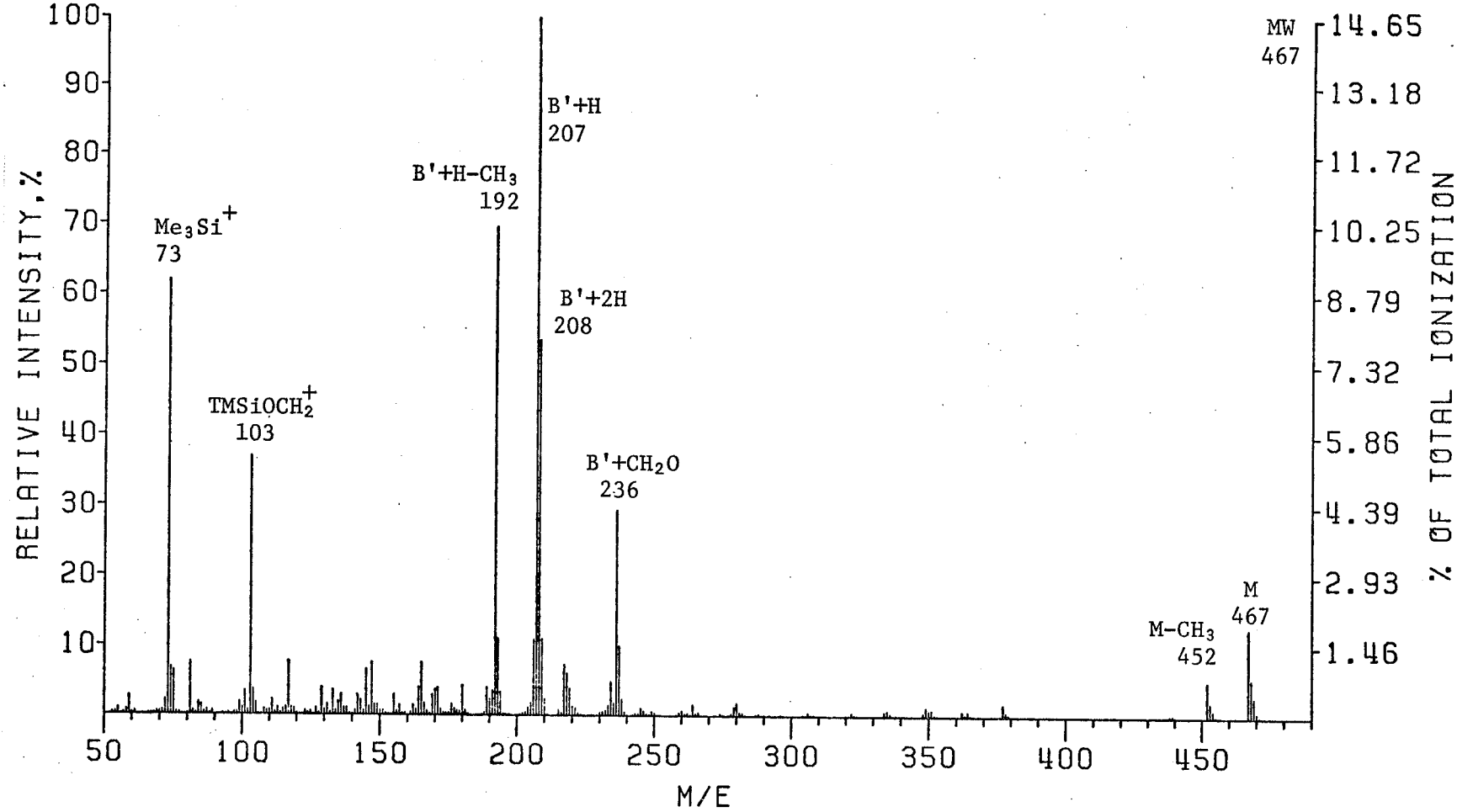


Figure 69. Mass spectrum of 3',5'bis-O-TMSi-N6-TMSi-deoxyadenosine (Veee) recorded on Finnigan MS at 70 eV.

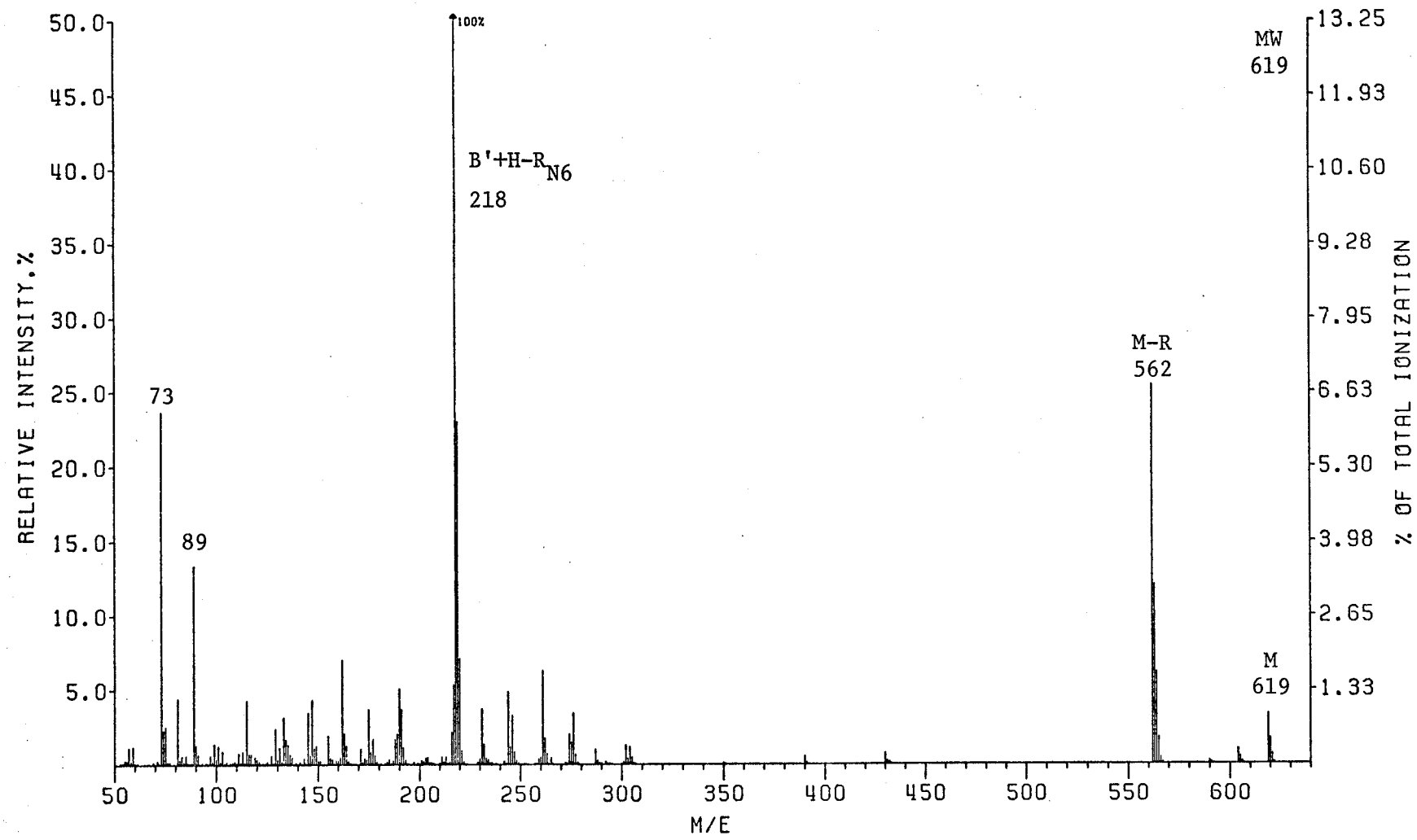


Figure 70. Mass spectrum of 3',5'bis-O-TBDMSi-N6-TMTBSi-deoxyadenosine (Vbbc) recorded on Finnigan MS at 70 eV.

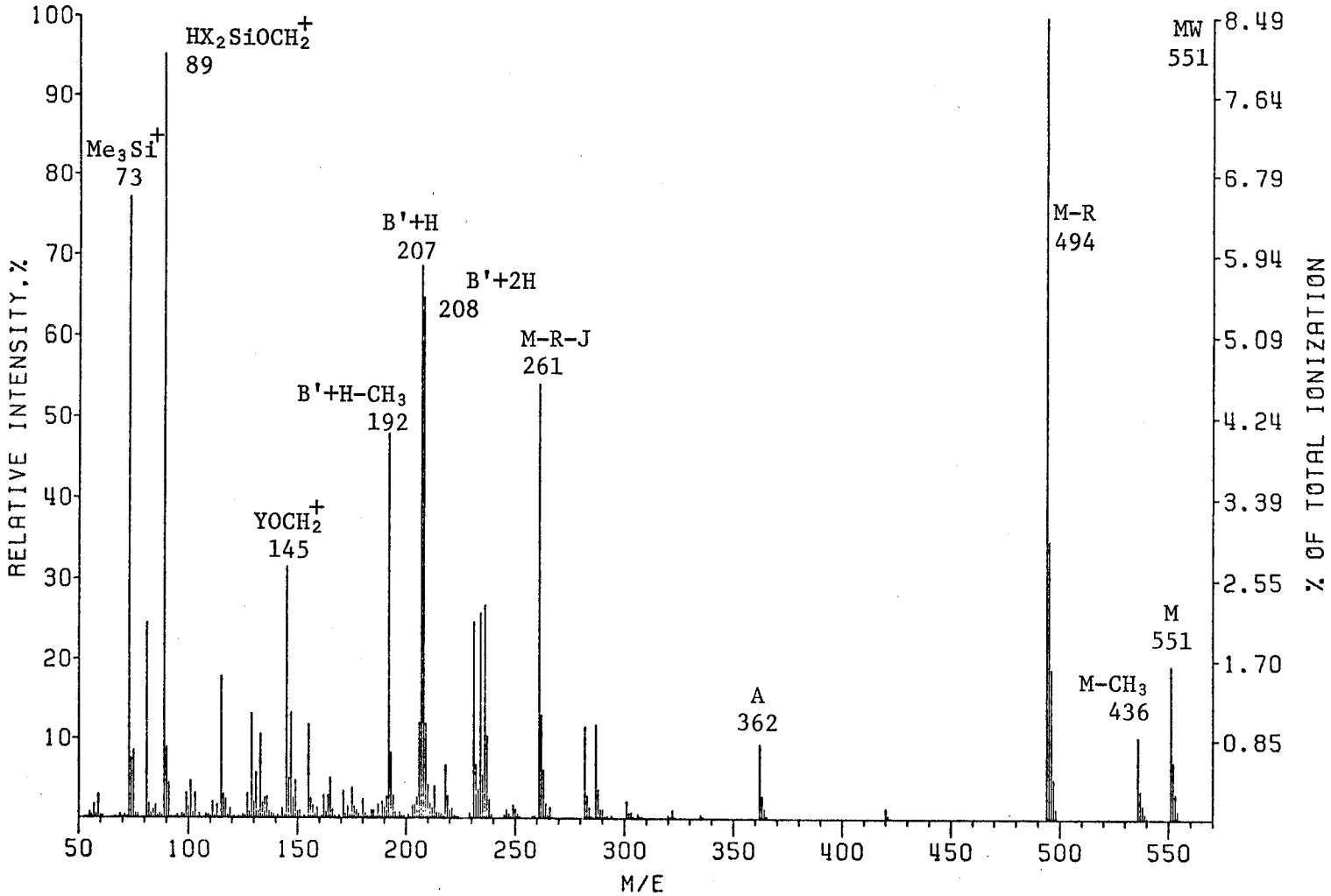


Figure 71. Mass spectrum of 3',5'bis-O-TBDMSi-N6-TMSi-deoxyadenosine (Vbbe) recorded on Finnigan MS at 70 eV.

TABLE XVa. Mass spectral data for 3',5'bis-O-TBDMSi-thymidine (Ibb).

Line #	Assignment[e]	Elemental Composition C-H-Si-N-O	Exact Mass Calculated	Exact Mass Measured	m/e	Rel.[f] Int.%	d-shift
1.	M	[22-42-2-2-5]	-	-	470	0	(1)
2.	$M-CH_3$	[21-39-2-2-5]	-	-	455	0.2	1
3.	M-R	18-33-2-2-5	413.193	413.196	413	12.	1
4.	$M-R-CH_2O$	17-31-2-2-4	383.182	383.183	383	1.3	1
5.	$M-R-C_2H_4O(44)$	16-29-2-2-4	369.167	369.168	369	1.2	1
6.	S	17-37-2-0-3	345.228	345.229	345	0.6	0
7.	$M-CH_3-BH$	16-33-2-0-3	329.197	329.198	329	1.0	0
8.	$M-CH_3-RX_2SiOH$	15-23-1-2-4	323.143	323.141	323	1.5	0(90%)
9.	$B+X_2Si+RX_2SiOH$	13-27-2-2-3	315.156	315.156	315	0.5	1
10.	$M-CH_3-J$	14-31-2-0-3	303.181	303.181	303	0.7	0
11.	$2RX_2Si+C_3H_3O_2(71)$	15-33-2-0-2	301.202	301.202	301	0.7	0
12.	[M-R-BH] or G	13-27-2-0-3	287.150	287.151	287	15.	0
13.	$[M-R-RX_2SiOH]$ or A	12-17-1-2-4	281.096	281.096	281	51.	0(90%)
14.	$J+RX_2Si$	13-23-1-2-2	267.153	267.153	267	1.2	1
15.	$[M-R-RX_2SiOH-H_2O]$ or $[A-H_2O]$	12-15-1-2-3	263.085	263.085	263	3.0	0
16.	M-R-J	11-25-2-0-3	261.134	261.135	261	7.8	0
17.	$B+H+RX_2Si$	11-21-1-2-2	241.137	241.137	241	5.6	1
18.	$M-R-J-CH_2O$	10-23-2-0-2	231.124	231.123	231	6.6	0
19.	B+RXSi	10-17-1-2-2	225.106	225.107	225	0.8	1
20.		8-13-1-2-3	213.070	213.070[c]	213	8.2	0
21.	$[S-RX_2SiOH]$ or K	11-21-1-0-2	213.131	213.131[a]			
22.	$B+C_5H_6O(82)$	10-11-0-2-3	207.077	207.077	207	4.4	1
23.	$[B+X_2Si+H_2O]$ or E	7-13-1-2-3	201.070	201.069	201	18.	1
24.	$RX_2SiOSiX_2$	8-21-2-0-1	189.113	189.113	189	2.7	0
25.	$RX_2Si+C_3H_4O_2(72)$	9-19-1-0-2	187.115	187.117	187	6.5	0
26.	$[B+X_2Si]$ or F	7-11-1-2-2	183.059	183.058	183	10.	1(50%)
27.	$B+C_3H_4(40)$	8- 9-0-2-2	165.066	165.067	165	3.8	1
28.	$[M-R-RX_2SiOH-BH]$ or [A-BH]	7-11-1-0-2	155.053	155.052	155	7.9	0
29.	$CH_3X_2SiOSiX_2$	5-15-2-0-1	147.066	147.060	147	12.	0
30.	RX_2SiOCH_2	7-17-1-0-1	145.105	145.102	145	60.	0

continued...

TABLE XVa. continued

Line #	Assignment[e]	Elemental Composition C-H-Si-N-O	Exact Mass Calculated	Exact Mass Measured	m/e	Rel.[f] Int.%	d-shift
31.	$HX_2SiOSiX_2$	4-13-2-0-1	133.151	133.150	133	15.	0
32.	$X_2SiOSi(CH_2)X$	4-11-2-0-1	131.035	131.035[b]	131	8.1	0
33.	$X_2Si+C_3H_5O_2(73)$	5-11-1-0-2	131.053	131.052[b]			
34.	$X_2Si+C_3H_3O_2(71)$	5- 9-1-0-2	129.037	129.037[b]	129	7.6	0
35.	$R(CH_2)SiOCH_2$	6-13-1-0-1	129.074	129.073[b]			
36.	B+2H	5- 7-0-2-2	127.051	127.052	127	2.9	1
37.	RX_2Si	6-15-1-0-0	115.095	115.094[a]	115	17.	0
38.		5-11-1-0-1	115.058	115.058[c]			
39.		4- 7-1-0-2	115.022	115.020[c]			
40.	$X_2Si+C_2H_3O(43)$	4- 9-1-0-1	101.042	101.042			0
41.	$R(CH_2)Si$	5-11-1-0-0	99.063	99.063[b]	99	3.0	0
42.	$X_2Si+C_2HO(41)$	4- 7-1-0-1	99.027	99.027[b]			
43.	$H(X_2SiOCH_2)$	3- 9-1-0-1	89.042	89.043	89	89.	0
44.	C_5H_5O or D	5- 5-0-0-1	81.034	81.033	81	55.	0
45.	X_2SiOH	-	-	-	75	17.	0
46.	CH_3X_2Si	-	-	-	73	100.	0
47.	HX_2Si	-	-	-	59	8.1	0

Base Peak as $\%\Sigma_{50}$ = 13.26

[a-c] Relative contribution to integer m/e abundance: a = major, b = medium, c = minor.

[e] Positive ions; sign left out for simplification.

[f] Low resolution relative intensity data recorded on Hitachi instrument at 50 eV.

TABLE XVb. Correlation of m/e values for prominent fragment ions of 3',5'bis-O-SCTASi derivatives of 2'-deoxynucleosides. Unless indicated by a footnote, intensity is comparable to that of Ibb.

Line #	Assignment	Compound: Ibb	Icc	Idd	IIbb	Vbba	IIIbb	IVbb
1.	M	470[c]	522[c]	494	456[c]	479	455	495
2.	$M-CH_3$	455	-	-	441	464	440	480
3.	M-R	413	465	451	399	422	398	438
4.	$M-R-CH_2O$	383	435	421	369	392[w]	368	408
5.	$M-R-C_2H_4O(44)$	369	421	407	355	378[c]	354[w]	394[c]
6.	S	345	397	369	345[c]	345[c]	345[c]	345[c]
9.	$B+X_2Si+RX_2SiOH$	315	367	353	301[a]	324[c]	300	340
11.	$2RX_2Si+C_3H_3O_2(71)$	301	353	325[a]	301[a]	301	301[b]	301
12.	G	287	339	325[a]	287	287	287	287
13.	A	281	307	307	267	290	266	306
14.	$J+RX_2Si$	267	293	279	253	276[w]	252	292
15.	$A-H_2O$	263	289	289	249	272	248	288[b]
16.	M-R-J	261	313	299	261	261	261	261
17.	$B+H+RX_2Si$	241	267	253	227	250	226	266
18.	$M-R-J-CH_2O$	231	283	269	231	231	231	231
21.	K	213	239	225	213	213[w]	213[b]	213
22.	$B+C_5H_6O(82)$	207	207	207	193	216	192	232[b]
23.	E	201	227	227[a]	187[a]	210	186	226
24.	$RX_2SiOSiX_2$	189	241	227[a]	189	189	189	189
25.	$RX_2Si+C_3H_4O_2(72)$	187	213	199[a]	187[a]	187	187[b]	187
26.	F	183	209	209	169	192[w]	168	208
27.	$B+C_3H_4(40)$	165	165	165	151	174	150[b]	190
28.	A-BH	155	181	181	155	155	155	155
29.	$CH_3X_2SiOSiX_2$	147	199	199[a]	147	147	147	147
30.	RX_2SiOCH_2	145	171	157[a]	145	145	145	145
31.	$HX_2SiOSiX_2$	133	185	185	133	133	133	133
33.	$X_2Si+C_3H_5O_2(73)$	131[a]	157	157[a]	131	131	131	131

continued...

TABLE XVb. continued

Line #	Assignment	Compound: Ibb	Icc	Idd	IIbb	Vbba	IIIbb	IVbb
34.	$X_2Si+C_3H_3O_2(71)$	129[a]	155	155	129	129	129	129
36.	B+2H	127	127[a]	127[a]	113	136[s]	112[s]	152[s]
37.	RX_2Si	115	141	127[a]	115	115	115	115
40.	$X_2Si+C_2H_3O(43)$	101	127[a]	127[a]	101	101	101	101
43.	$H(X_2SiOCH_2)$	89	115	115	89	89	89	89
44.	D	81	81	81	81	81	81	81
45.	X_2SiOH	75	101	101	75	75	75	75
46.	CH_3X_2Si	73	99[a]	99[a]	73	73	73	73
47.	HX_2Si	59	85	85	59	59	59	59

[a] Overlapping ion types that contribute significantly to relative intensity.

[b] Major isotopic contribution.

[c] Not present or not detected.

[w] Very weak compared to Ibb.

[s] Extra strong compared to Ibb.

TABLE XVc. Partial mass spectra of mixed SCTASi derivatives of thymidine (I).[a]

#	Assignment	Compound #: Ibc O-Substituent Y(5'): TBDMSi Z(3'): TMTBSi	Icb TMTBSi TBDMSi	Ibd TBDMSi TMIPSi	Idb TMIPSi TBDMSi	Icd TMTBSi TMIPSi	Idc TMIPSi TMTBSi
1.	M	496 (0)	496 (0)	482 (0.2)	482 (0.5)	508 (0)	508 (0)
3a.	$M-R_y$	439 (8.7)	439 (12.)	425 (3.1)	439 (1.3)	451 (10.)	465 (2.9)
3b.	$M-R_z$			439 (0.6)	425 (15.)	465 (3.4)	451 (14.)
4a.	$M-R_y-CH_2O$	409 (0.6)	409 (0.2)	395 (0.1)[b]	409 (0)	421 (0)	435 (0)
4b.*	$M-R_z-CH_2O$			409 (0.2)	395 (0.4)[b]	435 (0)	421 (0.5)[b]
5a.	$M-R_y-C_2H_4O(44)$	395 (0)	395 (1.0)	381 (0)	395 (0.4)[b]	407 (0)	421 (0.5)[b]
5b.	$M-R_z-C_2H_4O(44)$			395 (0.1)[b]	381 (0.7)	421 (0)	407 (0)
6.	S	371 (0)	371 (0)	357 (0.4)	357 (0.7)	383 (0)	383 (0)
9a.	$B+X_2Si_y+ZOH$	341 (0)	341 (0.5)	327 (0)	341 (0.7)	353 (0)	367 (0)
9b.	$B+X_2Si_z+ZOH$			341 (0)	327 (1.0)	367 (0)	353 (0)
11.	$Y+Z+C_3H_3O_2(71)$	327 (0)	327 (0.3)	313 (0.7)[b]	313 (2.0)[b]	339 (0.2)[b]	339 (0.2)[b]

continued...

TABLE XVc. continued

#	Assignment	Compound #: Y(5'): Z(3'):	Ibc TBDMSi TMTBSi	Icb TMTBSi TBDMSi	Ibd TBDMSi TMIPSi	Idb TMIPSi TBDMSi	Icd TMTBSi TMIPSi	Idc TMIPSi TMTBSi
12a.	$M-R_y-BH$		313 (3.5)	313 (10.)	299 (1.5)	313 (2.0)[b]	325 (1.1)	339 (0.2)[b]
12b.	$M-R_z-BH$				313 (0.7)[b]	299 (8.4)	339 (0.2)[b]	325 (0.6)
13a.*	$M-R_y-ZOH$		281 (49.)	307 (57.)	281 (52.)	307 (17.)	307 (31.)	307 (7.2)
13b.	$M-R_z-YOH$		307 (1.5)	281 (1.9)	307 (0.8)	281 (2.2)		
14a.	J+Y		267 (0)	293 (0)	267 (0.2)	279 (0)	293 (0.3)	279 (0.3)
14b.*	J+Z		293 (0)	267 (8.2)[b]	279 (1.8)	267 (1.2)	279 (4.0)	293 (0.3)
15a.*	$M-R_y-ZOH-H_2O$		263 (2.8)	289 (3.3)	263 (1.9)	289 (0.9)	289 (2.3)	289 (0.8)
15b.	$M-R_z-YOH-H_2O$		289 (0.5)	263 (0)	289 (0.05)	263 (0)		
16a.*	$M-R_y-J$		287 (4.6)	287 (11.)	273 (4.7)	287 (2.7)	299 (5.1)	313 (0.9)
16b.	$M-R_z-J$				287 (0.4)	273 (1.2)	313 (0.2)	299 (0.5)
17a.*	B+H+Y		241 (4.0)	267 (8.2)[b]	241 (0.9)	253 (6.8)	267 (3.1)	253 (5.2)
17b.	B+H+Z		267 (0)	241 (0.7)[c]	253 (0.3)	241 (0.6)	253 (1.5)	267 (0.5)

continued...

TABLE XVc. continued

#	Assignment	Compound #: Y(5'): Z(3'):	Ibc TBDMSi TMTBSi	Icb TMTBSi TBDMSi	Ibd TBDMSi TMIPSi	Idb TMIPSi TBDMSi	Icd TMTBSi TMIPSi	Idc TMIPSi TMTBSi
18a.*	$M-R_y-J-CH_2O$		257 (3.8)	257 (6.7)	243 (5.0)	257 (2.4)	269 (7.4)	283 (1.1)
18b.	$M-R_z-J-CH_2O$				257 (0.3)	243 (2.3)	283 (0.5)	269 (2.2)
21a.*	S-ZOH		213 (14.)[b]	239 (7.3)	213 (15.)	225 (14.)	239 (7.2)	225 (12.)
21b.	S-YOH		239 (0.6)	213 (0.9)[b]	225 (0.7)	213 (4.2)	225 (2.9)	239 (1.2)
22.	$B+C_5H_6O(82)$		207 (2.3)	207 (7.7)	207 (0.7)	207 (10.)	207 (1.9)	207 (3.5)
23a.	$B+X_2Si_y+H_2O$		201 (1.1)	227 (2.7)	201 (2.2)[b]	227 (7.1)	227 (10.)[b]	227 (44.)[b]
23b.*	$B+X_2Si_z+H_2O$		227 (46.)	201 (23.)	227 (6.0)	201 (39.)[b]		
24a.	X_2Si_y+OZ		215 (1.5)	215 (1.9)	201 (2.2)[b]	215 (0.7)	227 (10.)[b]	241 (0.8)
24b.	X_2Si_z+OY				215 (1.3)[c]	201 (39.)[b]	241 (1.2)	227 (44.)[b]
25a.	$Y+C_3H_4O_2(72)$		187 (1.0)	213 (0.9)[b]	187 (0.9)	199 (2.2)	213 (2.2)	199 (4.4)
25b.*	$Z+C_3H_4O_2(72)$		213 (14.)[b]	187 (7.0)	199 (3.0)	187 (6.7)	199 (6.3)	213 (5.4)

continued...

TABLE XVc. continued

#	Assignment	Compound #: Y(5'): Z(3'):	Ibc TBDMSi TMTBSi	Icb TMTBSi TBDMSi	Ibd TBDMSi TMIPSi	Idb TMIPSi TBDMSi	Icd TMTBSi TMIPSi	Idc TMIPSi TMTBSi
26a.	$B+X_2Si_y$		183 (7.6)	209 (7.6)	183 (7.0)	209 (4.6)	209 (12.)	209 (8.6)
26b.	$B+X_2Si_z$		209 (3.7)	183 (6.3)	209 (1.9)	183 (6.9)		
27.	$B+C_3H_4(40)$		165 (7.5)	165 (7.0)	165 (3.6)	165 (3.6)	165 (6.6)	165 (4.1)
28a.	$M-R_y-ZOH-BH$		155 (8.8)[b]	181 (9.0)	155 (7.2)[b]	181 (4.9)	181 (11.)	181 (8.1)
28b.	$M-R_z-YOH-BH$		181 (4.6)	155 (9.4)[b]	181 (1.8)	155 (7.2)[b]		
30a.*	$YOCH_2$		145 (100.)	171 (100.)	145 (100.)	157 (100.)[b]	171 (100.)	157 (100.)[b]
30b.	$ZOCH_2$		171 (18.)	145 (11.)	157 (16.)[b]	145 (5.3)	157 (28.)[b]	171 (10.)
33a.	$X_2Si_y+C_3H_5O_2(73)$		131 (1.7)	157 (2.0)	131 (1.3)	157 (100.)[b]	157 (28.)[b]	157 (100.)[b]
33b.*	$X_2Si_z+C_3H_5O_2(73)$		157 (5.9)	131 (9.2)	157 (16.)[b]	131 (9.0)		
34a.	$X_2Si_y+C_3H_3O_2(71)$		129 (9.1)	155 (9.4)[b]	129 (6.9)	155 (7.2)[b]	155 (11.)	155 (7.1)
34b.	$X_2Si_z+C_3H_3O_2(71)$		155 (8.8)[b]	129 (7.9)	155 (7.2)[b]	129 (5.1)		

continued...

TABLE XVc. continued

#	Assignment	Compound #: Y(5'): Z(3'):	Ibc TBDMSi TMTBSi	Icb TMTBSi TBDMSi	Ibd TBDMSi TMIPSi	Idb TMIPSi TBDMSi	Icd TMTBSi TMIPSi	Idc TMIPSi TMTBSi
36.	B+2H		127 (5.3)[b]	127 (3.4)[b]	127 (16.)[b]	127 (11.)[b]	127 (28.)[b]	127 (14.)[b]
37a.	Y^+		115 (20.)[b]	141 (3.2)	115 (21.)[b]	127 (11.)[b]	141 (5.1)	127 (14.)[b]
37b.	Z^+		141 (5.9)	115 (42.)[b]	127 (16.)[b]	115 (56.)[b]	127 (28.)[b]	141 (5.4)
40a.	$X_2Si_y+C_2H_3O$(43)		101 (5.9)[b]	127 (3.4)[b]	101 (3.9)[b]	127 (11.)[b]	127 (28.)[b]	127 (14.)[b]
40b.	$X_2Si_z+C_2H_3O$(43)		127 (5.3)[b]	101 (5.5)[b]	127 (16.)[b]	101 (4.9)[b]		
43a.*	$HX_2Si_y+OCH_2$		89 (62.)	115 (42.)[b]	89 (56.)	115 (56.)[b]	115 (60.)	115 (56.)
43b.	$HX_2Si_z+OCH_2$		115 (20.)[b]	89 (44.)	115 (21.)[b]	89 (16.)		
44.	C_5H_5O or D		81 (49.)	81 (68.)	81 (39.)	81 (45.)	81 (59.)	81 (36.)
45a.	X_2Si_y+OH		75 (5.3)	101 (5.5)[b]	75 (2.8)	101 (4.9)[b]	101 (12.)	101 (11.)
45b.	X_2Si_z+OH		101 (5.9)[b]	75 (6.3)	101 (3.9)[b]	75 (5.1)		

continued...

TABLE XVc. continued

#	Assignment	Compound #:	Ibc	Icb	Ibd	Idb	Icd	Idc
		Y(5'):	TBDMSi	TMTBSi	TBDMSi	TMIPSi	TMTBSi	TMIPSi
		Z(3'):	TMTBSi	TBDMSi	TMIPSi	TBDMSi	TMIPSi	TMTBSi
46a.	$CH_3X_2Si_y$		73 (35.)	99 (8.3)	73 (14.)	99 (10.)	99 (40.)	99 (28.)
46b.	$CH_3X_2Si_z$		99 (11.)	73 (43.)	99 (16.)	73 (26.)		
	Base Peak as $\%\Sigma_{50}$		[12.79]	[12.00]	[16.58]	[13.08]	[10.27]	[11.93]

[a] Results are expressed as m/e and (Rel. Int. %).

[b] Overlap of ion types.

[c] Significant interference by isotopic overlap.

[*] Intensities indicate that this is the predominant species of the two possibilities.

TABLE XVd. Mass spectral data for mixed TMSi/SCTASi derivatives of thymidine (I) and deoxyadenosine (V)[a].

#	Ion Type [Assignment][d]	Ibe	Ieb	Ibf	Ifb	Ice	Iec	Ide	Ied	Vbea	Veba
	Compound #:	Ibe	Ieb	Ibf	Ifb	Ice	Iec	Ide	Ied	Vbea	Veba
	O-Substituents Y(5'):	TBDMSi	TMSi	TBDMSi	dTMSi	TMTBSi	TMSi	TMIPSi	TMSi	TBDMSi	TMSi
	Z(3'):	TMSi	TBDMSi	dTMSi	TBDMSi	TMSi	TMTBSi	TMSi	TMIPSi	TMSi	TBDMSi
1.	M	428		437		454		440		437	
		(0)	(0)	(0)	(0)	(0.2)	(0.1)	(0.9)	(1.0)	(1.5)	(11.)
3.	M-R	371		380		397		397		380	
		(3.5)	(4.7)	(3.7)	(4.8)	(7.6)	(6.5)	(2.6)	(3.1)	(44.)	(48.)
4.	M-R-CH_2O	341		350		367		367		350	
	[M-R_z-CH_2O]	(0)	(0.3)	(0)	(0.5)	(0)	(1.1)	(0)	(0.4)	(0)	(0.8)
5.	M-R-C_2H_4O(44)	327		336		353		353		336	
		(0)	(0.5)	(0)	(0.6)	(0.3)	(0.3)	(0)	(0.1)	(0)	(0)
6.	S	303		312		329		315		303	
		(1.1)	(0.7)	(0.3)	(0.5)	(0.6)	(0.6)	(2.5)	(4.1)	(0)	(0)
9.	B+X_2Si+TMSiOH	273		282		299		299		282	
	[B+X_2Si_z+YOH]	(0)	(0.4)	(16.[c])	(0.6)	(0)	(0.3)	(1.1)	(0.1)	(0)	(2.0)
11.	RX_2Si+TMSi+$C_3H_3O_2$(71)	259		268		285		271[b]		259	
		(1.1)	(0.5)	(1.3)	(0.9)	(0.8)	(0.5)	(4.5)	(3.8[c])	(9.1)	(4.5)
12.	M-R-BH	245		254		271		271[b]		245	
		(4.5)	(10.)	(7.3)	(12.)	(5.3)	(4.2)	(4.5)	(3.8[c])	(17.)	(21.)

continued...

TABLE XVd. cont'd

	Ibe	Ieb	Ibf	Ifb	Ice	Iec	Ide	Ied	Vbea	Veba
Y(5'):	TBDMSi	TMSi	TBDMSi	dTMSi	TMTBSi	TMSi	TMIPSi	TMSi	TBDMSi	TMSi
Z(3'):	TMSi	TBDMSi	dTMSi	TBDMSi	TMSi	TMTBSi	TMSi	TMIPSi	TMSi	TBDMSi
13. M-R-TMSiOH [M-R_y-ZOH]	281 (55.)	(2.3)	281 (76.)	(0.9)	307 (72.)	(2.0)	307 (22.)	(0.3)	290 (23.)	(3.3)
14a. J+TMSi	225 (12.)	(0.2)	234 (21.)	(0.2)	225 (28.)	(1.3)	225[b] (33.)	(1.0)	234 (27.)	(2.6)
14b. J+RX_2Si	267 (0)	(0.2)	267 (0.4)	(0.4)	293 (0)	(0)	279 (0.3)	(0.6)	276 (0)	(0.2)
15. M-R-TMSiOH-H_2O [M-R_y-ZOH-H_2O]	263 (2.9)	(0.2)	263 (6.6)	(0.3)	289 (3.4)	(0)	289 (1.7)	(0.1)	272 (0.7)	(0)
16. M-R-J [M-R_y-J]	219 (7.9)	(0.9)	228 (9.7)	(0.8)	245 (12.)	(0.6)	245 (5.2)	(0.3)	219 (74.)	(3.3)
17a. B+H+TMSi	199 (1.8)	(1.7)	208 (2.2[c])	(1.5[c])	199 (3.5)	(0.8)	199[b] (3.1)	(4.3)	208 (2.3)	(5.9)
17b. B+H+RX_2Si	241 (0.6)	(0)	241 (0.9)	(0.1)	267 (2.9)	(0)	253 (6.6)	(0)	250 (0.6)	(1.6)
18. M-R-J-CH_2O [M-R_y-J-CH_2O]	189 (23.)	(2.3)	198 (34.)	(2.5)	215 (26.)	(1.9)	215 (10.)	(0.9)	189 (100.)	(5.3)
21a. S-TMSiOH [S-ZOH]	213 (15.)	(0.3)	213 (19.)	(0.6)	239 (8.0)	(0.2)	225[b] (33.)	(1.0)	213 (1.8)	(1.7)
21b. S-RX_2SiOH	171 (2.4)	(27.)	180 (3.4)	(23.)	171[b] (100.)	(35.)	171 (4.6)	(56.)	171 (2.4)	(8.7)
22. B+C_5H_6O(82)	207 (1.4)	(6.7)	207 (7.5)	(5.0)	207 (1.9)	(3.3)	207 (1.1)	(0.9)	216 (1.5[c])	(4.9)

continued...

TABLE XVd. cont'd

	Ibe	Ieb	Ibf	Ifb	Ice	Iec	Ide	Ied	Vbea	Veba
Y(5'):	TBDMSi	TMSi	TBDMSi	dTMSi	TMTBSi	TMSi	TMIPSi	TMSi	TBDMSi	TMSi
Z(3'):	TMSi	TBDMSi	dTMSi	TBDMSi	TMSi	TMTBSi	TMSi	TMIPSi	TMSi	TBDMSi
23. $B+X_2Si+H_2O$ [$B+X_2Si_z+H_2O$]	201 (1.4)	(29.)	201 (4.4)	(40.)	227 (5.1)	(46.)	227 (9.9)	(20.)	210 (1.5)	(100.)
24. $TMSiOSiX_2$	147 (17.)	(16.)	156 (22.[c])	(19.)	173 (14.)	(8.6)	159 (6.9[c])	(1.5)	147 (26.)	(36.)
25a. $TMSi+C_3H_4O_2(72)$ [Z+72]	145[b] (100.)	(1.2)	154 (22.)	(1.0)	145 (19.)	(1.7)	145 (15.)	(8.4)	145[b] (14.)	(2.4)
25b. $RX_2Si+C_3H_4O_2(72)$	187 (0.9)	(5.7)	187 (1.3[c])	(5.9)	213 (1.2)	(2.0)	199[b] (3.1)	(4.3)	187 (0.5)	(4.0)
26. $B+X_2Si$	183 (10.)	(4.6)	183 (15.)	(6.1)	209 (10.)	(3.1)	209 (5.9)	(2.0)	192 (6.8)	(8.0)
27. $B+C_3H_4(40)$	165 (5.6)	(1.6)	165 (8.4)	(2.0)	165 (8.3)	(2.4)	165 (3.4)	(1.1)	174 (5.5)	(3.9)
28. M-R-TMSiOH-BH	155 (9.6)	(4.4)	155 (18.[c])	(6.1)	181 (11.)	(3.4)	181 (4.8)	(2.6)	155 (15.)	(18.)
30a. $TMSiOCH_2$ [$YOCH_2$]	103 (33.)	(100.)	112 (40.)	(100.)	103 (43.)	(100.)	103 (16.)	(100.)	103 (67.)	(77.)
30b. RX_2SiOCH_2	145[b] (100.)	(1.2)	145 (100.)	(0.6)	171[b] (100.)	(35.)	157[b] (100.)	(8.1)	145[b] (14.)	(2.4)
33. $X_2Si+C_3H_5O_2(73)$	131 (3.1)	(9.6)	131 (2.1)	(7.4)	157 (4.7)	(4.2)	157[b] (100.)	(8.1)	131 (3.7)	(11.)
34. $X_2Si+C_3H_3O_2(71)$ [X_2Si_y+71]	129 (18.)	(2.9)	129 (12.)	(2.5)	155 (8.5)	(2.7)	155 (4.9)	(3.6)	129 (22.)	(7.7)

continued...

TABLE XVd. cont'd

	Ibe	Ieb	Ibf	Ifb	Ice	Iec	Ide	Ied	Vbea	Veba
Y(5'):	TBDMSi	TMSi	TBDMSi	dTMSi	TMTBSi	TMSi	TMIPSi	TMSi	TBDMSi	TMSi
Z(3'):	TMSi	TBDMSi	dTMSi	TBDMSi	TMSi	TMTBSi	TMSi	TMIPSi	TMSi	TBDMSi
36. B+2H	127 (4.5)	(1.7)	127 (6.7[c])	(1.7)	127[b] (4.2)	(2.2)	127[b] (7.9)	(11.)	136 (52.)	(84.)
37. RX_2Si	115 (6.0)	(7.9)	115 (7.0)	(6.7)	141 (3.3)	(3.5)	127[b] (7.9)	(11.)	115 (4.7)	(9.2)
40. $X_2Si+C_2H_3O(43)$	101 (4.1)	(3.1)	101 (3.2)	(2.6)	127[b] (4.2)	(2.2)	127[b] (7.9)	(11.)	101 (5.9)	(6.0)
43a. HMe_2SiOCH_2[e]	89 (63.)	(2.7)	98 (0.4)	(0.2)	89 (1.6)	(0.3)	89 (0.5)	(0.4)	89 (22.)	(4.5)
43b. HX_2SiOCH_2 $[HX_2Si_yOCH_2]$			89 (52.)	(1.4)	115 (37.)	(2.2)	115 (38.)	(2.2)		
44. C_5H_5O [D]	81 (49.)	(28.)	81 (43.)	(21.)	81 (76.)	(18.)	81 (13.)	(7.3)	81 (9.4)	(53.)
45. X_2SiOH	75 (6.8)	(4.7)	75 (5.0)	(3.8)	101 (5.8)	(3.3)	101 (3.3)	(2.7)	75 (7.5)	(9.5)
46a. CH_3X_2Si	73 (44.)	(24.)	73 (13.)	(11.)	99 (7.8)	(4.7)	99 (6.5)	(7.8)	73 (57.)	(50.)
46b. TMSi			82 (49.)	(15.)	73 (50.)	(17.)	73 (18.)	(8.3)		
Base Peak as $\%\Sigma_{50}$	[14.20]	[23.92]	[11.01]	[22.60]	[10.95]	[23.88]	[16.54]	[24.29]	[9.78]	[10.27]

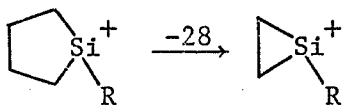
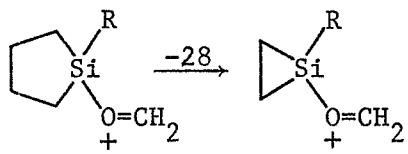
[a] Results expressed as m/e and (Rel.Int.%) for isomer pairs, as measured on Finnigan instrument at 70eV; [b] Ion type overlap; [c] Significant isotopic contribution from ion type of lower mass; [d] [Assignment] of major species as deduced from relative intensity data; [e] Me = CD_3 for dTMSi derivative.

TABLE XVI. Metastable transitions in the mass spectra of SCTASi derivatives of 2'-deoxynucleosides.[a]

Transition Supported	Compound: Ibb	Vbba	Icc	Idd
[M-R] → (M-R-RX_2SiOH) or A	413 → 281 (191.1, 191m)	422 → 290 (199.3, 199.5m)	465 → 307 (202.7, 203m)	451 → 307 (209.0, 209w)
A → [A-H_2O]	281 → 263 (246.2, 246m)		307 → 289 (272.1, 272m)	
[M-R] → [M-R-BH] or G		422 → 287 (195.2, 195w)		
[M-R] → [M-R-J]		422 → 261 (161.4, 161.5m)		
[M-R-J] → [M-R-J-CH_2O]	261 → 231 (204.4, 205vw)	261 → 231 (204.4, 204.5m)	313 → 283 (255.9, 256w)	
[M-R-J] → RX_2SiOCH_2		261 → 145 (80.6, 81m)	313 → 171 (93.4, 93.5w)	
K → RX_2SiOCH_2	213 → 145 (98.7, 99w)			
[RX_2SiOCH_2] → H(X_2SiOCH_2)	145 → 89 (54.6, 54.6s)	145 → 89 (54.6, 54.7s)	171 → 115 (77.3, 77.3s)	157 → 115 (84.2, 84.2s)
RX_2Si → CH_3X_2Si	115 → 73 (46.3, 46.4m)	115 → 73 (46.3, 46.4m)		

continued...

TABLE XVI. continued

Transition Supported	Compound: Ibb	Vbba	Icc	Idd
$RX_2Si \rightarrow HX_2Si$		115 → 59 (30.5, 30.5vw)		
	(NA)	(NA)	141 → 113 (90.6, 90.5w)	127 → 99 (77.2, 77.2m)[b]
	(NA)	(NA)	171 → 143 (119.6, 119.5w)	157 → 129 (106.0, 106w)

[a] See footnote a in Table XIIIa

[b] May also be assigned to $RX_2Si \rightarrow CH_3X_2Si$

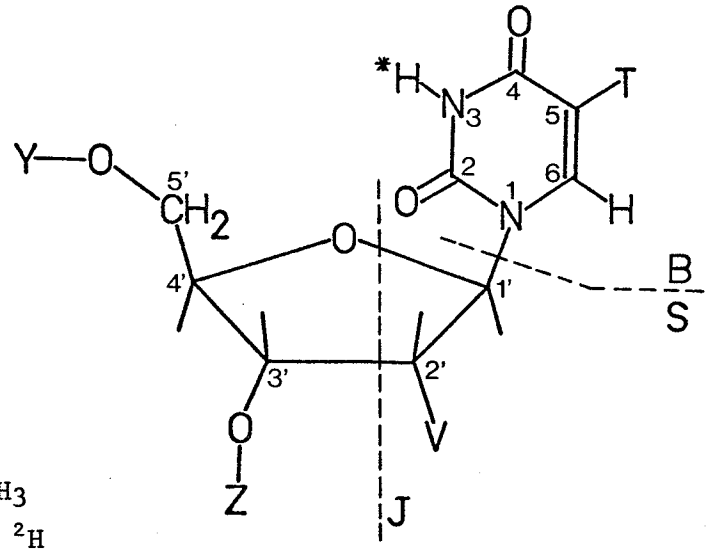
NA = not applicable

2. Discussion of Fragmentation Mechanisms

This section presents the proposed assignments of ion types, and mechanisms for their formation, for the partial, mixed, and fully O-silylated derivatives of deoxynucleosides. Since many of the fragmentation mechanisms are applicable to the ribonucleoside series, schemes have been generalized so that reference from later sections can be made. Scheme 19 illustrates the designation used for basic structural features. The symbols B and S are commonly used in the literature to represent the base and sugar fragments, respectively. J is proposed to represent BC_2H_2V, a portion of the nucleoside structure involved in several fragmentations. For most of the fragmentation schemes, the base is given as thymine ($T = CH_3$) or uracil ($T = H$). Most mechanisms apply to cytosine, adenine, and guanine nucleosides as well, unless very specific base functions are required. In the crystalline state, and in solution, thymidine and deoxyuridine exist predominantly in the diketo form (as in Scheme 19) rather than in either of the tautomeric enol alternatives (84). On vaporization, it is possible that the proportions of the less polar enol forms may increase, but for interpretation of mass spectra, there was little advantage found in considering these latter forms to be present. Also, either syn (as in Scheme 19) or anti conformations of the base are probable in ionic species due to their high energy content and the low energy barrier to rotation about the glycosyl bond.

The interpretations that follow are based upon a good deal of evidence, namely:

(a) High resolution measurements and subsequent calculation of elemental compositions of all important ions for Iba, Iab, and Ibb. (Tables XIIa and XVa)



Scheme 19. T = H or CH_3

H* = ^1H or ^2H

V = H, for deoxynucleosides
OW, for ribonucleosides

Y,Z,W = substituents a (^1H or ^2H) and b to f (Scheme 3)

(b) Metastable peaks that support proposed pathways (indicated by an asterisk (*) in schemes). (Tables XIIIa, XIIIb, and XVI)

(c) Deuterium labelling of labile hydrogens on free hydroxyls and amines of Iba, Iab, and Ibb. (Tables XIIa and XVa)

(d) Effective labelling of silicon substituents, R and X_2, through the examination of TBDMSi, TMTBSi, and TMIPSi derivatives (Tables XIIb XVb), as well as mixed SCTASi and TMSi/SCTASi derivatives (Tables XVc and XVd).

(e) Effective labelling of the base by examination of various nucleosides. (Thymidine and deoxyuridine were especially useful) (Tables XIIb and XVb). Correlations between base structures and fragment ion intensities also agree with proposed mechanisms.

(f) The interpretations are consistent over the entire series examined: partial, mixed, and fully protected derivatives of various nucleosides (including the ribonucleosides).

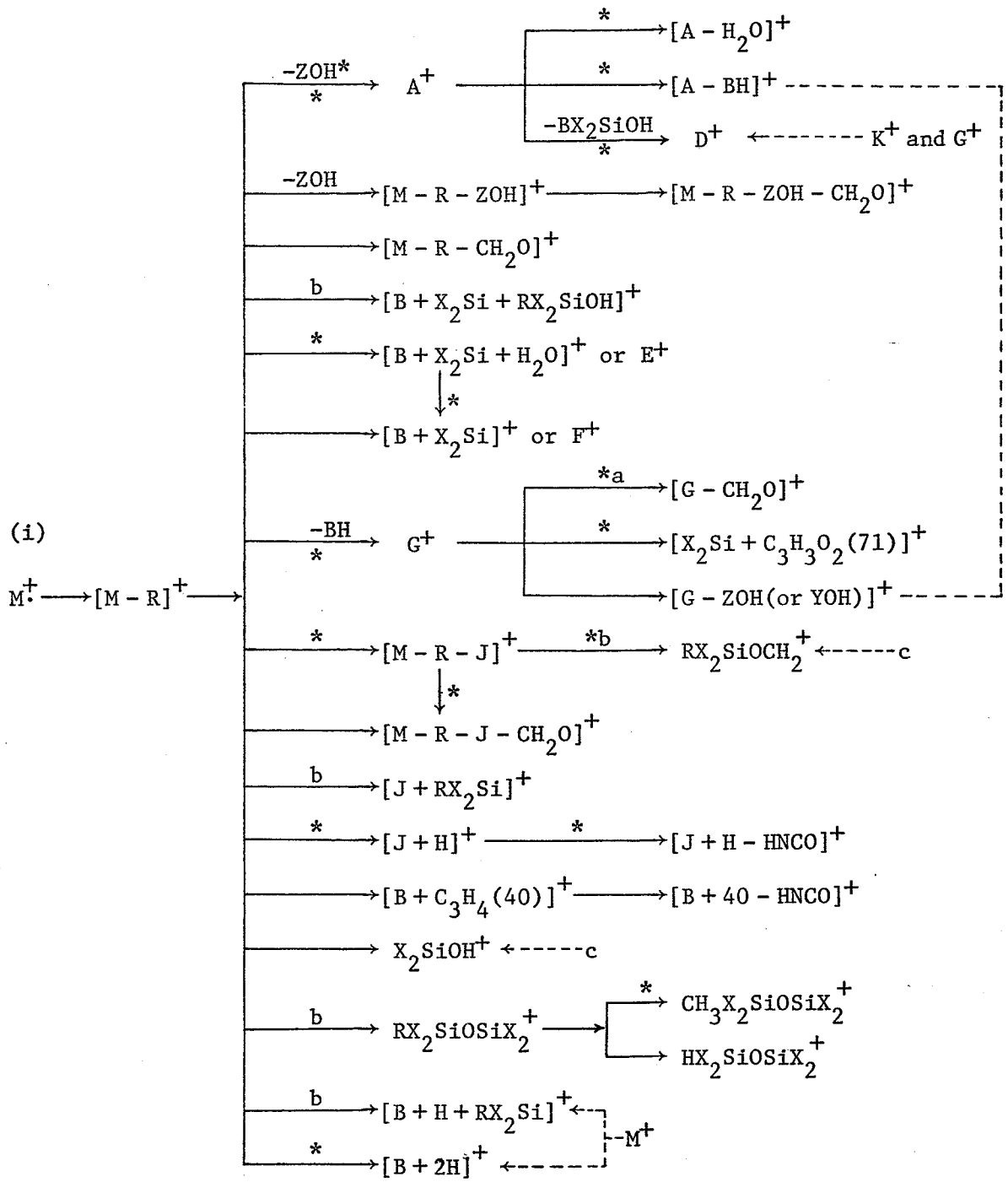
In the following discussion, principal ion types observed in the spectra of SCTASi derivatives of deoxynucleosides are described in an order related to their proposed precursors and mechanisms of formation. One mechanism is proposed in most cases; others could be depicted, but usually are unsuitable due to steric or energetic considerations (e.g., ring-opening to produce diradical species and subsequent rearrangement is a mechanism that can be suggested for many ions, but this is not expected to be energetically favorable when sterically-compatible concerted rearrangements are available). Ions are named according to their principal composition as determined by labelling experiments. If there is a certain amount of non-specificity, alternate compositions and mechanisms are proposed when important. Tables XII and XV give detailed assignments for most ions observed. Easy reference to these tables is made possible by numbering the ions according to line numbers in Tables XII and XV. The m/e values for Iba (or Iab) and Ibb are included to allow fast reference to mass spectra.

Scheme 20 illustrates the proposed genesis of important ions for SCTASi derivatives of deoxynucleosides. Many pathways (indicated by *) are supported by metastable peaks observed for various compounds (Tables XIII and XVI).

$\underline{M^+}$ [XII.1, Iba - m/e356; XV.1, Ibb - m/e470]

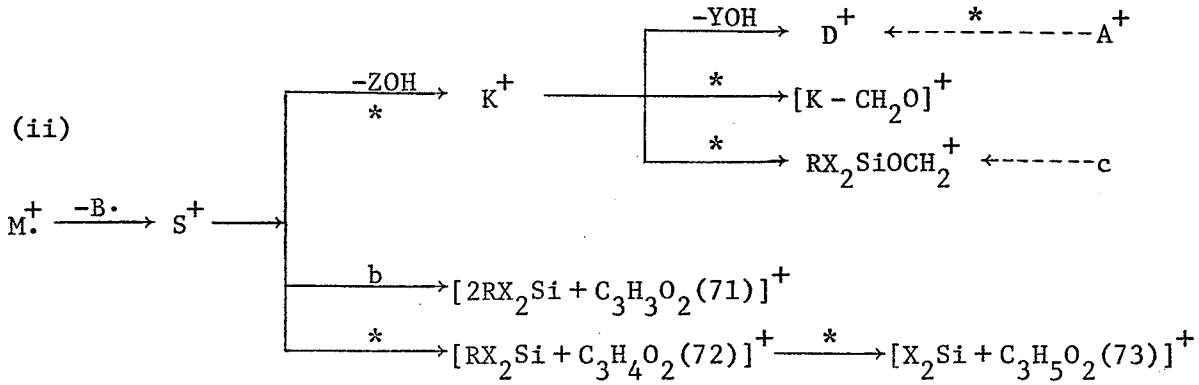
The molecular ion is undetectable for most derivatives, but does occur in low abundance for Idd, IIIab, IIIba, IVbb, Vaba, Vbaa, and Vbba. Its presence in the spectra of derivatives of III-V reflects the greater chance for ionization of their more aromatic bases (versus those of I and II) and subsequent stabilization of the molecular ion. Its occurrence for Idd is due to the lower tendency for $[M-R]^+$ formation in TMIPSi

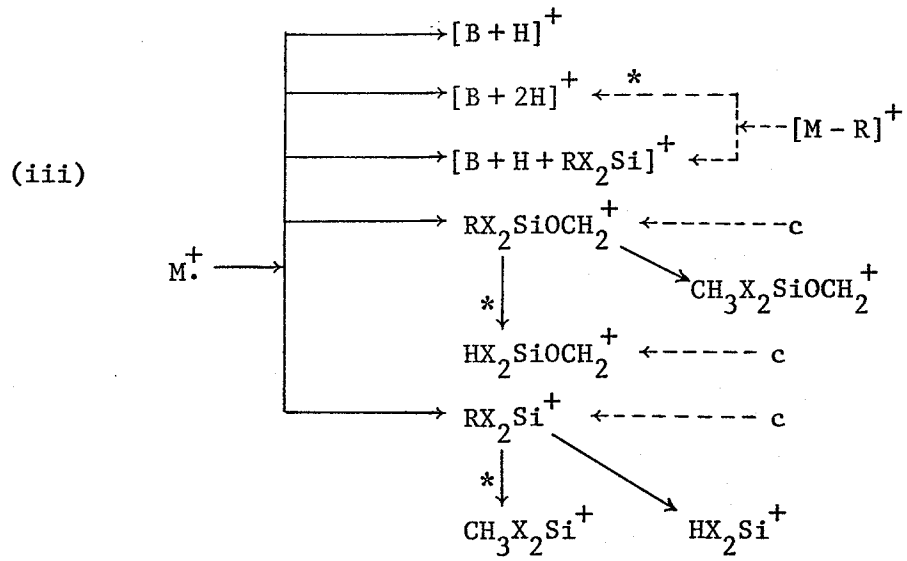
Scheme 20. Summary of the genesis of ions in the mass spectra of SCTASi derivatives of deoxynucleosides as determined by metastable evidence (*) and labelling studies. Some of the ions are observed only for partial silyl derivatives ([a]) or for fully silylated derivatives ([b]). Other sources are possible for some ([c]).



continued....

Scheme 20 (continued)



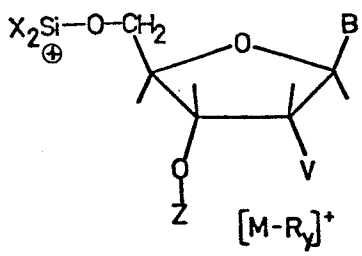


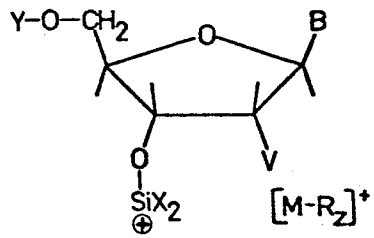
derivatives. The molecular weights of these derivatives are best determined from the $[M-R]^+$ ion.

a) Ions formed by charge localization on silicon by loss of an alkyl group.

$[M-CH_3]^+$ [XII.2, Iba - m/e341; XV.2, Ibb - m/e455]

This ion is observed at low intensity for TBDMSi and mixed TMSi/SCTASi derivatives. Its absence for TMTBSi and TMIPSi derivatives indicates that it is formed by elimination of a methyl radical from a TBDMSi or TMSi group. The decomposition of $[M-CH_3]^+$ results in a series of fragments of low abundance that parallel those produced from $[M-R]^+$. These are identified in Tables XII and XV and are not discussed any further.





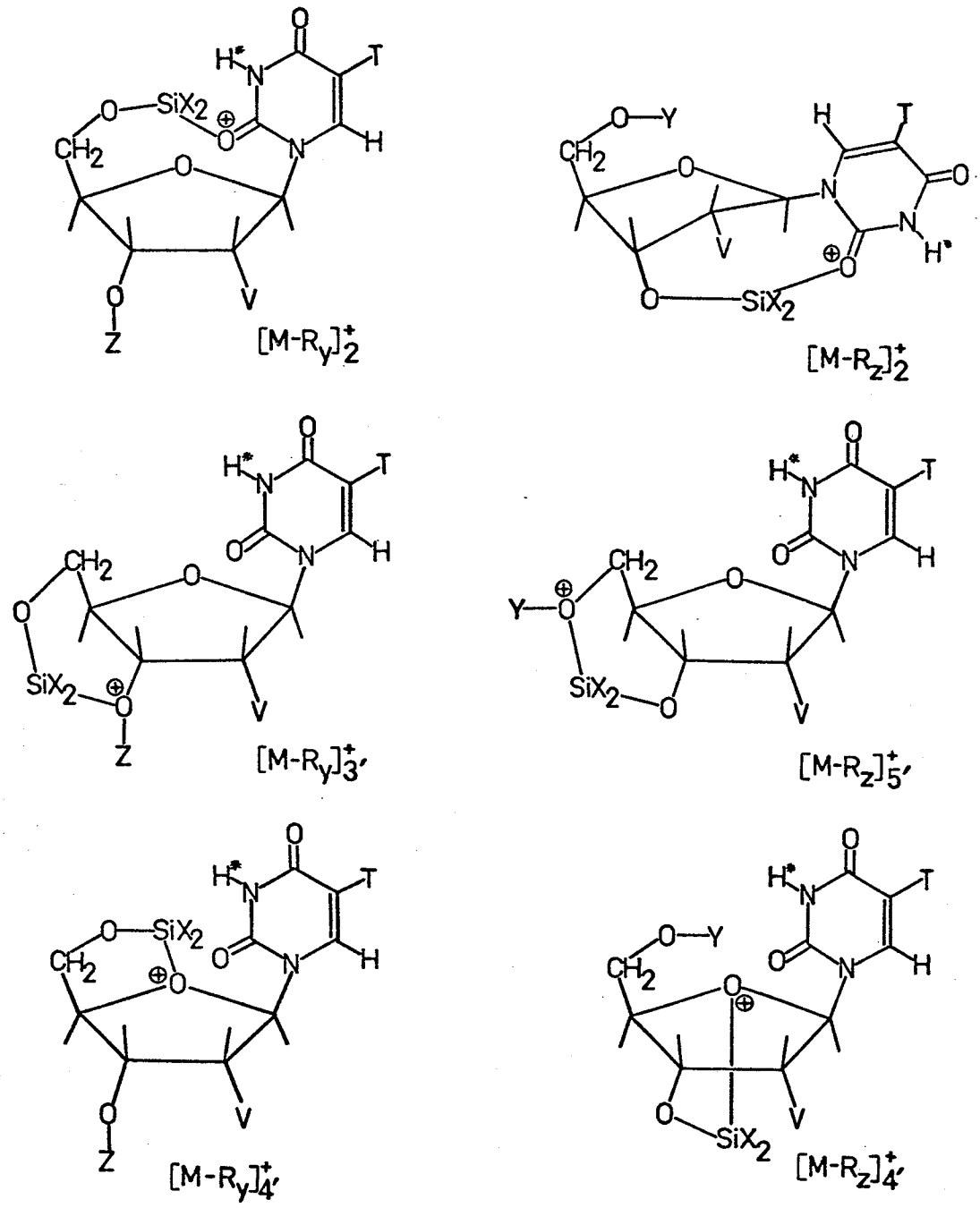
$[M-R]^+$ [XII.5, Iba - m/e299; XV.3, Ibb - m/e413]

An intense $[M-R]^+$ ion is observed in all spectra. There are two different species possible: $[M-R_y]^+$ and $[M-R_z]^+$, where the subscripts indicate the origin of the R substituent as either the 5'- or 3'-O-silyl group respectively. For partial derivatives, only one species can be present, but for the bis-SCTASi derivatives both exist, although not necessarily in equal proportions. For mixed derivatives, the order of preference for R group elimination, tBu > iPr > Me, leads to a predominance of one species. The very different spectra of isomeric partial and mixed

silyl derivatives are due to the fact that $[M-R]^+$ is the ultimate precursor of most other ions and that each type of $[M-R]^+$ directs the further fragmentation of the ion along different pathways.

Fragmentation mechanisms are best rationalized in terms of an initial attack of the electrophilic "siliconium ion" upon an electron-dense center in the molecule to produce a cyclic "silyloxonium" ion. Then, a concerted rearrangement of electrons leads to a specific fragmentation. For deoxynucleoside derivatives, there are six cyclic silyloxonium ions possible as illustrated in Scheme 21. These have been determined by examining CPK molecular models in terms of steric accessibility of the interacting functions. For $[M-R_y]^+$, models indicate that the siliconium ion center can approach the 2, 3', or 4' oxygen atoms to within van der Waals distances. Similarly, for $[M-R_z]^+$, the 5' and 4' oxygens are quite accessible. The $[M-R_z]_2^+$ ion is more difficult to illustrate on paper, but models show that the silicon atom can approach O_2 almost to within van der Waals distances. For bond formation between these atoms, only slight distortion from the position of minimum potential energy of this conformation is required, without the development of large steric interferences. In an excited ion, therefore, it appears that the formation of $[M-R_z]_2^+$ is quite probable. The interaction of either siliconium ion with O_4 is very difficult unless ring-opening occurs, and therefore $[M-R_y]_4^+$ and $[M-R_z]_4^+$ need not be considered as precursors for major ions.

Whether these cyclic silyloxonium ions exist for very long or are just formed prior to fragmentation is not known, although it appears that they have fairly stable ion structures.

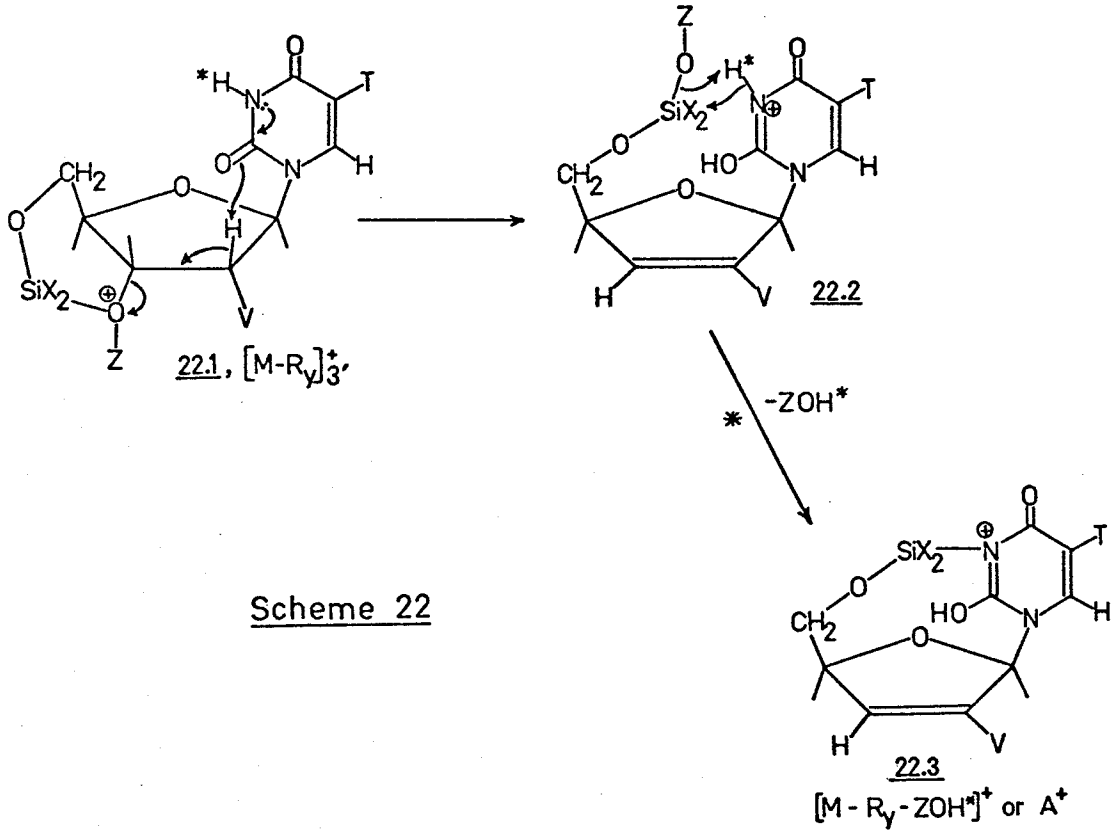


Scheme 21. Structures of cyclic silyloxonium ions of 2'-deoxynucleoside (V = H) derivatives. These are useful precursors in fragmentation schemes and are produced by the attack of a siliconium ion on sterically accessible electron-dense centers within the molecule.

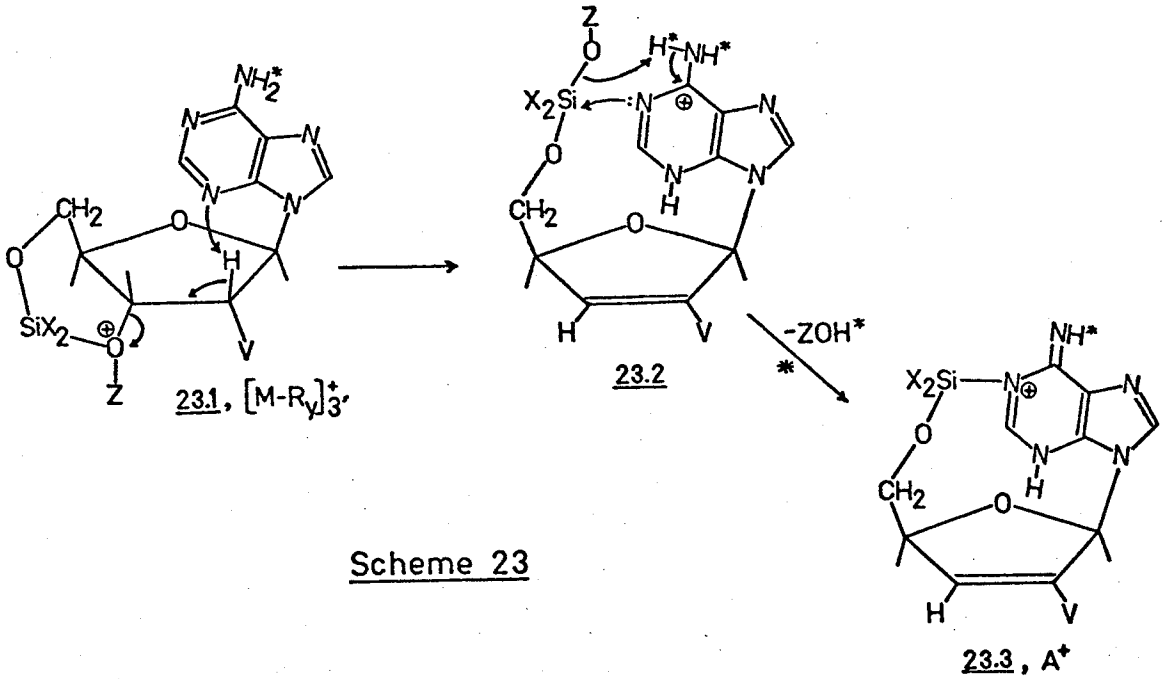
$[M - R_y - ZOH^*]^+$ or A^+ [XII.7 and XV.13; Iba and Ibb - m/e281]

This is one of the most useful ions in the spectra of partial and mixed derivatives of deoxynucleosides for identification of isomers. Thus, in the case of partial silyl derivatives, $[M - R - H_2O]^+$ is a prominent ion in the spectra of 5'-O-silyl compounds, but is very weak in the case of the 3'-O-silyl isomers. For the fully protected derivatives, A^+ is present in high abundance only when $Y = RX_2Si$. The loss of ZOH from $[M - R]^+$ is confirmed by metastable peaks in the spectra of most compounds where the ion is prominent. Labelling of the labile base hydrogen in Ibb, as well as the 3' hydroxyl hydrogen in Iba, indicates loss of ZOH^*.

In Scheme 22, a mechanism is proposed for this process, based on studies with CPK space-filling models. The models show that if the sugar ring remains intact, $O_{3'}$ does not come sufficiently close to the labile hydrogen of the base for hydrogen transfer to occur. On the other hand, as discussed previously, the $O_{3'}$ atom is readily accessible to the electrophilic 5'-O-siliconium ion so that the cyclic silyloxonium ion, $[M - R_y]^+_{3'}$, should be readily formed. Also, the $H_{2'}$ is in a favorable position for transfer to O_2 of the base via a six-membered cyclic transition state. In a concerted process, such a transfer would assist fission of the $C_{3'} - O_{3'}$ bond resulting in transfer of the OZ group to the silicon atom as shown by 22.2. This process is also facilitated by the ability of the base to accommodate the positive charge. Intermediate 22.2 may then readily lose ZOH^* to give A^+, with structure 22.3. This mechanism is also possible for other types of bases, as illustrated in Scheme 23 for deoxyadenosine derivatives, although it is not quite as favorable in this case as with thymine or uracil as shown by the lower intensity of A^+ relative to that of $[M - R]^+$.



Scheme 22



Scheme 23

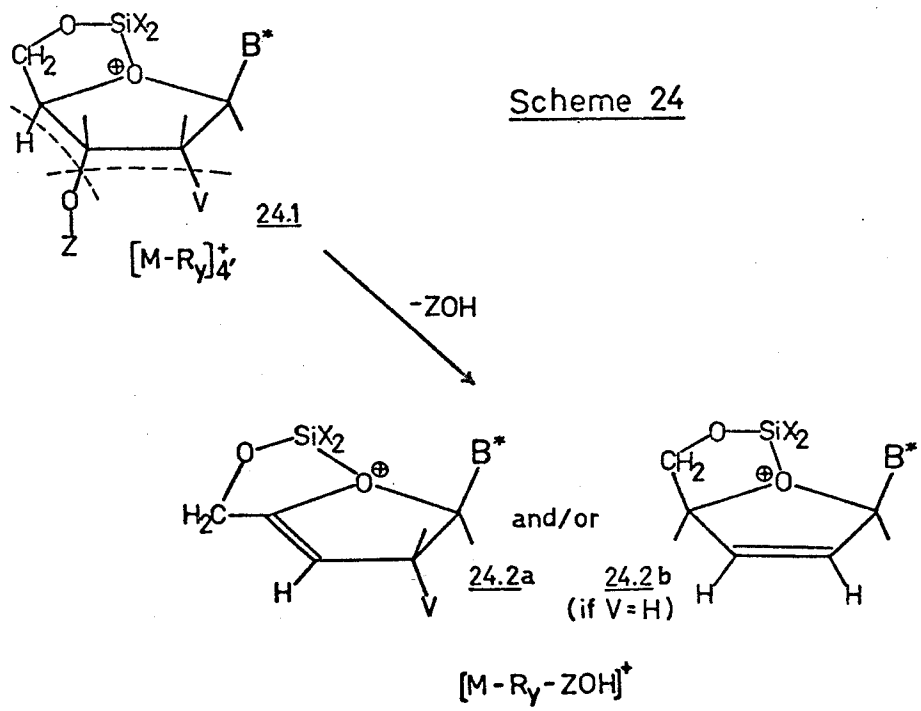
The apparently simple decompositions shown in Scheme 24, which eliminate ZOH or YOH, evidently compete unfavorably with the sequence 22.1 to 22.3, presumably due to lack of the appropriate initiating step and other more facile decompositions of silyloxonium ions 24.1 and 24.3. However, these mechanisms do explain a certain amount of non-specificity. Thus $[M-R-ZOH]^+$ would account for the 20% retention of the labile base hydrogen in $[M-R-H_2O]^+$ of Iba. In the case of 3'-O-silyl derivatives, the weak $[M-R-H_2O]^+$ corresponds to $[M-R-YOH]^+$ since labelling indicates retention of the labile base hydrogen. Similarly, the sequence 24.3 to 24.4 would account for the small peaks corresponding to $[M-R-YOH]^+$ for mixed silyl derivatives.

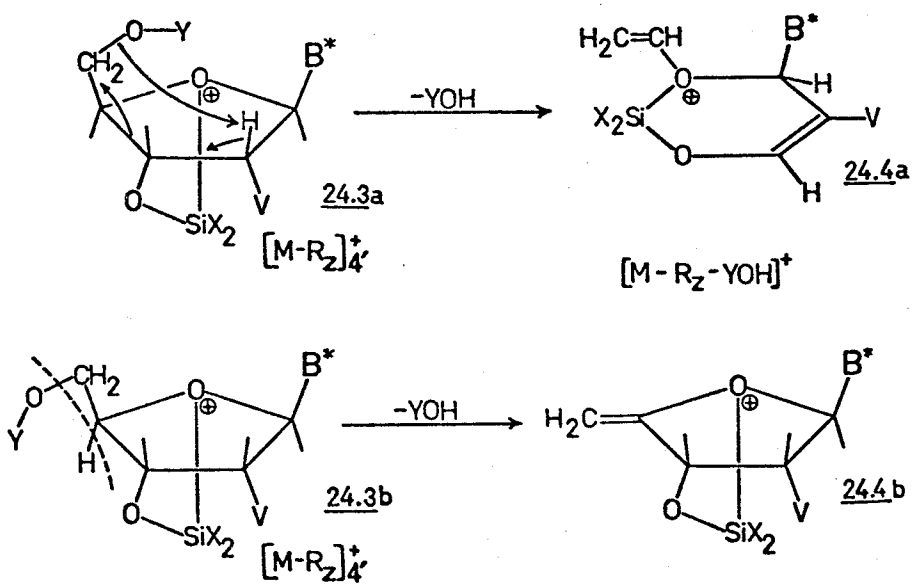
A^+ is the precursor for some other important ions in the spectra of SCTASi-deoxynucleosides.

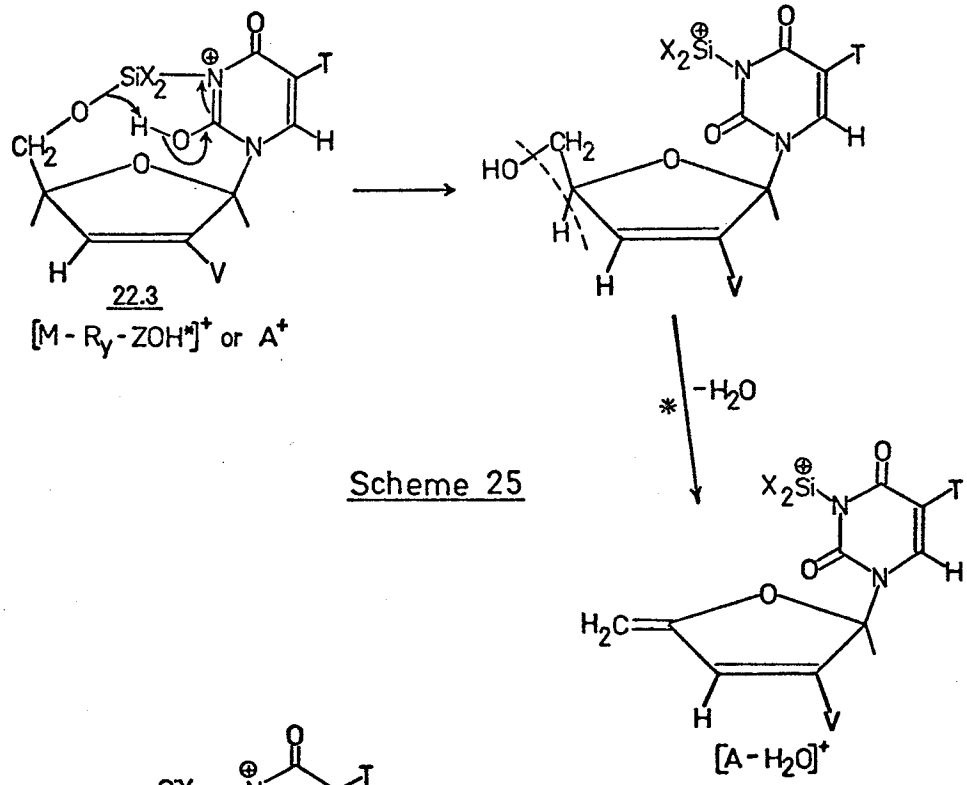
$[A-H_2O]^+$ [XII.10 and XV.15, Iba and Ibb - m/e263]

This ion is observed exclusively for compounds with a 5'-O-RX_2Si group, although it is quite weak for the purine nucleosides, IV and V. Metastable peaks in Iba, Ibb and Icc confirm A^+ as the precursor and labelling indicates no retention of labile hydrogens. The origin of the oxygen and two hydrogen atoms constituting the molecule of water lost is not known. Schemes 25 and 26 illustrate proposed mechanisms that could account for $[A-H_2O]^+$, and suggest loss of $H_{2'}$, $O_{5'}$, and $H_{4'}$. Models confirm that all rearrangements involve feasible conformations, although it is expected that the second mechanism would be more favorable sterically (but not necessarily energetically). The requirement of specific pyrimidine base functions would account for the low abundance of $[A-H_2O]^+$ for the purine nucleosides.

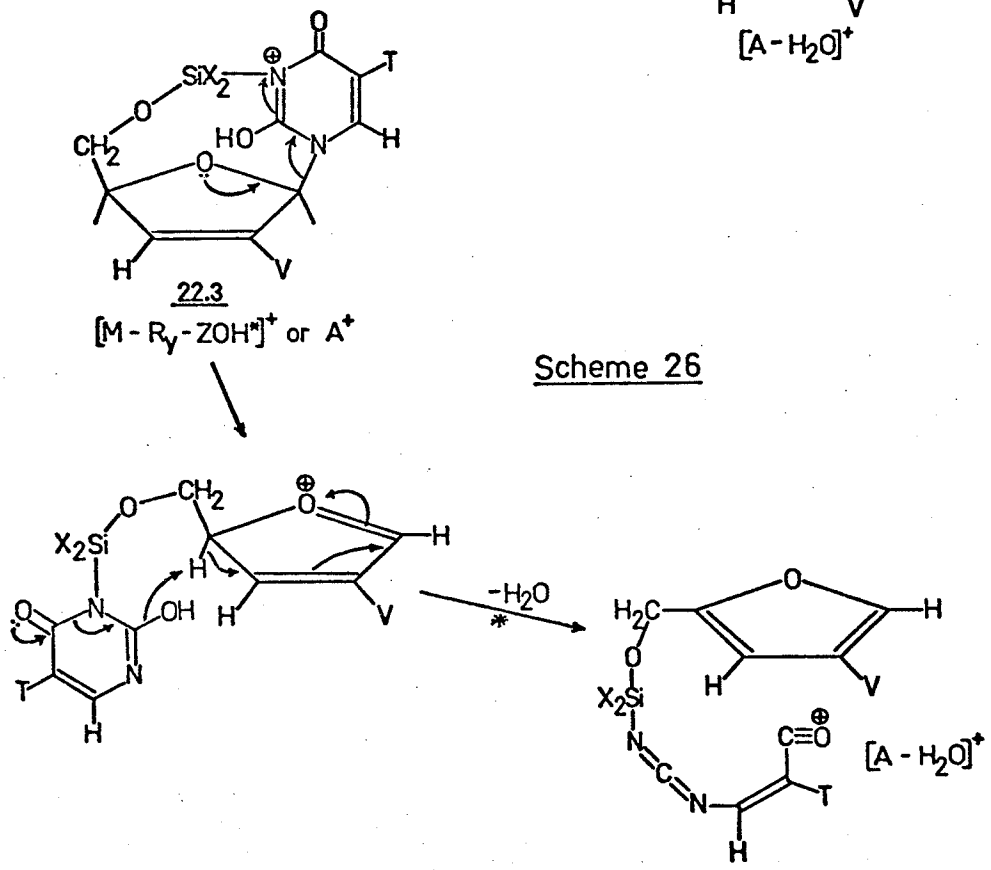
Scheme 24


24.1
$[M-R_y]^{+}_{4'}$
-ZOH
24.2a
and/or
24.2b
(if V=H)
$[M-R_y-ZOH]^{+}$

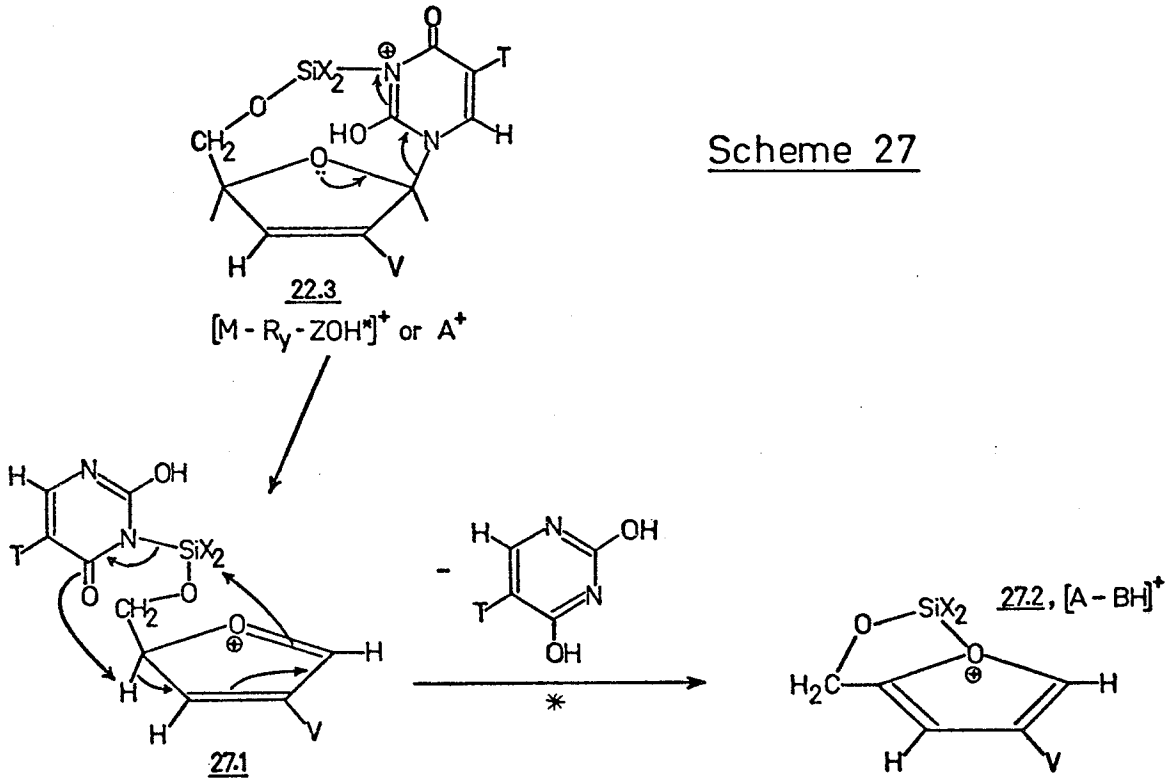

24.3a
$[M-R_z]^{+}_{4'}$
-YOH
24.4a
$[M-R_z-YOH]^{+}$
24.3b
$[M-R_z]^{+}_{4'}$
-YOH
24.4b



Scheme 25



Scheme 26



Scheme 27

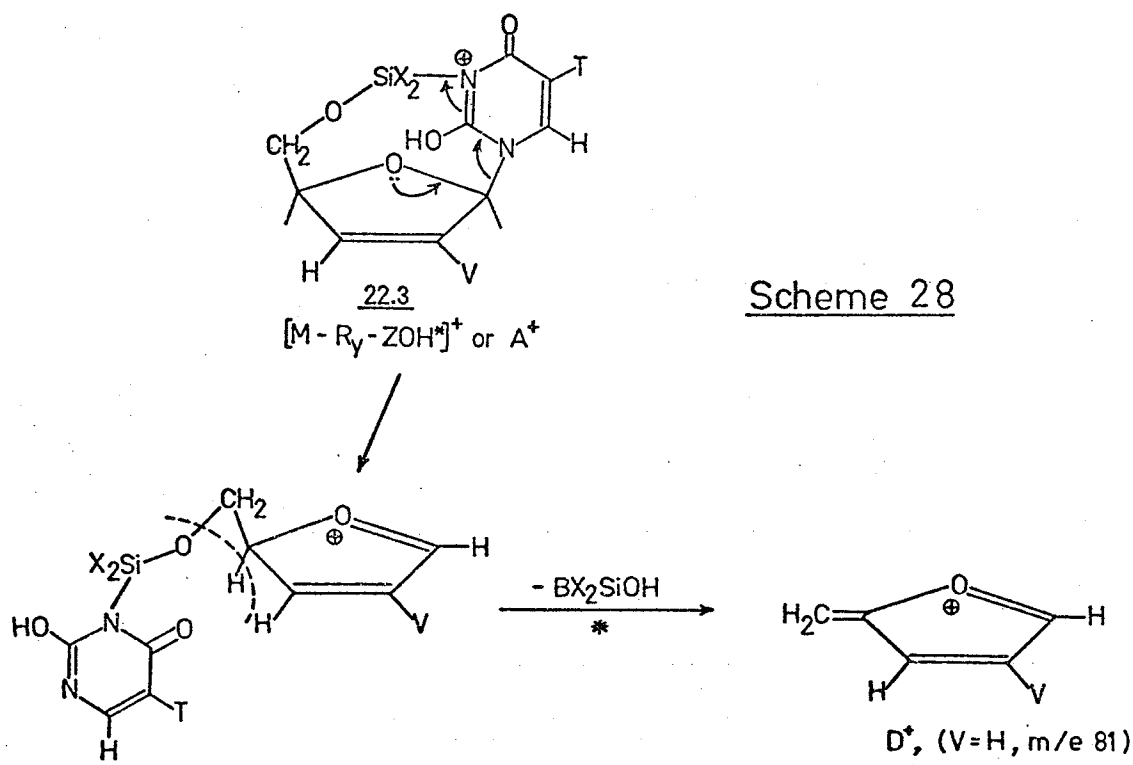
$[A - BH]^+$ [XII.33 and XV.28, Iba and Ibb - m/e155]

An ion corresponding to $[M - R - H_2O - BH]^+$ is observed for partial silyl derivatives, where it is more abundant for 5'-O-silyl isomers. For Vbaa, a metastable peak indicates formation from A^+; and d-labelling of Iba and Iab indicates no retention of labile hydrogens. In the case of fully protected derivatives, the corresponding ion $[M - R - RX_2SiOH - BH]^+$ is observed to be predominantly $[M - R_y - ZOH - BH]^+$. Scheme 27 presents a possible mechanism that involves formation of intermediate 27.1 by a favorable transfer of the positive charge from the base to the conjugated sugar residue, followed by rearrangement to $[A - BH]^+$ through a sterically favorable transition state.

However, this is not the only source for this ion. Some may be due to loss of ZOH from $[M - R_y - BH]^+$, and the small amount of non-specific fragmentation may be loss of YOH from $[M - R_z - BH]^+$.

$C_5H_5O^+$ or D^+ [XII.62 and XV.44, m/e81]

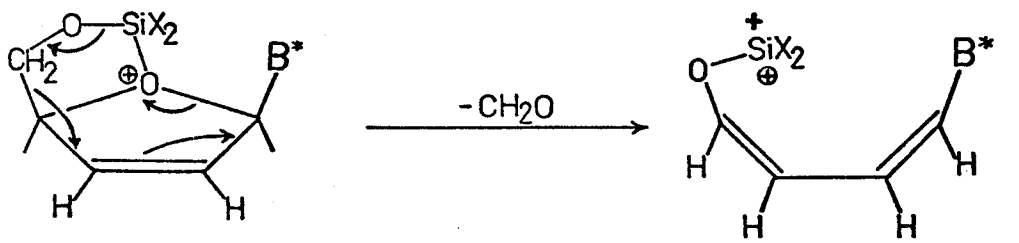
An ion of mass 81, $C_5H_5O^+$, is observed in all deoxynucleoside derivatives' spectra, but is of low intensity for ribonucleosides. However, there is an analog for the latter of composition $C_5H_4VO^+$, where V = OW (see Schemes 4 and 19). It is characteristically more abundant for those derivatives with a 5'-O-RX_2Si function (exceptions: Veba vs. Vbea). Its composition corresponds to $[S - ZOH - YOH]^+$, and as discussed later, a certain amount of this ion may be formed from S^+ or $[S - ZOH]^+$. Nevertheless, a metastable peak in Iba identifies A^+ as a precursor for D^+. Scheme 28 suggests a possible mechanism. An additional source of this fragment may be $[M - R_Z - BH]^+$ (see later), which would explain the greater abundance of m/e81 for Veba than for Vbea.



Scheme 28

$\underline{[M - R_y - ZOH - CH_2O]^+}$ [XII.11, Iba (and Ibb) - m/e251]

A weak ion corresponding to $[M - R - H_2O - CH_2O]^+$ is observed in the spectra of partial silyl derivatives, almost exclusively for 5'-O-SCTASi isomers, while the analogous ion, $[M - R - RX_2SiOH - CH_2O]^+$, is very weak in fully protected derivatives. There is no metastable peak to indicate the origin of this ion. The retention of the labile base hydrogen indicates that A^+ is not the precursor. It is possible that the small amount of $[M - R_y - ZOH]^+$ (see Scheme 24) is a precursor and a suggested mechanism, involving rearrangement of a cyclic silyloxonium ion, is as follows:

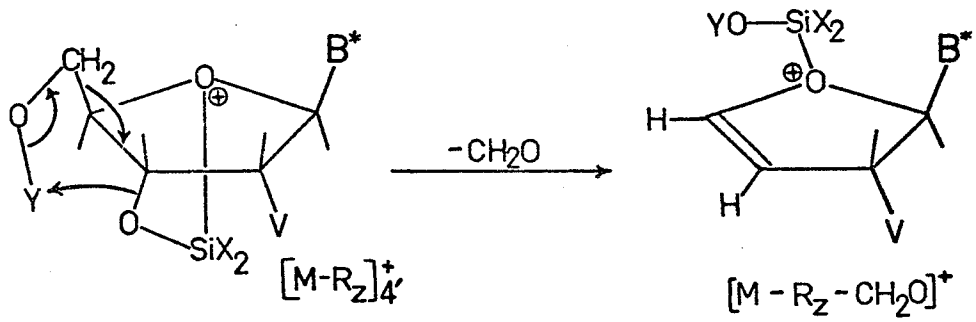


$[M - R_y - ZOH]^+$
24.2b, when V = H

$[M - R_y - ZOH - CH_2O]^+$

$[M - R_z - CH_2O]^+$ [XII.8, Iab - m/e269; XV.4, Ibb - m/e383]

This is a weak ion observed almost exclusively for those derivatives with a 3'-O-SCTASi function. A mechanism consistent with this and the labelling results is as follows:



$[B + X_2Si + RX_2SiOH]^+$	[XV.9, Ibb - m/e315]
$[B + X_2Si + H_2O]^+$ or E^+	[XII.19 and XV.23, Iab and Ibb - m/e201]
$[B + X_2Si]^+$ or F^+	[XII.25 and XV.26, Iab and Ibb - m/e183]

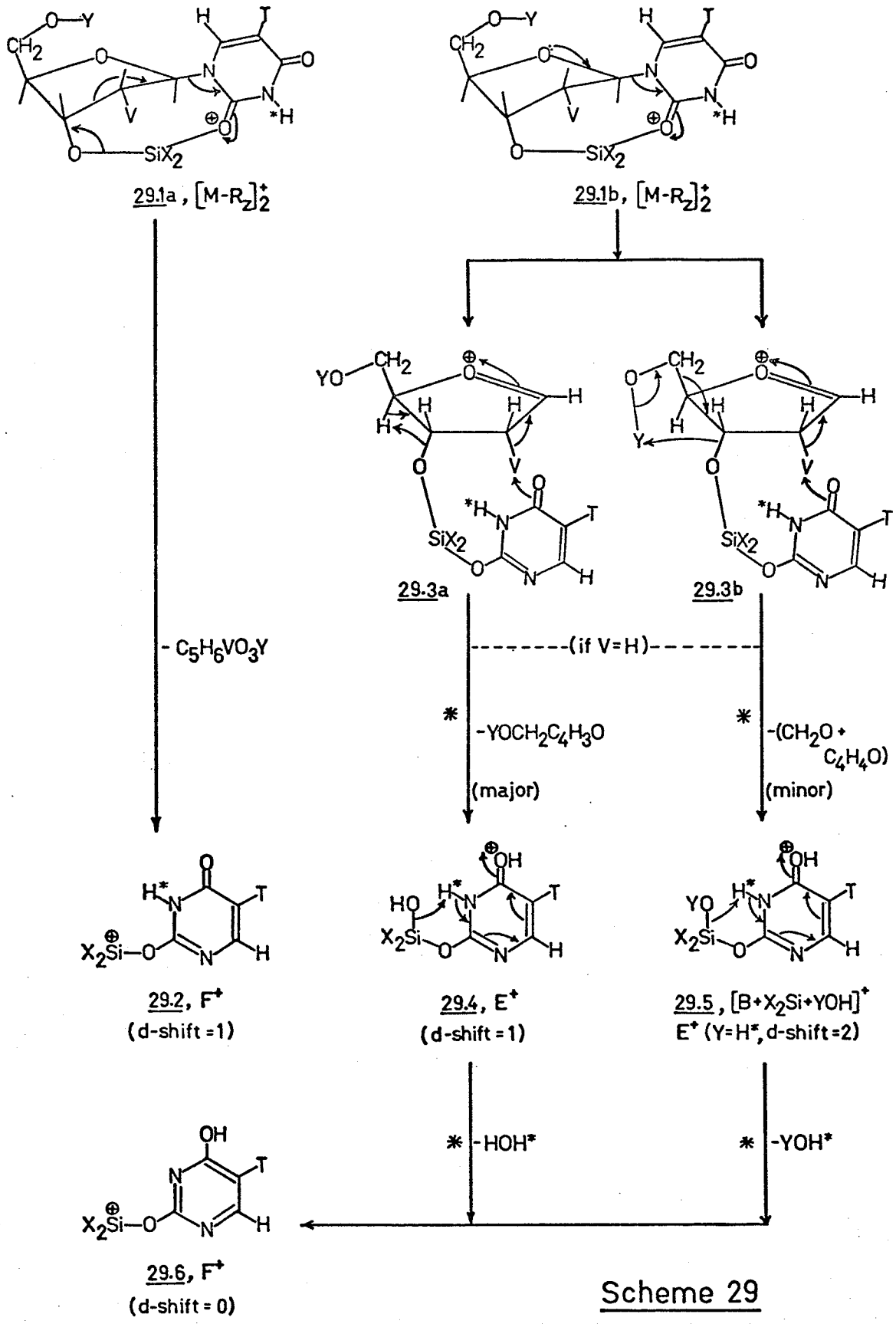
It is appropriate to discuss the origin of these ions together, since they are produced by the interaction of a siliconium ion with the nucleobase.

An ion corresponding to $[B + X_2Si + H_2O]^+$, or E^+, is characteristically prominent in the spectra of partial and fully protected derivatives with a 3'-O-SCTASi function, but quite weak for derivatives with only a 5'-O-SCTASi group. Thus, it is very useful for distinguishing isomers, as well as identifying the nucleobase. Metastable peaks for Iab and Vaba indicate that $[M - R]^+$ is the precursor for E^+. In the fully O-silylated derivatives, an analogous ion of low intensity is observed corresponding to $[B + X_2Si + RX_2SiOH]^+$. Mixed derivatives indicate that it is exclusively $[B + X_2Si_z + YOH]^+$. Although not very useful for structural elucidation, it does help

support the proposed mechanisms. Deuterium labelling of labile hydrogens gave a great deal of information on the mechanism of formation. For the partial derivatives, E^+ showed different d-shifts for the two isomers. The 3'-O-silyl derivative showed only partial (≃15%) incorporation of a second labile hydrogen into this fragment (one is already attached to the base), while the 5' isomer displayed a predominant shift of 2 (75%). These E^+ fragments with shifts of 2 correspond to $[B+X_2Si_z+YOH]^+$ and $[B+X_2Si_y+ZOH]^+$ respectively.

Another ion of composition $[B+X_2Si]^+$ or F^+ is prominent for all derivatives. In the spectra of 3'-O-silyl derivatives, Iab and Vaba, a metastable peak indicates formation, at least in part, from E^+ by loss of water. The labelling results for F^+ (for Iab, Iba, and Ibb) show partial loss of the labile base hydrogen (d-shift = 0(≃50%)). (An ion corresponding to $[B+C_3H_6O]^+$ is also present as a minor component of the ion at m/e183 in the mass spectrum of Iba, but should not cause much interference in the labelling results.)

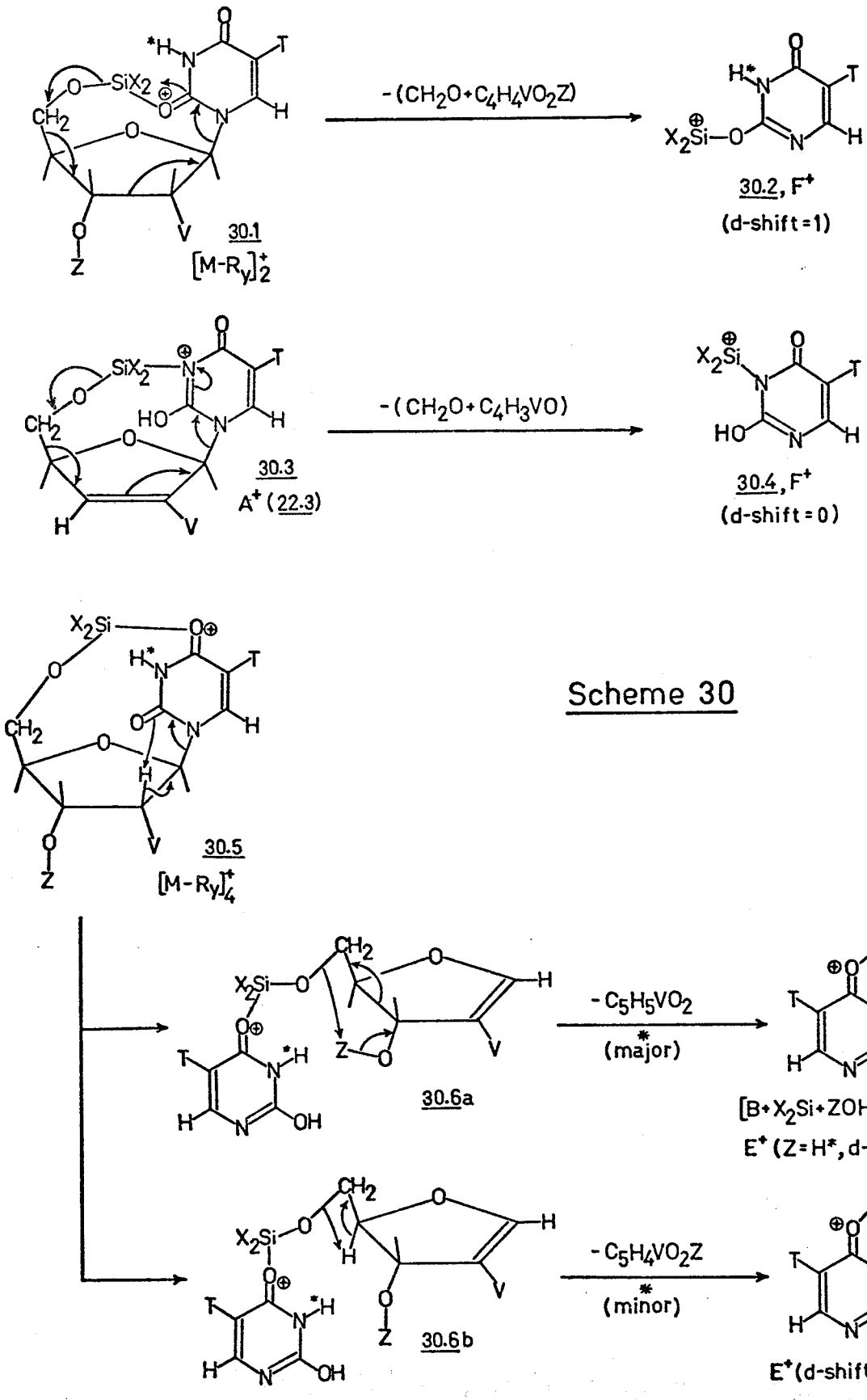
Proposals for the formation of these ions for the 3'-O-SCTASi isomers are given in Scheme 29. The cyclic silyloxonium ion $[M-R_z]_2^+$ can decompose directly to $[B^*+X_2Si]^+$ by a sequence of concerted electron shifts (29.1a to 29.2). This would explain the portion of F^+ fragment in which the labile base hydrogen is retained. The intermediate, 29.3, can be formed from $[M-R_z]_2^+$ by fission of the glycosyl linkage with transfer of the charge to the ring oxygen. This can then lead, via 29.3a to 29.4 and 29.3b to 29.5, to E^+ and $[B+X_2Si+YOH]^+$ fragment ions, respectively. The former is the major pathway accounting for a predominant d-shift = 1 for partial derivatives. (It should be noted that neither of these fragments are observed for ribonucleoside derivatives, where V = OW.) The elimination


29.1a, [M-R_Z]+2
29.1b, [M-R_Z]+2
29.3a
29.3b
- C5H6VO3Y
(if V=H)
-YOCH2C4H3O
(major)
-(CH2O + C4H4O)
(minor)
29.2, F+
(d-shift = 1)
29.4, E+
(d-shift = 1)
29.5, [B+X2Si+YOH]+
E+ (Y=H*, d-shift=2)
-HOH*
-YOH*
29.6, F+
(d-shift = 0)

Scheme 29

of HOH* or YOH* from these ions would result in F^+, with no deuterium label (29.4 to 29.6 is supported by a metastable peak).

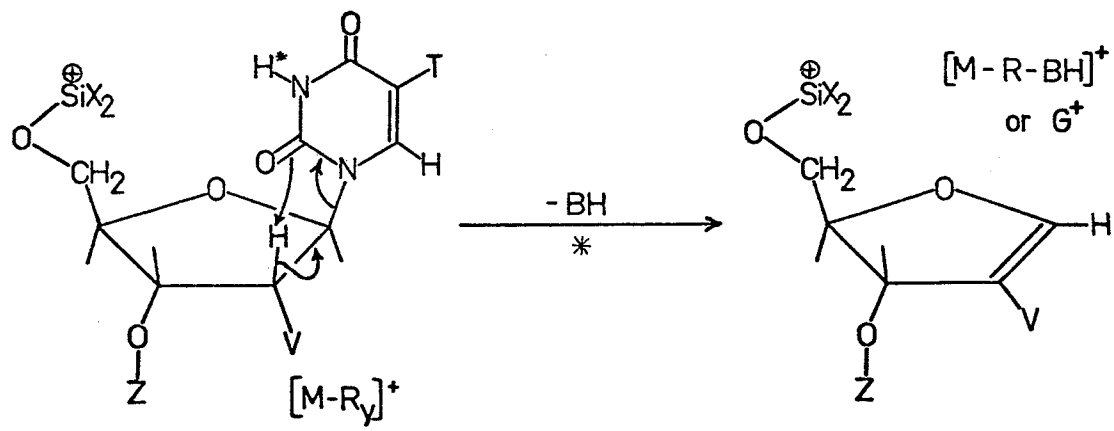
Scheme 30 illustrates the proposed mechanisms for formation of E^+ and F^+ for 5'-O-silyl derivatives. Decomposition of $[M-R_y]_2^+$ (30.1 to 30.2) results in F^+ with retention of the labile base hydrogen. To explain the portion of F^+ that does not retain the labile hydrogen, it is proposed that A^+ (structure 22.3) decomposes via a concerted rearrangement of electrons to give 30.4. The formation of E^+ for 5'-O-silyl derivatives requires the cyclic silyloxonium ion, $[M-R_y]_4^+$ (30.5), and decomposition to 30.7 or 30.8 via 30.6. Labelling indicates that 30.6a to 30.7 is the more favored pathway. However, CPK models indicate that conformations leading to the interaction of the Si and O_4 are of very low probability, which could explain the low abundance of E^+ for this series of isomers. Only certain skeletal conformations can allow close approach of these atoms, without the development of large, non-bonded repulsions between $O_{5'}$ or $H_{5'}$ and the ring of the base, or between the T substituent of the base and the X substituents on Si. It is interesting to note that when T is changed from CH_3 in thymine to H in uracil, the probability of Si-O_4 interaction increases and the intensity of E^+ becomes larger.


30.1
[M-Ry]+2
-(CH2O+C4H4VO2Z)
30.2, F+
(d-shift=1)
30.3
A+ (22.3)
-(CH2O+C4H3VO)
30.4, F+
(d-shift=0)
30.5
[M-Ry]+4
30.6a
-C5H5VO2
(major)
[B+X2Si+ZOH]+, 30.7
E+ (Z=H*, d-shift=2)
30.6b
-C5H4VO2Z
(minor)
E+(d-shift =1), 30.8

Scheme 30

$[M-R-BH]^+$ or G^+	[XII.26, Iab - m/e173; XV.12, Ibb - m/e287]
$[G-CH_2O]^+$	[XII.38, Iab - m/e143]
$[X_2Si+C_3H_3O_2(71)]^+$	[XII.42 and XV.34, Iab and Ibb - m/e129]

An ion corresponding to $[M-R-BH]^+$ or G^+ is observed for all SCTASi derivatives examined. Metastable peaks in Vbaa and Vbba indicate that $[M-R]^+$ is the precursor. The simplest explanation for this ion is given by the mechanism in Scheme 31, where the O_2 of the base and H_2, interact in a McLafferty rearrangement, resulting in elimination of BH.



Scheme 31

Although $[M-R_y]^+$ is used as a precursor in this scheme, $[M-R_z]^+$ or any of the cyclic silyloxonium ions in Scheme 21 (except $[M-R_y]_2^+$ and $[M-R_z]_2^+$) would also suffice.

However, this mechanism does not explain the variations in intensities between 3'- and 5'-O-silyl isomers (G^+ is more abundant for 3'-O-SCTASi derivatives), the metastable-supported decompositions of G^+ to $[G-CH_2O]^+$ (for Iba, Vbaa) and to $[X_2Si+C_3H_3O_2(71)]^+$ (for Iab and Vaba), and the unexpected deuterium-labelling results for the partial 3'-O-TBDMSi derivative, Iab. The latter indicates almost no retention of the labile

sugar hydrogen in G^+ (i.e., B + Y is eliminated). The 5'-O-TBDMSi derivative, Iba, on the other hand, does not eliminate B + Z, and there is no ion analogous to $[M - R - BY]^+$ (i.e., $[M - R - (B + RX_2Si)]^+$) for the fully protected derivatives.

The $[G - CH_2O]^+$ ion is observed in appreciable intensity only for the partial silyl derivatives. The labelling results parallel those of G^+ (d-shift = 0 for Iab, = 1 for Iba), as do the relative intensities for isomers.

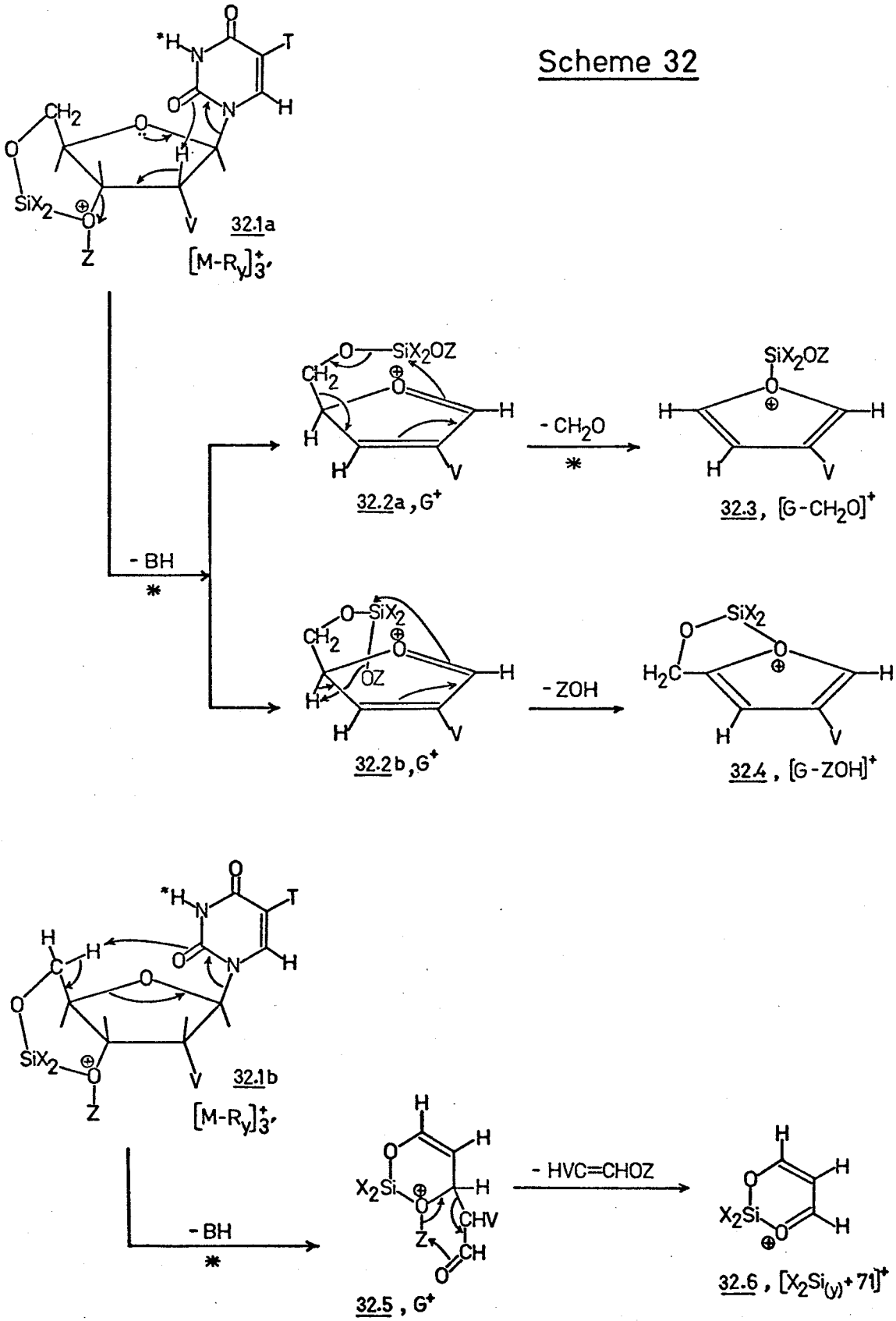
The $[X_2Si + 71]^+$ ion is observed for all derivatives, but the isomer intensity variations for partial and fully protected derivatives do not parallel each other. For the partial derivatives, it is more abundant for 3'-O-SCTASi isomers (i.e., $[X_2Si_z + 71]^+$ favored), but in the case of the mixed silyl derivatives it is more intense for the 5'-O-SCTASi isomers (i.e., mostly $[X_2Si_y + 71]^+$). All labelling results indicate that no labile hydrogen is retained.

Schemes 32 and 33 suggest some mechanisms which can explain the above observations. Also, shown in Scheme 32 is a possible mechanism for the formation of $[G - ZOH]^+$ which was mentioned earlier as an alternative for $[A - BH]^+$.

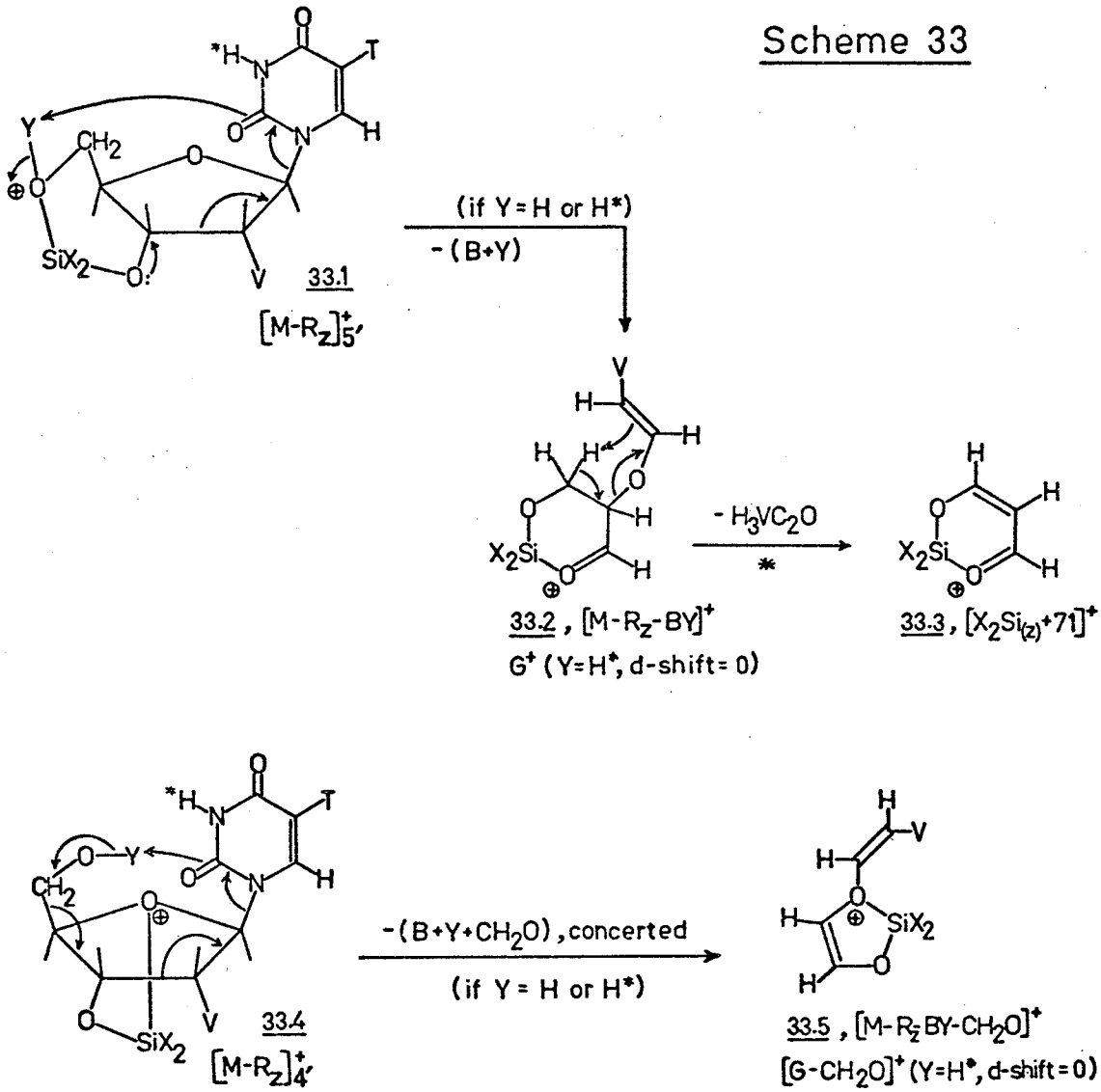
The special behavior of partial 3'-O-silyl derivatives with respect to G^+ and $[G - CH_2O]^+$ is probably due to the fact that, unlike the 5'-O-substituted derivatives, the labile 5'-hydroxyl hydrogen is available for transfer to the base with the result that the transfer of $H_{2'}$ to the base, while still observed, becomes relatively less important.

For the fully derivatized compounds, the mechanism in Scheme 31 may make a substantial contribution to G^+ since pathways in Scheme 33 are inhibited when the 5'-OH is protected (Y ≠ H or H^*). Also, as mentioned

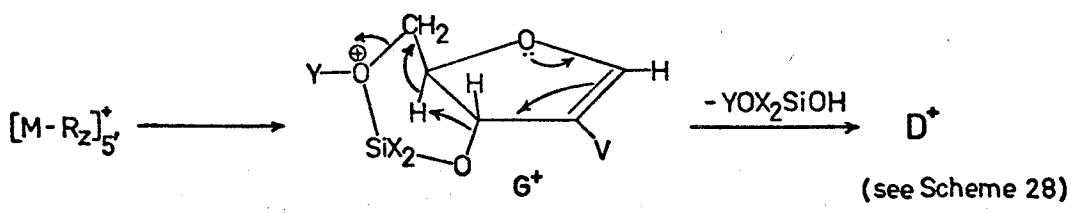
Scheme 32


32.1a
[M-R$_y$]$^+_3$,
- BH
*
32.2a, G$^+$
- CH$_2$O
*
32.3, [G-CH$_2$O]$^+$
32.2b, G$^+$
- ZOH
32.4, [G-ZOH]$^+$
32.1b
[M-R$_y$]$^+_3$,
- BH
*
32.5, G$^+$
- HVC=CHOZ
32.6, [X$_2$Si$_{(y)}$+71]$^+$

Scheme 33



Scheme 34



earlier, ion D^+ may be formed in part from G^+, as shown in Scheme 34.

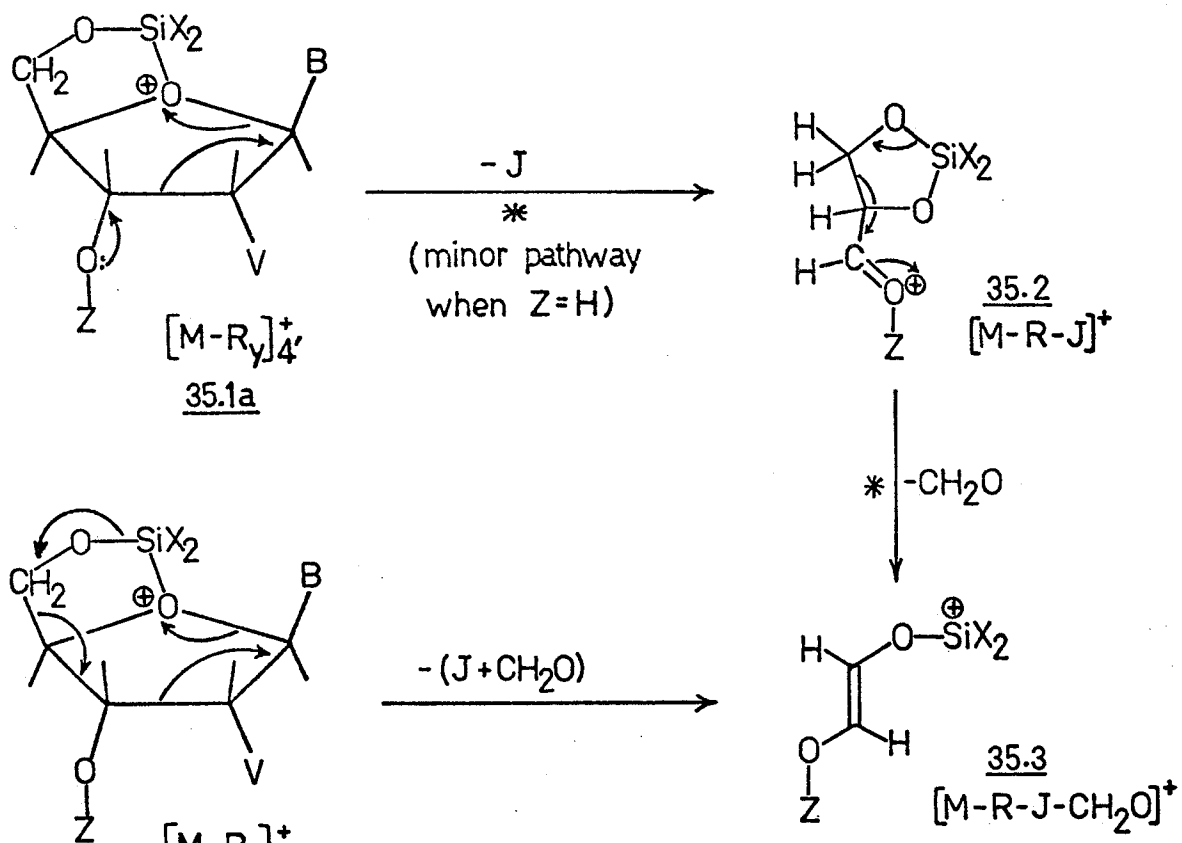
$[M-R-J]^+$	[XII.35, Iab - m/e147; XV.16, Ibb - m/e261]
$[M-R-J-CH_2O]^+$	[XII.47, Iab - m/e117; XV.18, Ibb - m/e231]

These ions are useful for distinguishing isomeric substituted derivatives, but the observed intensity variations are quite different for partial and fully silylated derivatives.

In the case of partial derivatives, $[M-R-J]^+$ is prominent for 3'-O-SCTASi derivatives of I and II but almost absent for all 5'-O-SCTASi isomers (i.e., $[M-R_z-J]^+ >> [M-R_y-J]^+$). Labelling experiments show the retention of one labile hydrogen. $[M-R-J-CH_2O]^+$ is observed for both 3'- and 5'-O-silyl derivatives, although it is more prominent for the former derivatives of I-III (about equal for IV and V). One labile hydrogen is retained and a metastable peak for Iab indicates that this ion is produced from $[M-R-J]^+$ by loss of CH_2O.

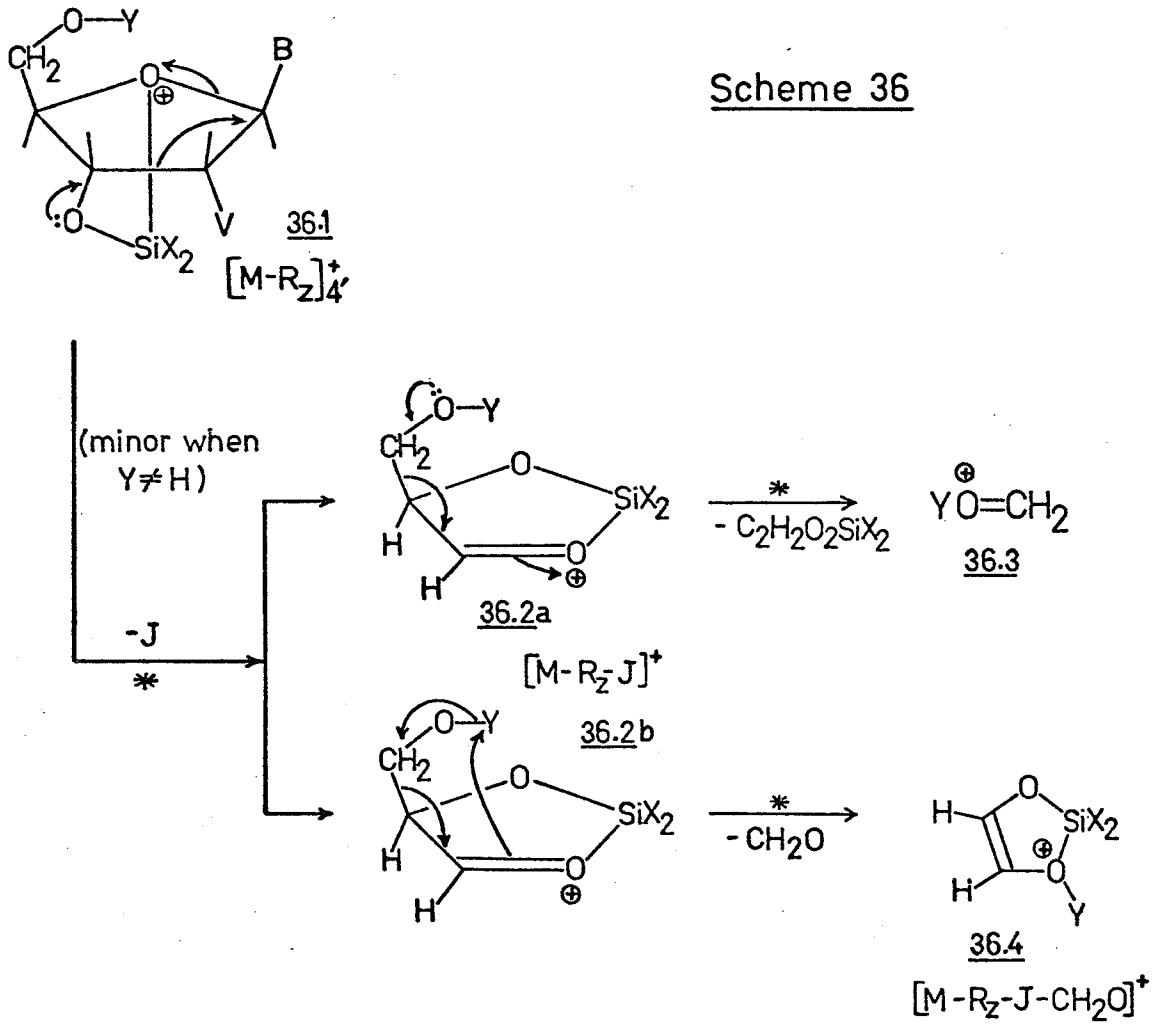
For the 3',5'bis-O-silylated derivatives, there is an opposite trend. Both ions are prominent only for compounds with a 5'-O-SCTASi function (i.e., these ions are attributed almost entirely to $[M-R_y-J]^+$ and $[M-R_y-J-CH_2O]^+$). Metastable peaks for various compounds support the sequential loss of J and CH_2O from $[M-R]^+$. In addition, a metastable peak for Vbba and Icc indicates decomposition of $[M-R-J]^+$ to $RX_2SiOCH_2^+$.

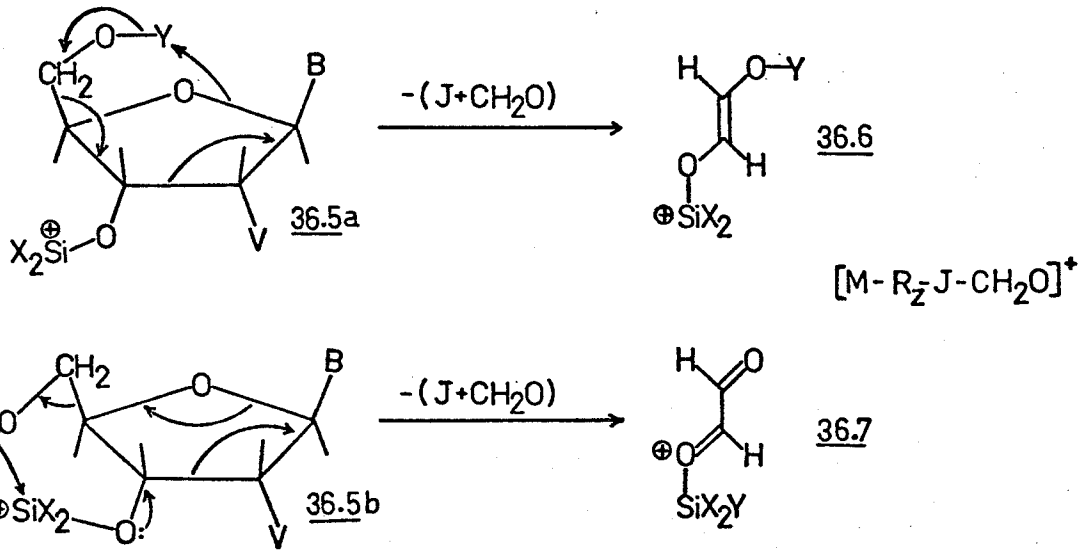
The mechanisms in Schemes 35 and 36 are proposed to explain these results. The reason for the different behaviors of mono- and bis-silyl derivatives may be the enhanced ability of a silyloxy group to take up the charge versus a hydroxy group. Thus, for the partial 5'-O-silyl derivatives, the sequence 35.1a → 35.2 → 35.3 is suppressed. It is possible that $[M-R_y-J-CH_2O]^+$ is produced directly from $[M-R]^+$ by a concerted elimination of $J+CH_2O$ (35.1b → 35.3).


O—SiX2
CH2
B
V
Z
[M-Ry]+4'
35.1a
- J
*
(minor pathway
when Z=H)
35.2
[M-R-J]+
* -CH2O
35.1b
- (J+CH2O)
35.3
[M-R-J-CH2O]+

Scheme 35

Scheme 36


36.1
[M-R_Z]+4'
(minor when Y≠H)
-J
*
36.2a
[M-R_Z-J]+
36.2b
* - C2H2O2SiX2
36.3
* - CH2O
36.4
[M-R_Z-J-CH2O]+


36.5a
-(J+CH2O)
36.6
[M-R_Z-J-CH2O]+
36.5b
-(J+CH2O)
36.7

$(J+RX_2Si)^+$	[XII.9 and XV.14, Iab and Ibb - m/e267]
$(J+H)^+$	[XII.34, Iab - m/e153]
$(J+H-HNCO)^+$	[XII.52, Iab - m/e110]

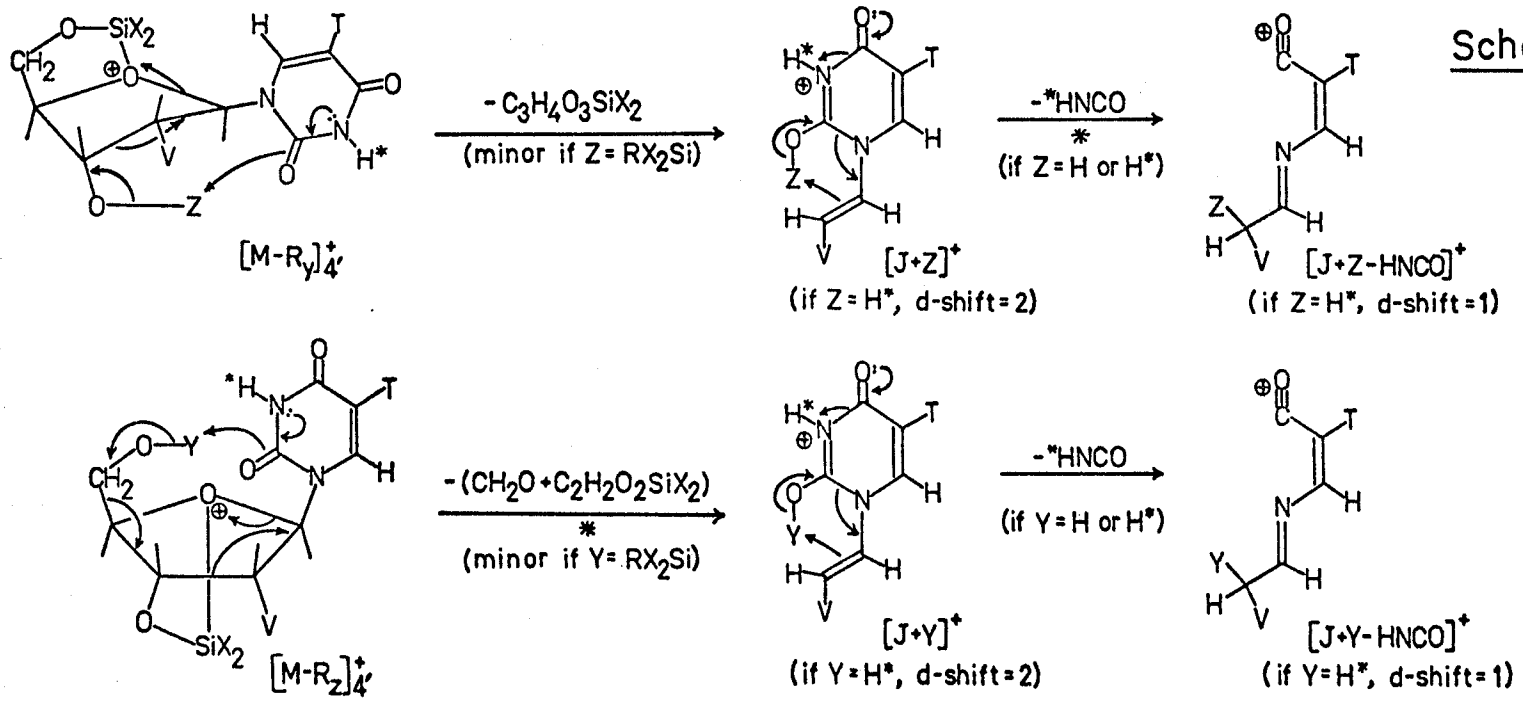
A fairly intense ion corresponding to $[B+28]^+$, or $[J+H]^+$, is observed for all partial silyl derivatives. It is usually more abundant for 3'-O-silyl versus 5'-O-silyl isomers, although it is not of much value for isomer identification since this order is reversed for deoxyadenosine (V). A metastable peak for Iab indicates that $[M-R]^+$ is the precursor for this ion. A d-shift of 2 for both isomers signifies incorporation of the labile sugar hydrogen (i.e., the ions can be designated as $[J+Z]^+$ and $[J+Y]^+$ for the 5'- and 3'-O-silyl isomers respectively). For bis-silylated I and II, $[J+H]^+$ is not present to any extent, but is observed for Vbba (m/e162).

An analogous ion, $[J+RX_2Si]^+$, is present for all derivatives, although it is usually of quite low intensity. It is predominantly $[J+Z]^+$ as indicated by its greater abundance for 3'-O-silyl derivatives and by results with mixed silyl isomers. For the latter, the abundance of $[J+RX_2Si]^+$ depends inversely on steric crowding in the silyl group, being quite abundant when Z = TMSi. This reflects the greater migratory aptitude of TMSi. However, no silyl group matches the lability of an hydroxyl hydrogen, as evidenced by the far greater abundance of $[J+H]^+$ for partial derivatives.

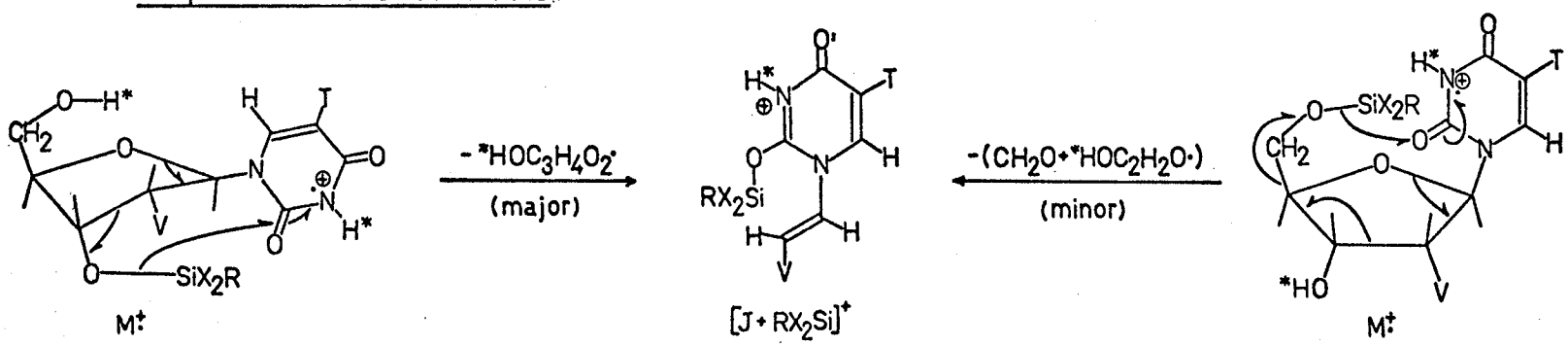
Scheme 37 suggests mechanisms for the formation of these ions. Cyclic silyloxonium ions are proposed as precursors, except in the case of mono-silyl derivatives where $[J+RX_2Si]^+$ must originate from the molecular ion.

Another ion, $[J+H-HNCO]^+$, is observed for mono-silyl derivatives of pyrimidine nucleosides (I-III). It is formed by loss of H^*NCO from

Scheme 37



For partial O-SCTASi derivatives:

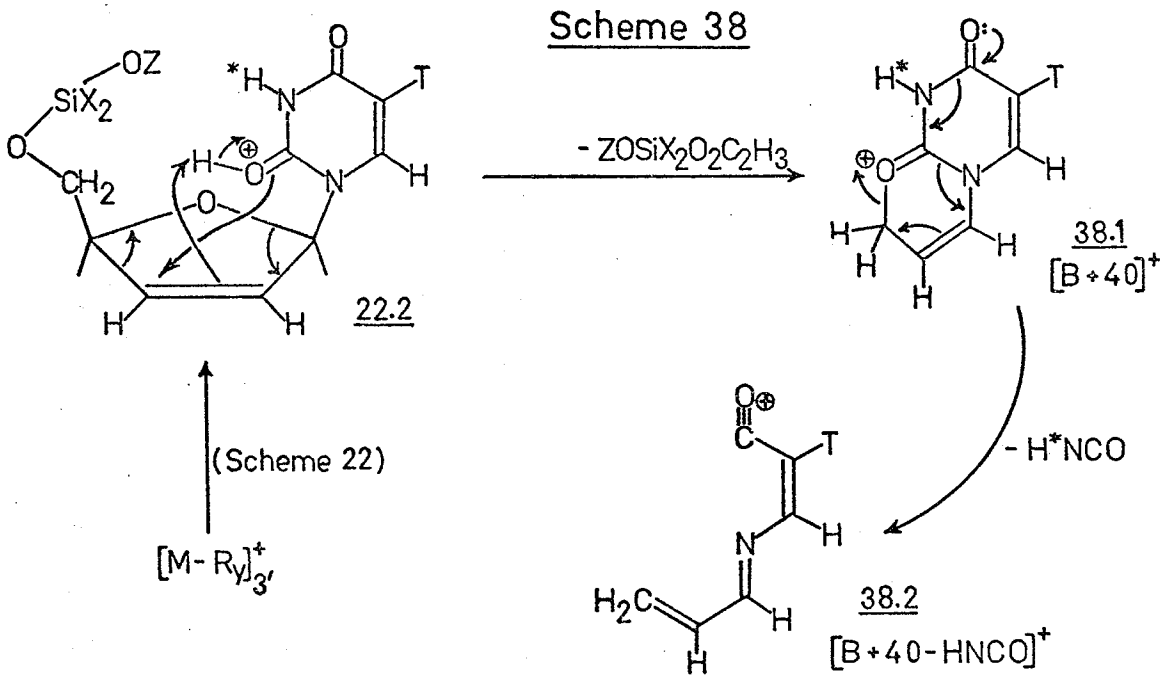


$[J+H^*]^+$ as shown by a metastable peak for Iba and labelling results. Scheme 37 suggests a mechanism and ion structure.

$[B+C_3H_4(40)]^+$ [XII.30 and XV.27, Iba and Ibb - m/e165]

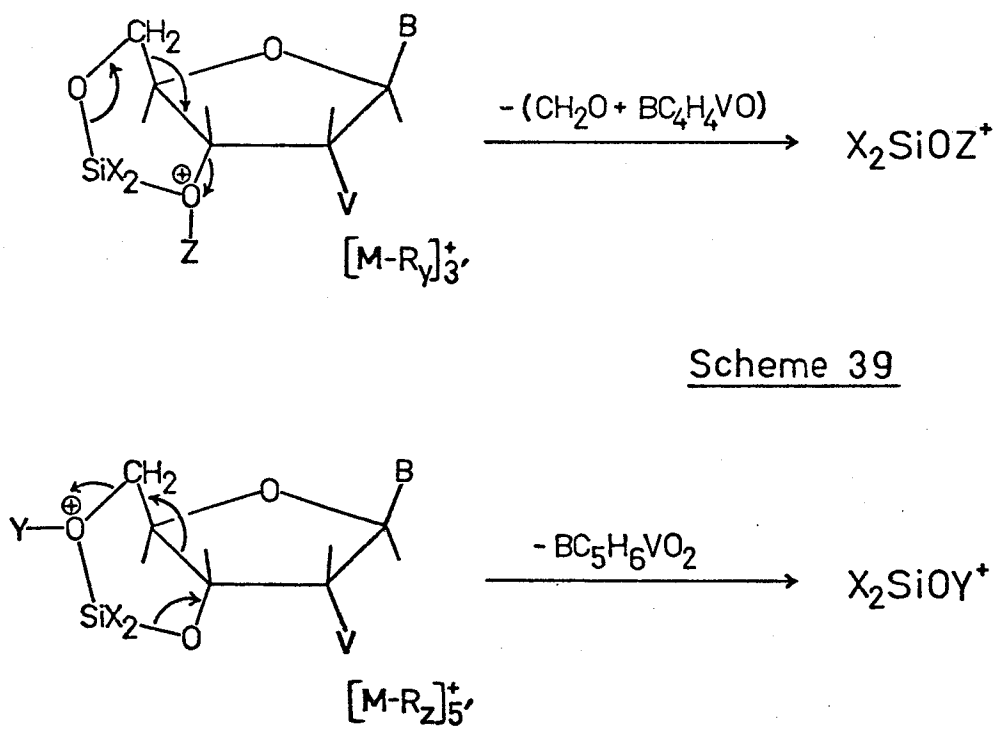
This is a medium intensity ion observed for partial and full derivatives of I and II with a 5'-O-SCTASi goup, and retains only the labile hydrogen of the base. It is proposed that $[M-R_y]^+$ is a possible precursor, although there is no supporting metastable peak. The decomposition may proceed as in Scheme 38, through the intermediate 22.2 that was proposed previously for the formation of A^+. Formation of a bond between O_2 and $C_{3'}$, migration of a hydrogen to $C_{3'}$, coupled with fission of the $C_{1'}$-$O_{4'}$ and $C_{3'}$-$C_{4'}$ bonds, in a concerted process, leads to the stable ion product 38.1, with elimination of a stable neutral species. A similar ion structure was proposed in the mass spectra of cyclonucleosides (244). It is also possible that $[B+40]^+$ then decomposes by loss of H^*NCO to give 38.2. Labelling confirms no retention of labile hydrogen.

Scheme 38



X_2SiOH^+ [XII.63 and XV.45, Iab and Ibb - m/e75]

This ubiquitous ion is observed in the spectra of most SCTASi derivatives of nucleosides, although it is more intense for partial derivatives. Labelling results and metastable peaks indicate more than one method of genesis. Many proposals can be made in which the siliconium group abstracts a hydrogen from elsewhere in the molecule. One type of mechanism that accounts for the greater abundance of X_2SiOH^+ in partial derivatives is that given for X_2SiOY^+ and X_2SiOZ^+ (where Y and Z = H or H^*) in Scheme 39.

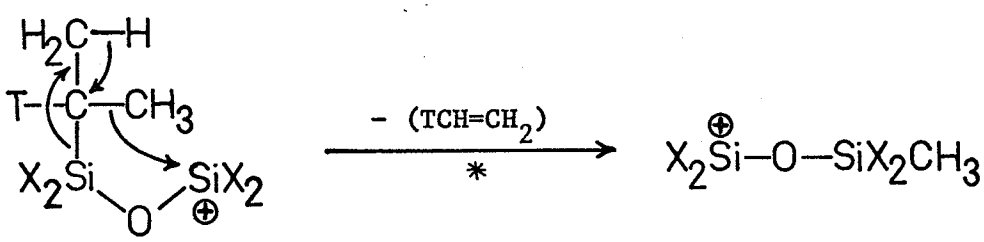


Scheme 39

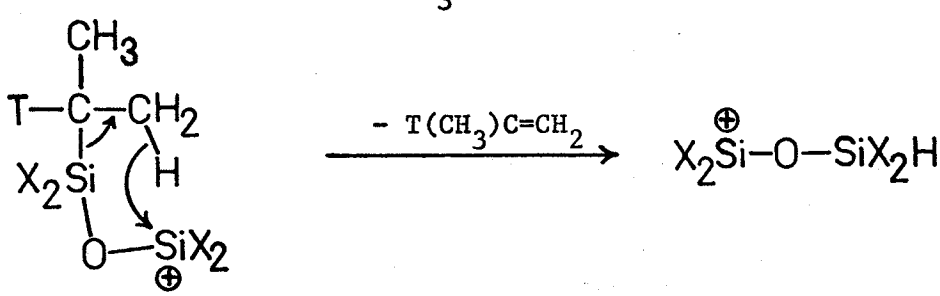
$RX_2SiOSiX_2^+$	[XV.24, Ibb - m/e189]
$CH_3X_2SiOSiX_2^+$	[XV.29, Ibb - m/e147]
$HX_2SiOSiX_2^+$	[XV.31, Ibb - m/e133]

An ion analogous to X_2SiOH^+ is observed in the spectra of fully silylated derivatives--$RX_2SiOSiX_2^+$. Mixed labelling studies indicate both X_2SiOY^+ and X_2SiOZ^+, and these probably arise from $[M-R]^+$ by the fragmentations in Scheme 39.

Far more intense fragment ions are observed corresponding to $CH_3X_2SiOSiX_2^+$ and $HX_2SiOSiX_2^+$. These are probably formed from $RX_2SiOSiX_2^+$ by rearrangement of the R alkyl group as shown in Scheme 40. Evidence for this is gained from the study of other compounds, such as di-<u>tert</u>-butyltetramethylsiloxane (see Figure 13b), where m/e189 rearranges to m/e147 as indicated by a metastable peak. $CH_3X_2SiOSiX_2^+$ appears to be a very stable ion product and its TMSi analog, m/e147, is observed in the spectra of many poly-TMSi derivatives. Examples of hydrogen and alkyl rearrangements similar to those proposed in Scheme 40 have been discussed in the literature (205,206).



(T = H, CH_3) <u>Scheme 40</u>



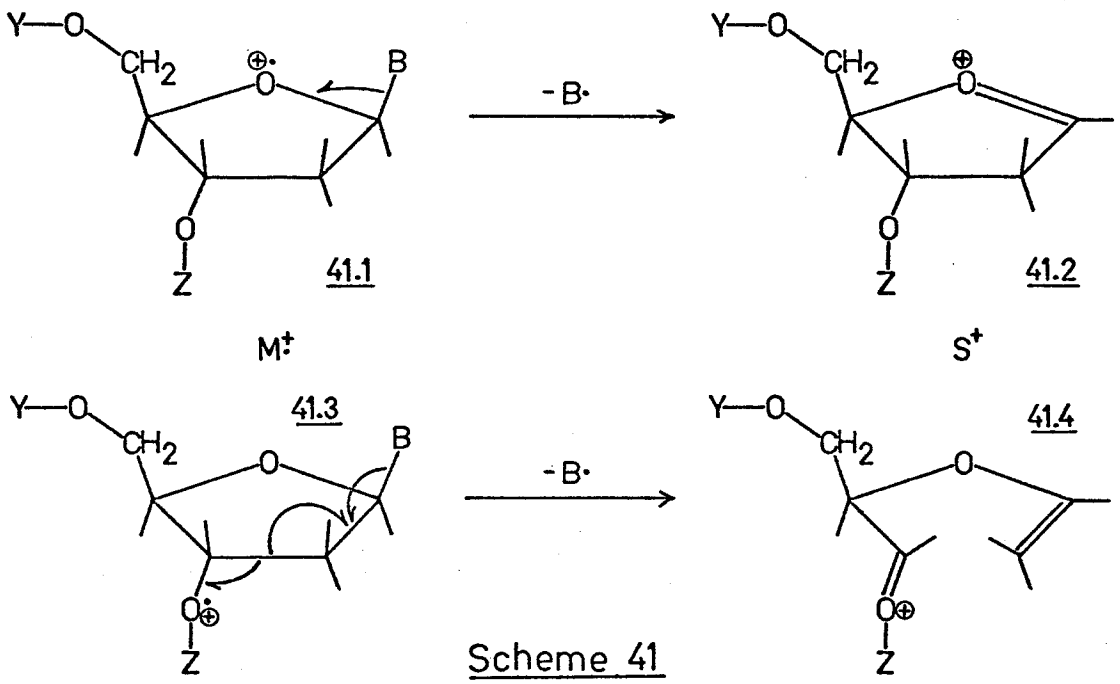
b) Ions formed by charge localization on the sugar ring

Among these ions there are significant intensity variations between isomeric partial or mixed derivatives. They are generally of lower abundance for fully silylated compounds, and are particularly weak for compounds III-V. The latter fact may be due to stronger glycosyl bonds or perhaps the greater ability of these nucleosides to accommodate a charge on their base moiety.

For the 5'-O-silyl partial derivatives, the abundance of S^+ and some of its decomposition ions (K^+, $[K-CH_2O]^+$, and $[RX_2Si+72]^+$) is generally quite low, while all are of moderate to high abundance for the 3'-O-silyl isomers. In contrast, $[S-H_2O]^+$ is more intense for 5'-O-silyl derivatives. (Because of the low intensity of all these fragments for the ribonucleoside series, and the probable involvement of $H_{2'}$ in several of these fragmentations, V is designated as H in Schemes 41 to 44) Ions corresponding to S^+, K^+, $[K-CH_2O]^+$, and D^+ are also present in the spectra of 2'-deoxynucleosides themselves, where similar assignments have been made (15).

S^+ [XII.13, Iab - m/e231; XV.6, Ibb - m/e345]

The sugar fragment, S^+, is of low abundance for both 5'-O-silyl and fully silylated derivatives, but is characteristically intense for for 3'-O-silyl deoxynucleosides. The low intensity for the former compounds indicates either that it is not formed as readily as for the 3'-O-silyl compounds, or that it undergoes decomposition more readily. The latter is unlikely, unless ion D^+ is formed almost entirely from S^+. Earlier evidence has indicated that it is not. However, the commonly proposed mechanism of charge localization on the ring oxygen followed by expulsion of B (Scheme 41, 41.1 → 41.2) does not explain the intensity



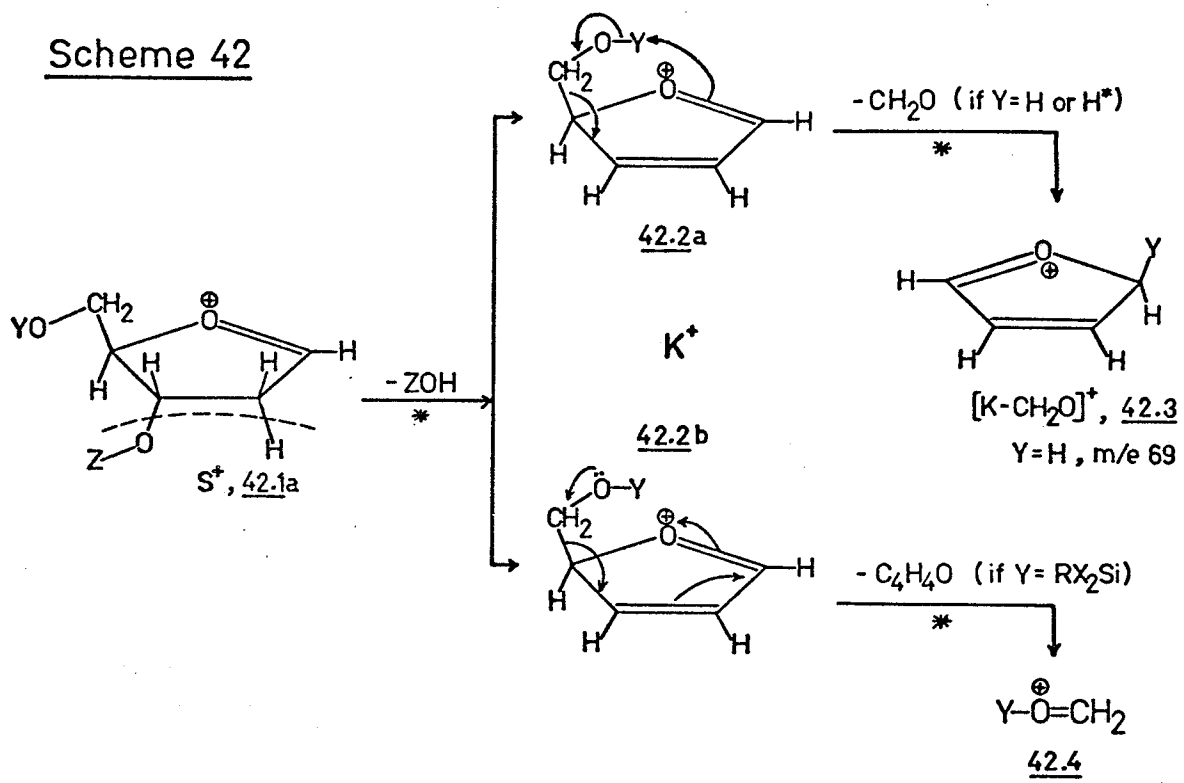
Scheme 41

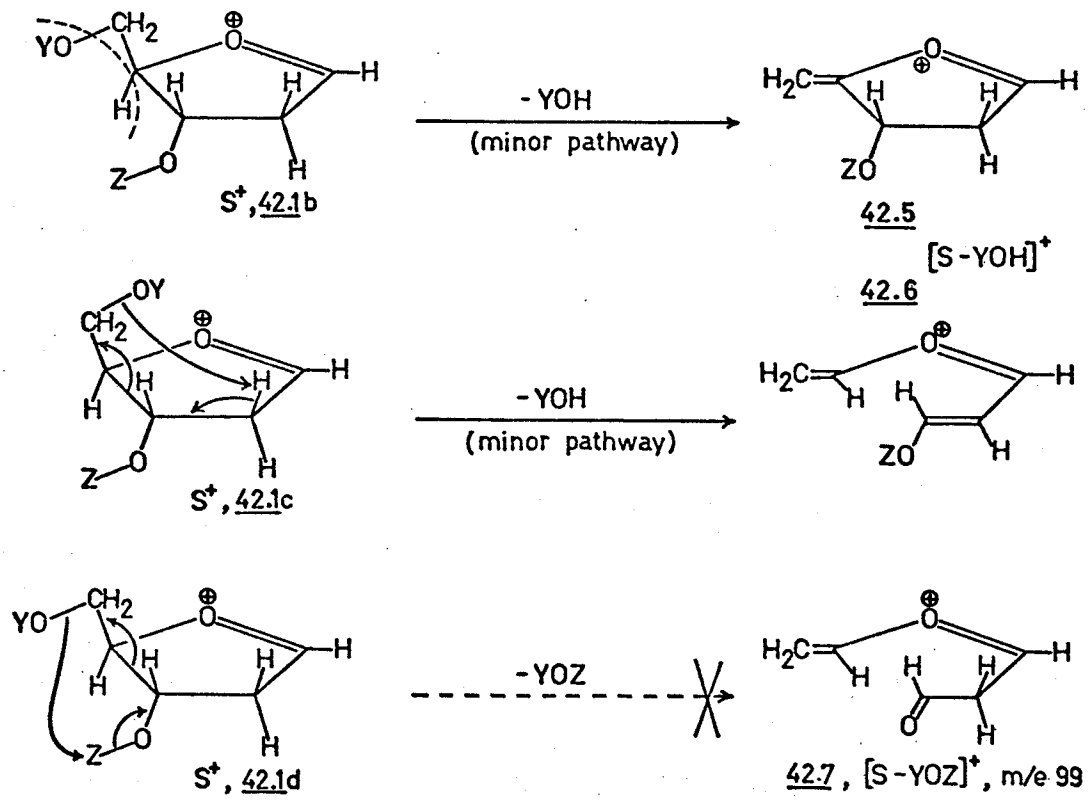
variations. It is proposed that, for 3'-O-silyl derivatives, an alternative mechanism shown in Scheme 41 (41.3 → 41.4) can account for these observations and, in addition, for the formation of $[RX_2Si+72]^+$ as discussed later. Localization of the charge on the 3'-silyloxy function can induce α-cleavage of the $C_{2'}$-$C_{3'}$ bond and a concerted elimination of B. Charge localization on the 5'-silyloxy function on the other hand, leads to formation of $YOCH_2^+$ as discussed later.

$[S-ZOH]^+$ or K^+ [XII.15, Iba - m/e213; XII.59, Iab - m/e99; XV.21, Ibb - m/e213]

The ions $[S-H_2O]^+$ for 5'-O-silyl deoxynucleosides and $[S-RX_2SiOH]^+$ for 3'-O-silyl and bis-silyl derivatives are best explained by a predominent loss of ZOH from S^+. This fragmentation is supported by metastable peaks in the spectra of compounds Iab and Iba. Deuterium labelling experiments show no retention of the labile sugar hydrogen in $[S-H_2O]^+$, but predominant retention in $[S-RX_2SiOH]^+$.

Scheme 42

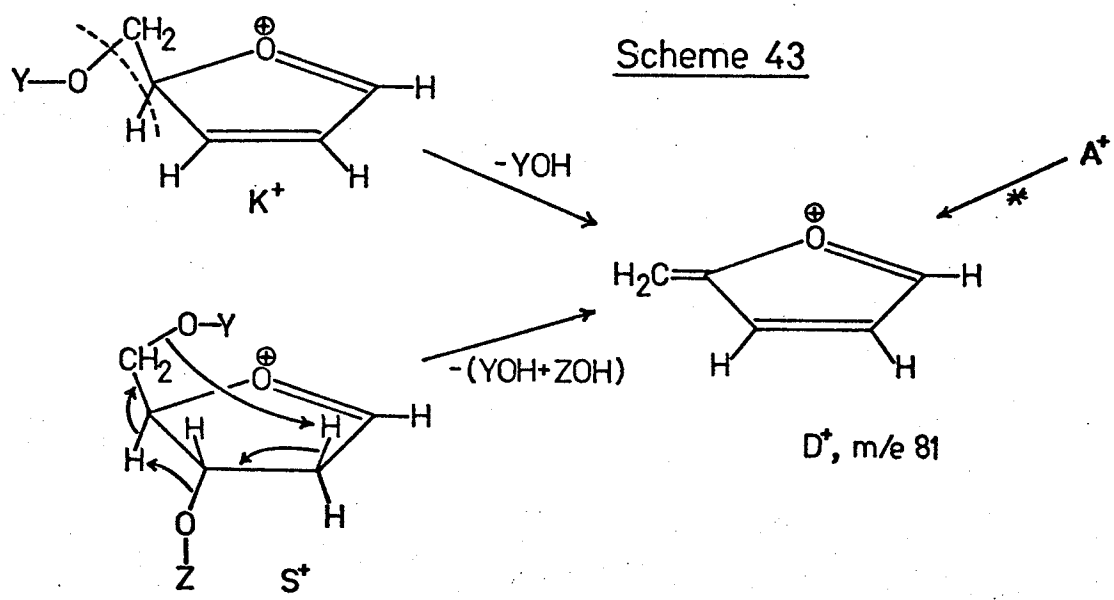

S+, 42.1a
- ZOH
42.2a
K+
42.2b
- CH2O (if Y=H or H*)
[K-CH2O]+, 42.3
Y=H, m/e 69
- C4H4O (if Y= RX2Si)
Y-O=CH2
42.4


S+, 42.1b
- YOH
(minor pathway)
42.5
[S-YOH]+
42.6
S+, 42.1c
- YOH
(minor pathway)
S+, 42.1d
- YOZ
42.7, [S-YOZ]+, m/e 99

The fully protected derivatives, especially mixed SCTASi and mixed TMSi/STASi, support the ion composition as being predominantly $[S-ZOH]^+$, hereafter designated as K^+, with minor contributions from $[S-YOH]^+$. In addition, for Ibb a metastable peak indicates the decomposition of K^+ to $YOCH_2^+$.

The fragmentations in Scheme 42 are consistent with these observations. The sequences, 42.1b → 42.5 and 42.1c → 42.6, account for minor contributions from $[S-YOH]^+$. It is also indicated by labelling and mixed derivatives that $[S-YOZ]^+$, 42.1d → 42.7, is not formed to any extent. The conjugation of double bonds in 42.2 is expected to make K^+ more stable than these alternatives.

The further decomposition of K^+ by loss of YOH, or the concerted loss of YOH + ZOH from S^+ to give m/e81 (ion D^+), as shown in Scheme 43, may also occur but there is no metastable support. The metastable supported decomposition $A^+ \rightarrow D^+$ is probably the major pathway in the case

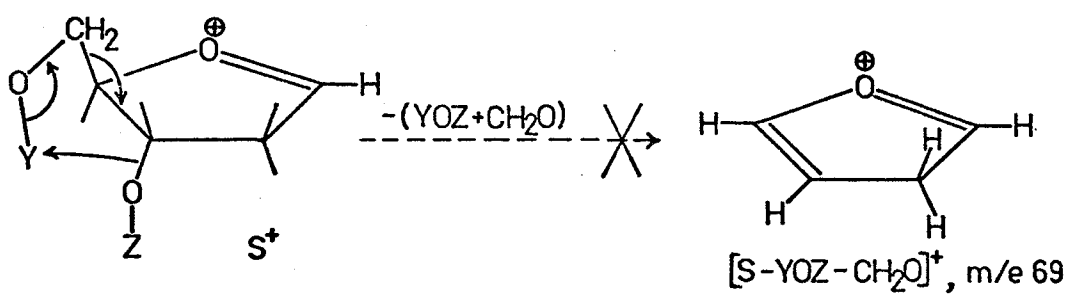


of 5'-O-silyl and bis-silyl derivatives, but the non-specific portion observed in 3'-O-RX_2Si derivatives can be accounted for by this scheme.

$[K - CH_2O]^+$ [XII.66, Iab - m/e69]

The ion assigned as $[K - CH_2O]^+$, is abundant only in the case of the partial 3'-O-silyl derivatives. A metastable peak for Iab indicates formation from K^+ by loss of CH_2O, and d-labelling shows retention of the labile sugar hydrogen. The mechanism given for 42.2a → 42.3 is consistent with these observations. The fact that Y does not migrate when it is a silyl group may be due to the lower energy of a Si-C bond versus that of a Si-O bond, steric hindrance from the bulky alkyl substituents, and possibly poor competition with the pathway leading to $YOCH_2^+$.

The following mechanism must be of low probability as well, possibly due to lack of conjugation in the resulting ion:



$[2RX_2Si + C_3H_3O_2(71)]^+$	[XV.11, Ibb - m/e301]
$[RX_2Si + C_3H_4O_2(72)]^+$	[XII.23 and XV.25, Iab and Ibb - m/e187]
$[X_2Si + C_3H_5O_2(73)]^+$	[XII.40 and XV.33, Iab and Ibb - m/e131]

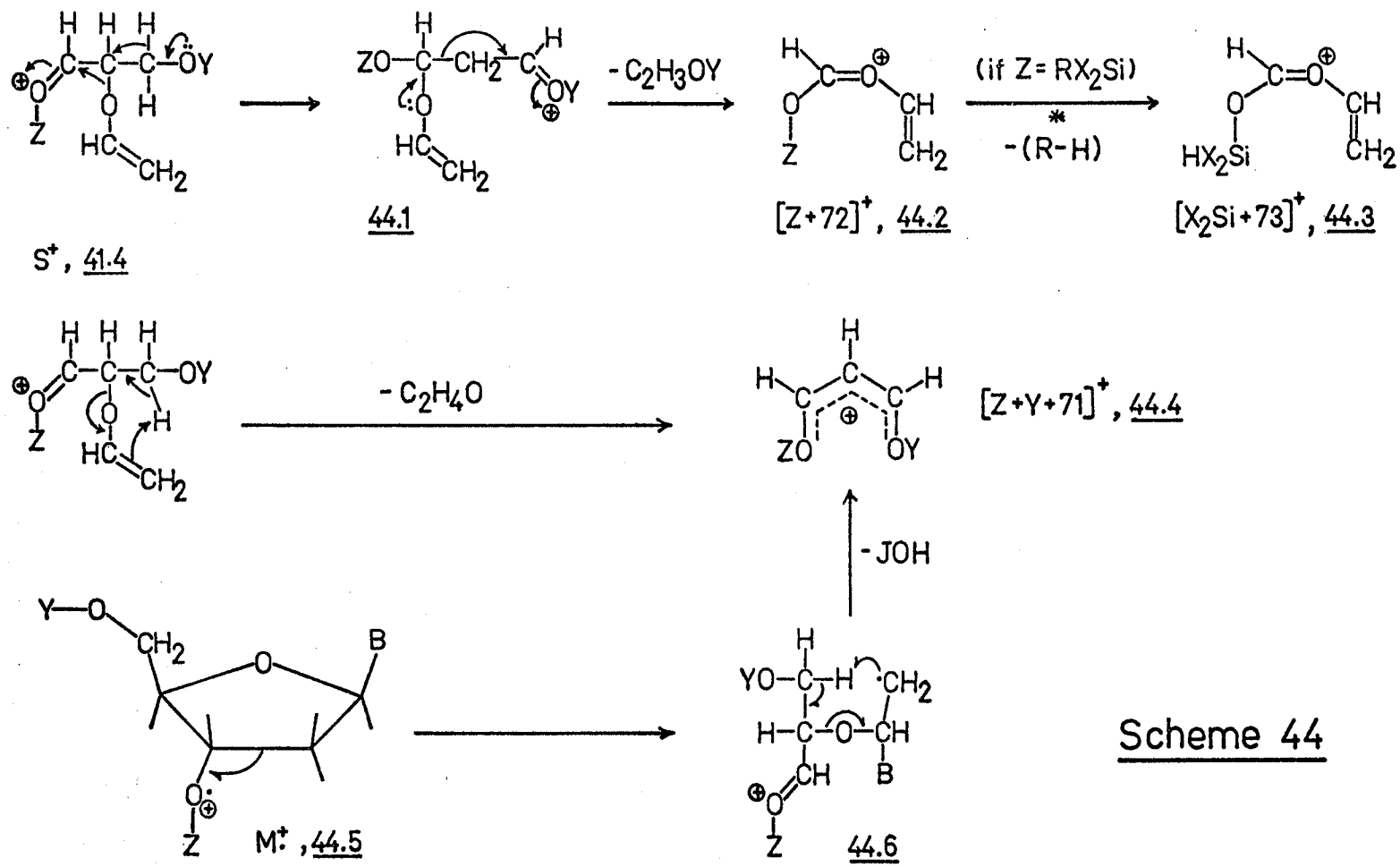
Ions assigned as $[RX_2Si + C_3H_4O_2(72)]^+$ and $[X_2Si + C_3H_5O_2(73)]^+$ are observed for both the partially and fully protected derivatives. In the case of the partial derivatives, $[RX_2Si + 72]^+$ is much more abundant for the 3'-O-silyl isomers, where only partial (30%) retention of one labile

hydrogen is observed (i.e., primarily $[Z+72]^+$). For the 5'-O-silyl isomers, one labile hydrogen is retained (i.e., primarily $[Z+Y+71]^+$). These could correspond to $[M-JOY]^+$ and $[M-JOH]^+$ respectively, but metastable peaks in Iab indicate the sequence, $S^+ \rightarrow [RX_2Si+72]^+ \rightarrow [X_2Si+73]^+$, which establishes the main precursor as S^+. As expected from this sequence, $[X_2Si+73]^+$ is also more abundant for the 3'-O-silyl isomers.

For the fully protected derivatives, ions are present corresponding to $[RX_2Si+72]^+$, $[X_2Si+73]^+$, and $[2RX_2Si+71]^+$, although the latter is not very abundant. Mixed derivatives indicate that the first is mostly $[Z+72]^+$, while the latter corresponds to $[Z+Y+71]^+$. $[Z+72]^+$ is especially abundant when Z = TMSi, while in the same instance, $[X_2Si+73]^+$ is quite weak.

Mechanisms consistent with these observations are presented in Scheme 44. It is proposed that the open ring form of S^+ (41.4), earlier suggested to be more abundant for 3'-O-silyl derivatives, decomposes to structures 44.2 and 44.4 to give $[Z+72]^+$ and $[Z+Y+71]^+$, respectively. Further rearrangement of $[Z+72]^+$ to $[X_2Si+73]^+$ is possible by elimination of (R-H), 44.2 → 44.3. Incidentally, when Z = H and Y = RX_2Si, the resulting fragment ion 44.3 is $C_3H_5O_2^+$, or m/e73, which is observed in the high resolution spectra of both Iba and Iab. (It overlaps with $CH_3X_2Si^+$ in the low resolution spectra of TBDMSi derivatives.)

The enhanced abundance of $[Z+72]^+$ for 3'-O-TMSi-5'-O-RX_2Si-derivatives is likely due to the fact that localization of charge on the 3'-OTMSi group will lead to greater α-cleavage of the sugar ring (Scheme 41) to produce a greater proportion of the open-ring form of S^+ (41.4), than would localization on a 3'-OTBDMSi group (which would lead primarily


S+, 41.4
44.1
- C2H3OY
[Z+72]+, 44.2
(if Z = RX2Si)
-(R-H)
[X2Si+73]+, 44.3
- C2H4O
[Z+Y+71]+, 44.4
- JOH
M+, 44.5
44.6

Scheme 44

to $[M-R]^+$).

It is possible that these ions may also be formed from M^+ by loss of JOH via the sequence 44.5 → 44.6 → 44.4.

c) Other ion types

$[B+H+RX_2Si]^+$	[XV.17, Ibb - m/e241]
$[B+2H]^+$	[XII.43 and XV.36, Iab and Ibb - m/e127]

$[B+2H]^+$ is a fragment ion which is commonly observed in the mass spectra of deoxynucleosides (15). For the SCTASi derivatives of I and II it is quite weak, but more abundant for such derivatives of III-V, especially deoxyadenosine (V). Genesis could be attributed to initial charge localization on the base and subsequent fragmentation of the molecular ion, but in the case of Vbaa and Vaba, metastable peaks indicate $[M-R]^+$ as a precursor. Labelling of Iab and Iba shows partial incorporation of the labile sugar hydrogen (i.e., $[B+H+Y]^+$ or $[B+H+Z]^+$).

The spectra of fully protected derivatives display an analogous ion, $[B+H+RX_2Si]^+$, that mixed silyl group labelling indicates is predominantly $[B+H+Y]^+$. $[B+H+TMSi]^+$ has been observed previously with TMSi derivatives of nucleosides (15).

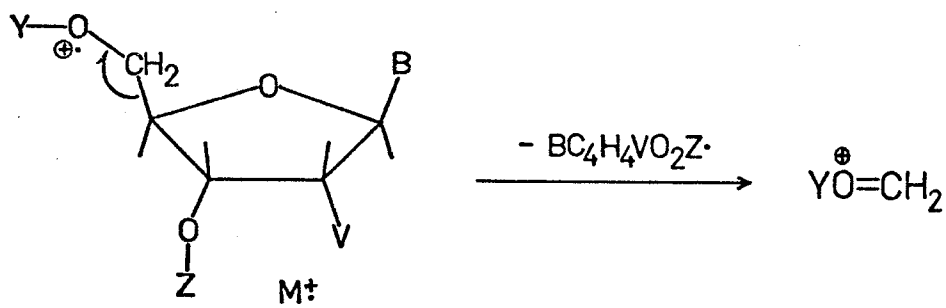
Other base-containing fragments such as $[B+H]^+$ and $[B+30]^+$ that are observed in spectra of underivatized nucleosides are usually in low abundance for SCTASi derivatives.

$YOCH_2^+$	[XII.36 and XV.30, Iba and Ibb - m/e145]
$CH_3X_2SiOCH_2^+$	[XII.55, Iba and Ibb - m/e103]
$H(X_2SiOCH_2)^+$	[XII.61 and XV.43, Iba and Ibb - m/e89]

$RX_2SiOCH_2^+$ is an intense ion in the spectra of nucleoside derivatives with a 5'-O-RX_2Si function and can be described as $YOCH_2^+$. In the spectrum of 3'-O-TBDMSi-thymidine (Iab), there is a moderately intense

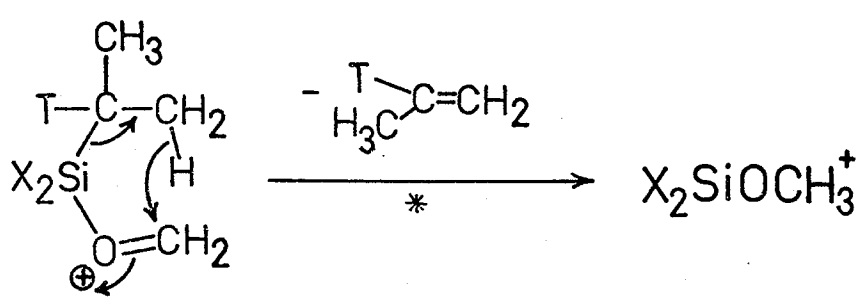
ion at m/e145, but the major component corresponds to $[X_2Si + C_4H_7O_2(87)]^+$ the genesis of which has not yet been established. $YOCH_2^+$ is valuable for the characterization of isomeric mixed silyl derivatives; for example, m/e145 ($TBDMSiOCH_2^+$) is the base peak for Ibe, but is very weak for Ieb, where m/e103 ($TMSiOCH_2^+$) is the base peak.

The major precursor for this ion is probably the molecular ion, where localization of the charge on O_5, can lead to α-cleavage of the $C_{4'}$-$C_{5'}$ bond:

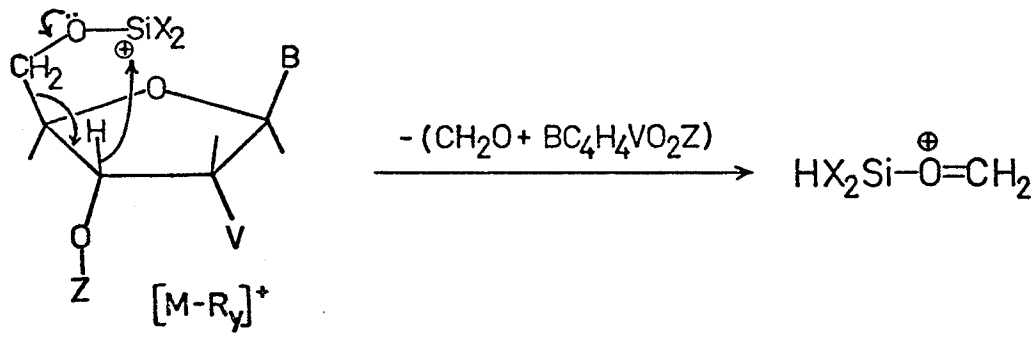


However, other precursors have been indicated by metastable peaks: K^+ (Scheme 42) and $[M - R - J]^+$ (Scheme 36 for bis-silyl derivatives).

Metastable peaks for several derivatives indicate further decomposition of $YOCH_2^+$ to $X_2SiOCH_3^+$, presumably through the following rearrangement (similar to that proposed for $HX_2SiOSiX_2^+$, Scheme 40):

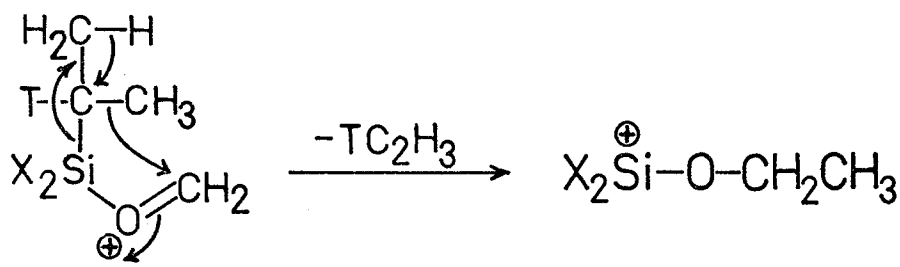


Another source for this ion may be decomposition of $(M-R)^+$ by a mechanism such as:



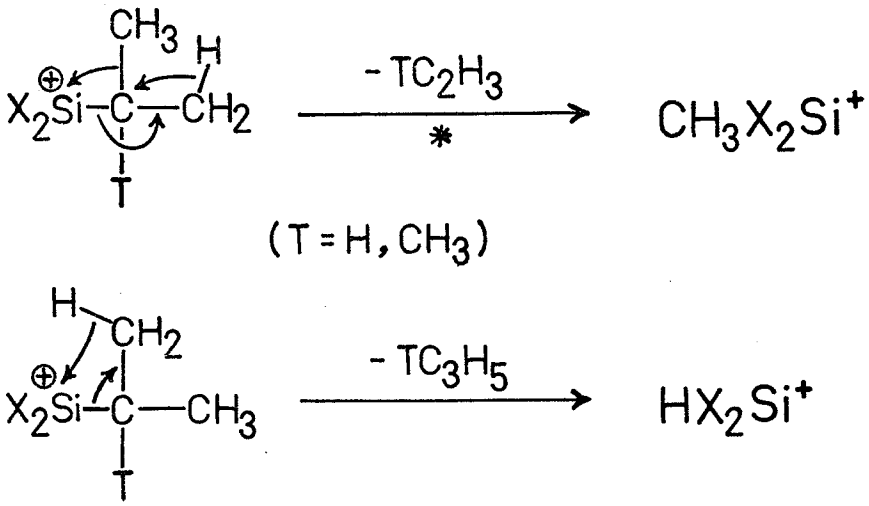
For convenience, these ions are designated as $H(X_2SiOCH_2)^+$.

Another ion that probably has $YOCH_2^+$ as precursor is $CH_3(X_2SiOCH_2)^+$. This can be derived by a mechanism similar to that proposed for $CH_3X_2SiOSiX_2^+$ (Scheme 40):



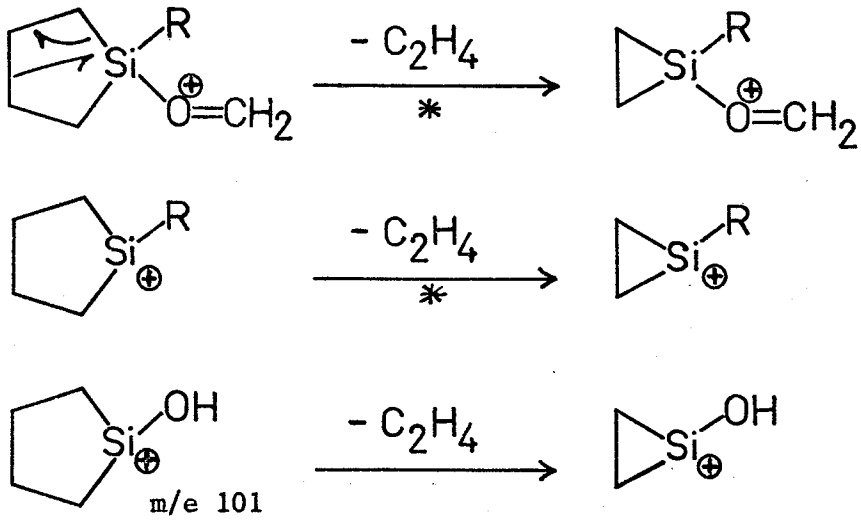
RX_2Si^+	[XII.48 and XV.37, Iba and Ibb - m/e115]
$CH_3X_2Si^+$	[XII.64 and XV.46, Iba and Ibb - m/e73]
HX_2Si^+	[XII.67 and XV.47, Iba and Ibb - m/e59]

These ions are observed in the spectra of all SCTASi derivatives. RX_2Si^+ is presumably formed by simple fission of a silicon-oxygen bond, and the others result from rearrangement of the R group as shown below. $CH_3X_2Si^+$ is most abundant, especially for TBDMSi derivatives (m/e73, Me_3Si^+) where it is often the base peak. A metastable peak for several derivatives signifies RX_2Si^+ as the precursor.



d) Fragmentations of the tetramethylene ring

A number of fragments are unique for TMIPSi and TMTBSi derivatives and involve fragmentation of the cyclo-tetramethylene ring on the silicon, with elimination of the alkene C_2H_4. These ions are usually not very prominent, but sometimes they can interfere with other ions, especially in the low m/e range. Some common fragment ions that are observed are listed below. Analogous fragmentations are observed in the mass spectra of germa-cyclopentanes where only C-2 and C-3 are incorporated into the ethylene produced (Ge is position 1) (245).



R = iPr, m/e 129
= tBu, m/e 143

R = iPr, m/e 99
= tBu, m/e113

m/e 73

C. SCTASi Derivatives of Ribonucleosides

1. Results

The mass spectral data for partial, mixed, and fully O-silylated derivatives of ribonucleosides, and a discussion of the general aspects of the mass spectra, are presented in this section. Following this, Section C2 provides detailed interpretations of the principal fragment ions of 2',3',5'tris-O-silyl derivatives.

a) Partial-O-SCTASi Derivatives

For ribonucleosides, the partial O-TBDMSi derivatives of uridine only have been studied. Since detailed labelling experiments have not been performed, any interpretations must be considered tentative and mechanisms will not be discussed.

Figures 72 to 74 present the spectra of the three mono-TBDMSi-uridine derivatives, VIbaa, VIaba, and VIaab, while Figures 75 to 77 give the spectra of the three bis-TBDMSi isomers, VIbba, VIbab, and VIabb. All six compounds have distinctive spectra. Table XVII summarizes the important ions in the spectra and gives some tentative assignments.

Although these uridine derivatives performed well during mass spectrometry, the direct analysis of partial derivatives in general is not entirely suitable. The migration of the 2'- or 3'-O-silyl group occurs during GC, preventing the application of GC/MS, and there is always the possibility of a slight amount of thermally induced or electron-impact induced migration of silyl groups during analysis by solid probe mass spectrometry. Further derivatization appears to be the best general approach to this problem. In addition, the application of GC and MS to the partial derivatives of the more labile nucleosides (cytidine and guanosine) will definitely require this approach.

b) Mixed Ac/TBDMSi Derivatives

Treatment of the partial O-TBDMSi uridines with acetic anhydride yielded mixed Ac/TBDMSi derivatives with good GC behavior (some isomers being separable), stability for TLC isolation, and very distinctive mass spectra (Figures 78 to 83). Table XVIII summarizes the important ions and tentative assignments. Acetylation appears to be the best procedure for characterizing the partial O-TBDMSi uridines by GC/MS. The suitability for other nucleosides still needs to be examined.

c) Mixed TMSi/TBDMSi Derivatives

Trimethylsilylation of the partial O-TBDMSi uridines gave mixed TMSi/TBDMSi derivatives. Although isomers could not be separated by GC, they have good volatility (which should allow extension to the more labile nucleoside derivatives), and GC/MS-SIR should be feasible considering their distinctive mass spectra (Figures 84 to 89). Table XIXc provides detailed assignments of major fragment ions. These data were invaluable for the elucidation of the tris-O-SCTASi-ribonucleosides' fragmentation mechanisms.

d) 2',3',5'tris-O-SCTASi Derivatives

The spectra of per-O-SCTASi ribonucleosides (Figures 90 to 94) demonstrate the potential value of these derivatives for gas phase analytical chemistry. The spectrum of 2',3',5'tris-O-TBDMSi-uridine (VIbbb, Figure 90) is typical, with a very abundant $[M-R]^+$ ion and a number of medium intensity fragment ions that provide considerable structural information. The latter is substantiated by the sensitivity of the fragmentation pattern towards changes in the base, as demonstrated by the spectra of VIbbb and VIIbbb (Figures 90 and 93), and substitution in the sugar ring, as demonstrated by the partial TBDMSi and mixed Ac/TBDMSi and TMSi/TBDMSi uridine derivatives.

The analogous 2',3',5'tris-O-TMSi derivatives of uridine (VIeee) and 5-methyluridine (VIIeee), on the other hand, have spectra that are dominated by sugar fragments (especially m/e217) and that are rather insensitive to changes in the base. It should be noted that trimethylsilylation is usually performed at high temperatures to yield the 2',3',5',4-tetrakis-O-TMSi derivative, VIeeee, that has a spectrum (Figure 97) not very different from that of VIeee. (The tetrakis-O-SCTASi derivatives could not be formed (see Chapter V).)

It is interesting to examine the spectrum of VIbbb at low electron energy (20eV, Figure 98). While the relative abundance of $[M-R]^+$ is increased dramatically (useful for SIR work), several ions (m/e147, 133, 101, 99, 75, and 73) are suppressed. This usually indicates that the latter ions are formed in multistep fragmentations requiring high internal energy.

Tables XIXa and XIXb present detailed assignments of ion types for the various derivatives studied. Fragmentation mechanisms are discussed in Section C2.

e) N,O-perSCTASi Derivatives

The fully N,O-silylated ribonucleosides also have potential analytical value. The spectra of 2',3',5'tris-O-TBDMSi-N6-TBDMSi-adenosine, VIIIbbbb (Figure 99), and 2',3',5'tris-O-TBDMSi-N6-TMSi-adenosine, VIIIbbbe, (Figure 100), have very abundant $[M-R]^+$ ions. The former also has the $[B'+H-R_{N6}]^+$ fragment ion that was observed for the analogous deoxyadenosine derivative (Scheme 18). Figure 101 gives the spectrum of the TMSi derivative, VIIIeeee, for comparison.

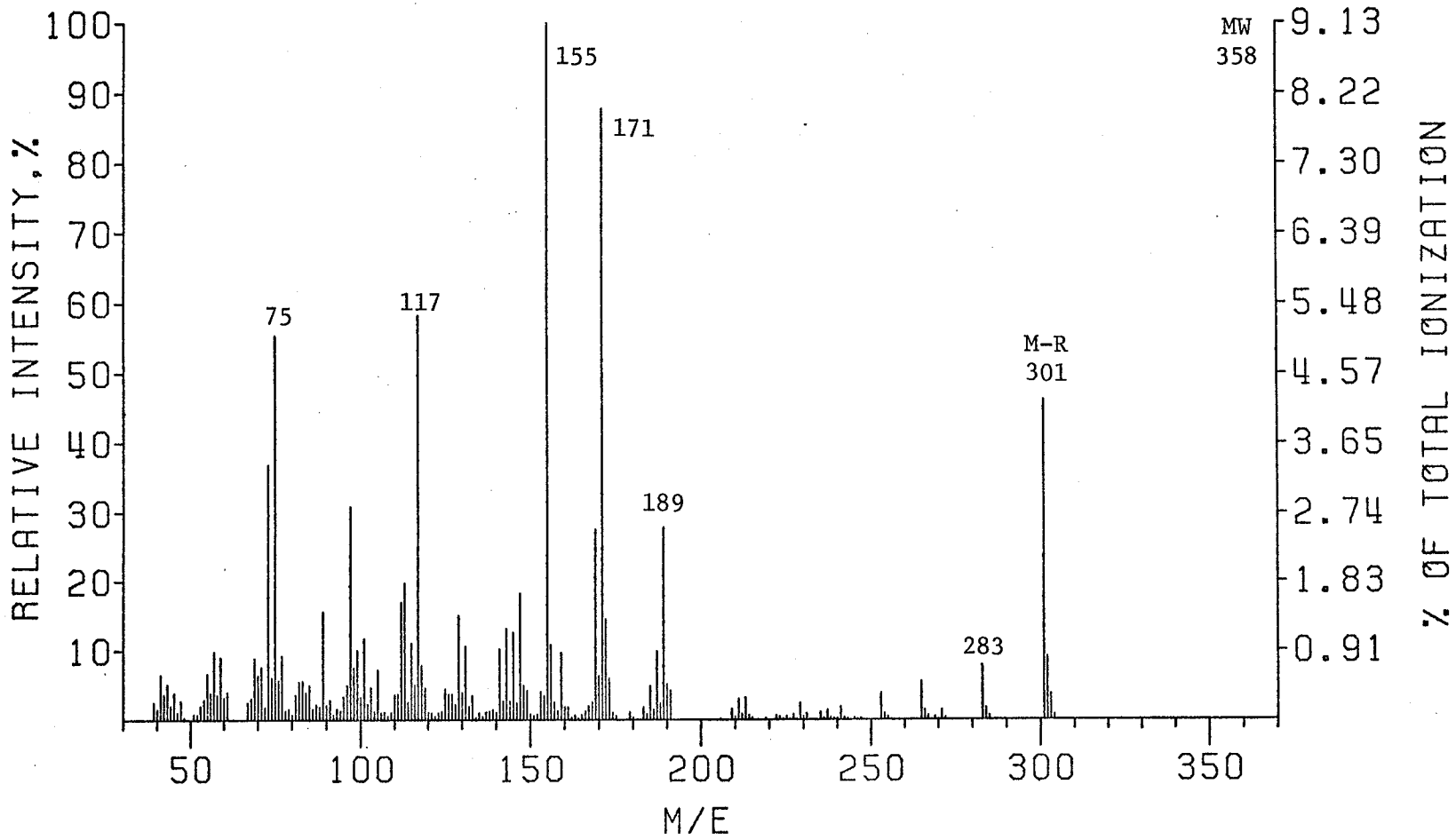


Figure 72. Mass spectrum of 5'-O-TBDMSi-uridine (VIbaa) recorded on Finnigan MS at 70 eV.

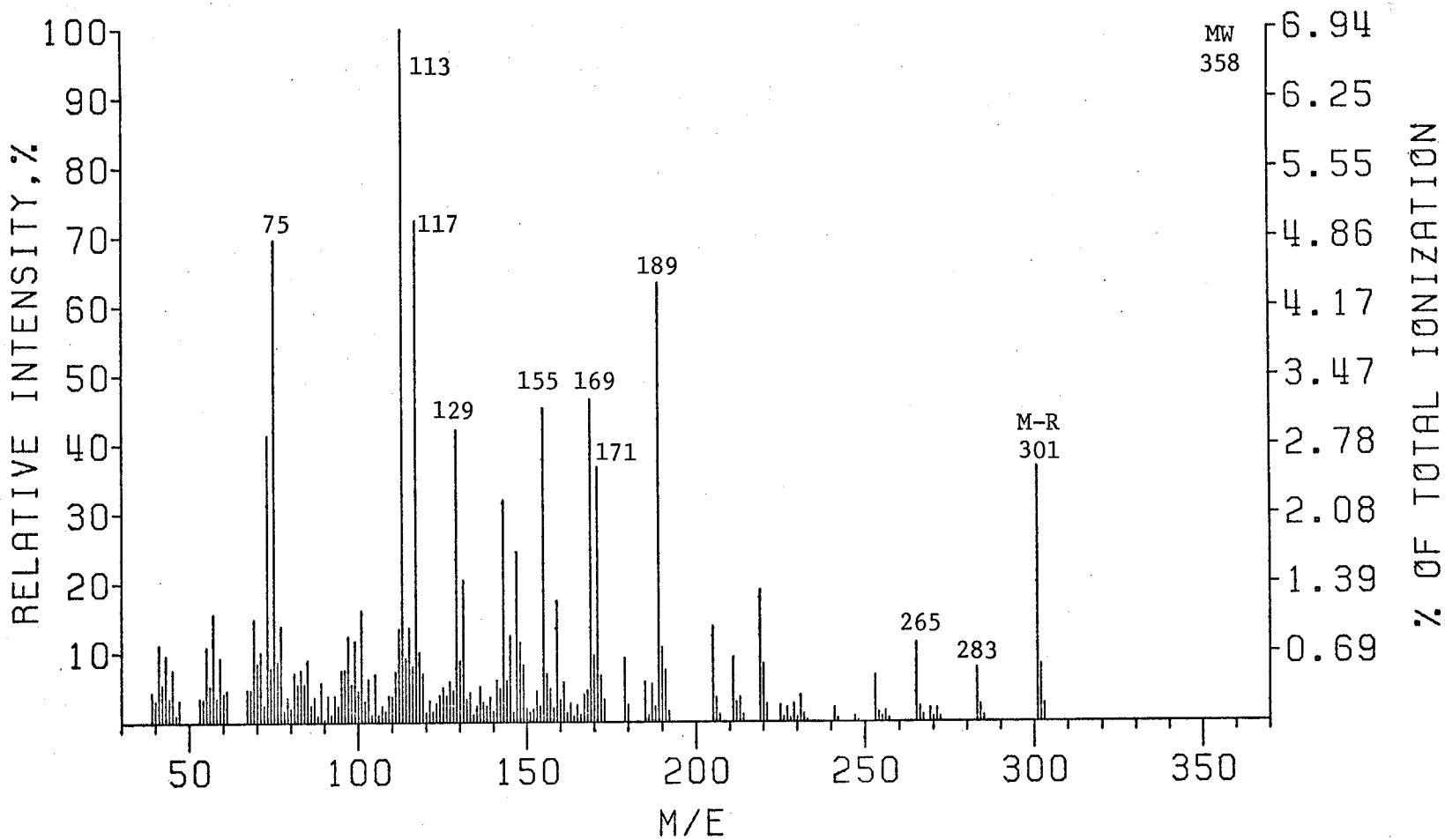


Figure 73. Mass spectrum of 3'-O-TBDMSi-uridine (VIaba) recorded on Finnigan MS at 70 eV.

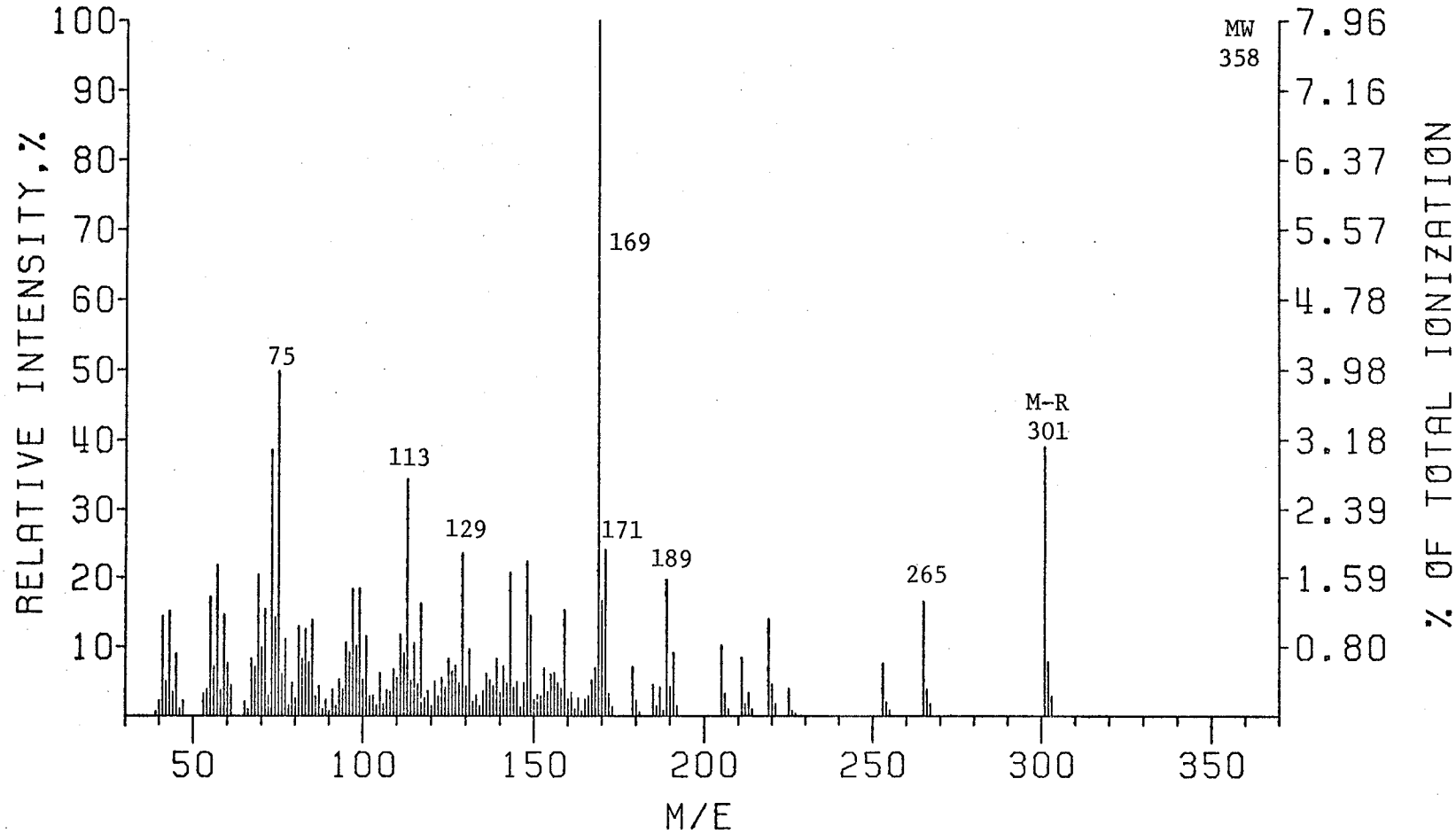


Figure 74. Mass spectrum of 2'-O-TBDMSi-uridine (VIaab) recorded on Finnigan MS at 70 eV.

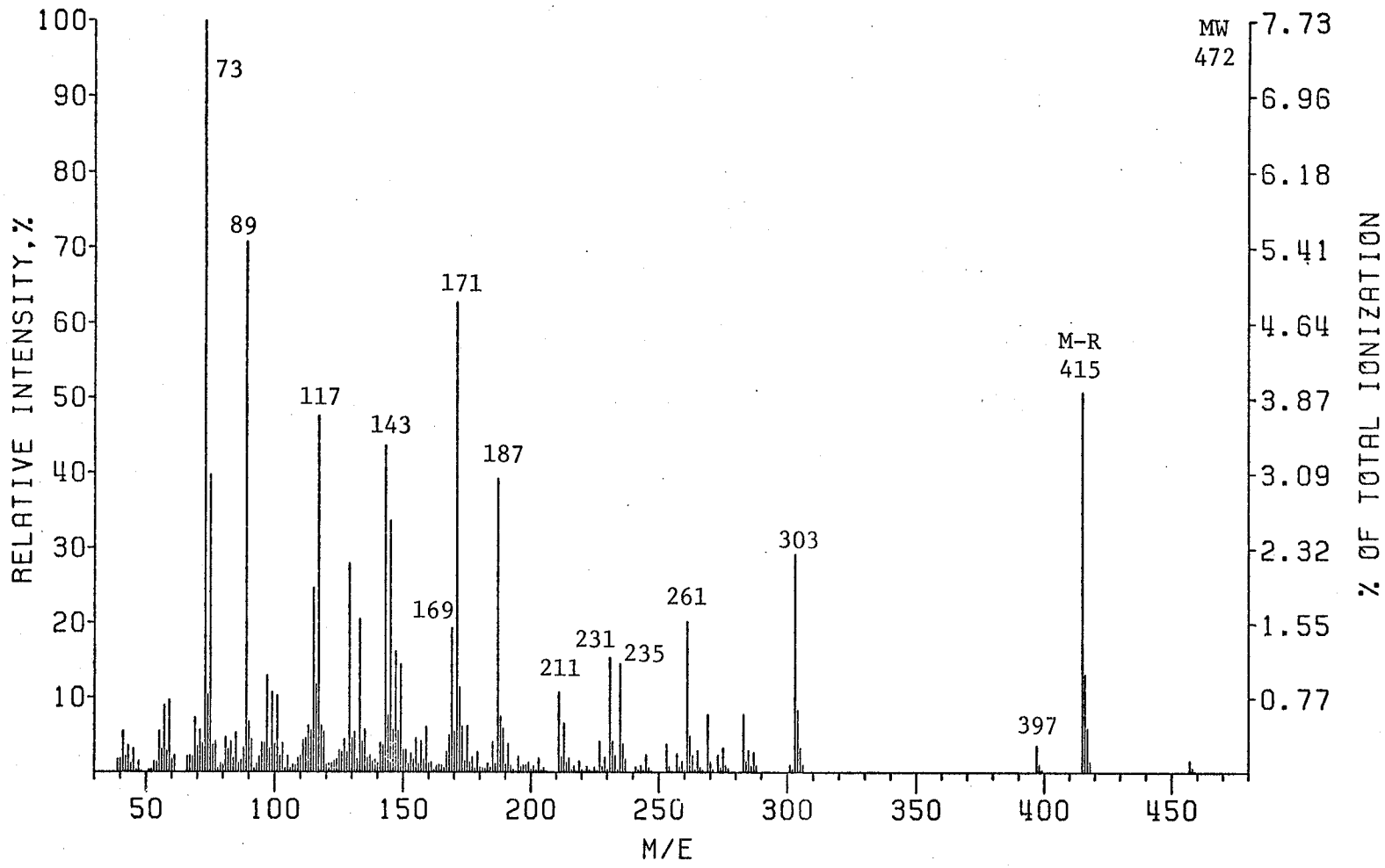


Figure 75. Mass spectrum of 3',5'bis-O-TBDMSi-uridine (VIbba) recorded on Finnigan MS at 70 eV.

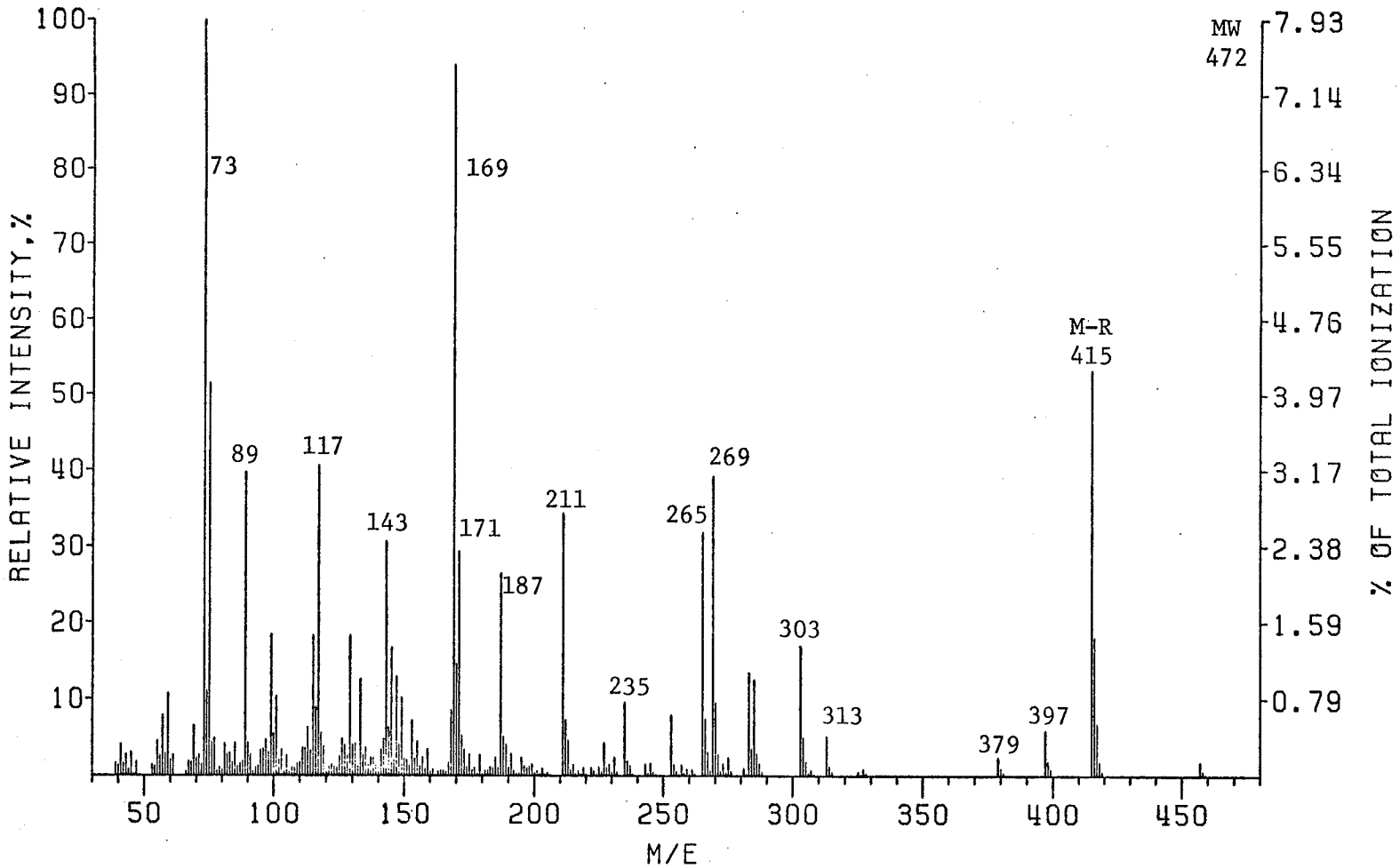


Figure 76. Mass spectrum of 2',5'bis-O-TBDMSi-uridine (VIbab) recorded on Finnigan MS at 70 eV.

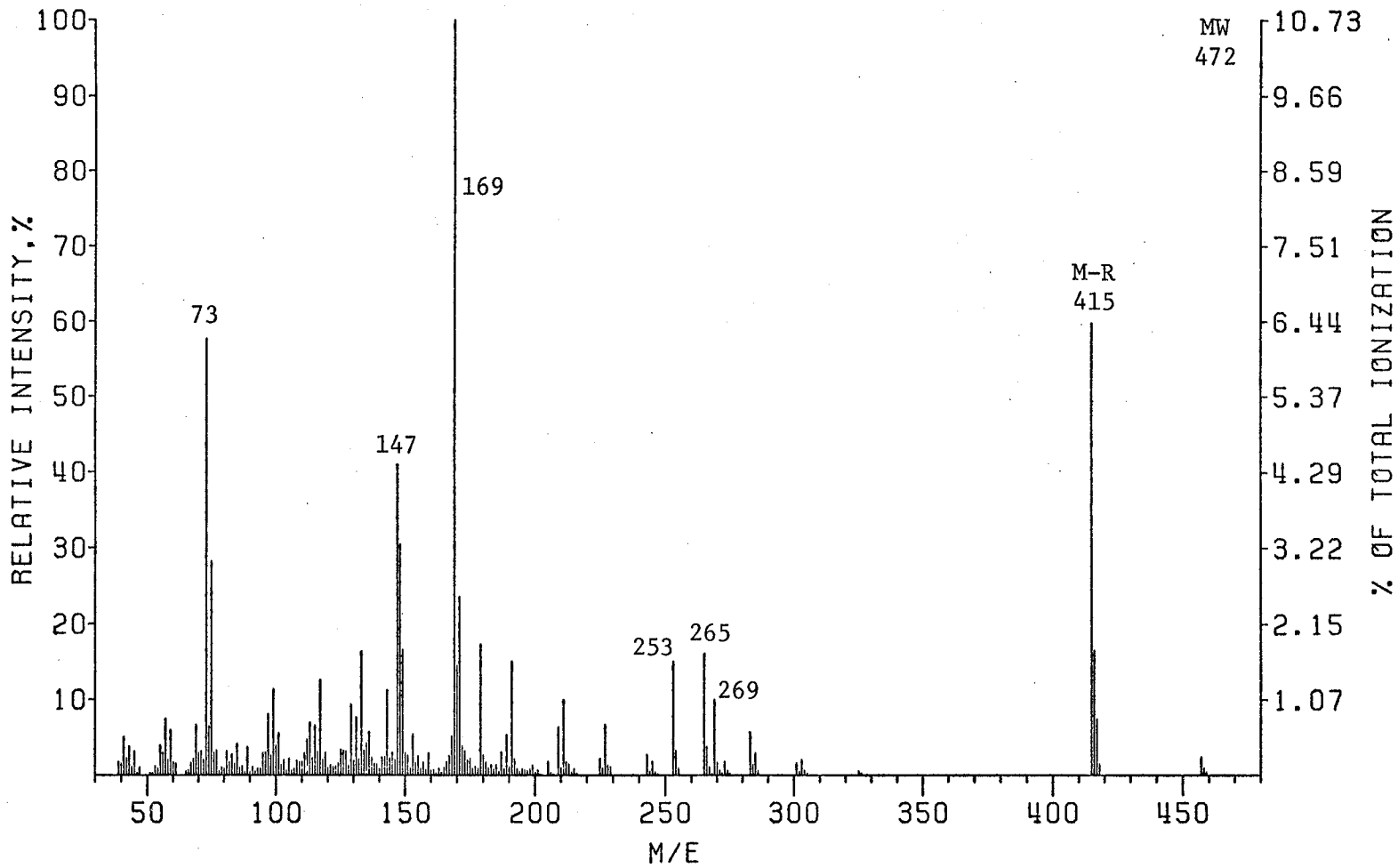


Figure 77. Mass spectrum of 2',3'bis-O-TBDMSi-uridine (VIabb) recorded on Finnigan MS at 70 eV.

TABLE XVII. Important fragment ions in the spectra of partial O-TBDMSi derivatives of uridine (VI).[a]

Assignment	m/e	mono-TBDMSi VIbaa	VIaba	VIaab	m/e	bis-TBDMSi VIbba	VIbab	VIabb
M	358	0	0	0	472	0	0	0
M-R	301	46.	37.	39.	415	51.	53.	60.
$M-R-H_2O$	283	7.7	7.8	0	397	3.5	5.8	0
$M-R-RX_2SiOH$	NA				283	7.8	13.	5.6
$M-R-2H_2O$	265	5.6	11.	16.	379	0	2.3	0
$M-R-RX_2SiOH-H_2O$	NA				265	2.9	32.	16.
$M-R-H_2O-CH_2O$	253	3.9	6.7	7.7	367	0	0	0
$B+CHO+X_2Si+RX_2Si$	NA				313	0	5.1	0
M-R-BH	189	28.	63.	20.	303	29.	17.	2.0
$M-R-BH-H_2O$	171	88.	37.	24.	285	2.9	12.	2.9
$M-R-BH-RX_2SiOH$	NA				171	63.	29.	24.
(J+H) $BC_2H_3ORX_2Si$	269	0.5[b]	1.8[b]	0	269	7.7	39.	10.
(J+H) BC_2H_4O	155	100.	45.	5.9	155	4.5	4.4	2.6
$C_5H_4O_2RX_2Si$ and $(J-R_W)$	211	3.0	9.4	8.4	211	11.	34.	10.
$B+C_5H_4O(80)$	191	4.3[b]	7.5[b]	9.2	191	3.8	2.8	15.
$B+X_2Si+H_2O$	187	9.9	5.5	4.2	187	39.	26.	3.0
$B+X_2Si$	169	28.	46.	100.	169	19.	94.	100.
(M-R-J):								
$M-R-BC_2H_3O$	147	18.	25.	4.7	261	20.	0.8	0
$M-R-BC_2H_2ORX_2Si$	NA				147	16.	13.	41.
$CH_3X_2SiOSiX_2$	NA							
$(M-R-J-CH_2O)$:								
$M-R-BC_3H_5O_2$	117	58.	72.	16.	231	15.	2.4	0
$M-R-BC_3H_4O_2RX_2Si$	NA				117	48.	41.	13.
$X_2Si+C_3H_3O_2(71)$	129	15.	42.	24.	129	28.	18.	9.3
B+2H	113	20.	100.	34.	113	6.2	6.3	6.9
HX_2SiOCH_2	89	16.	5.8	2.4	89	71.	40.	3.7
X_2SiOH	75	55.	70.	50.	75	40.	51.	28.
CH_3X_2Si	73	37.	41.	39.	73	100.	100.	58.
$\%\Sigma_{40}$		9.13	6.94	7.96		7.73	7.93	10.7

[a] Data given as relative intensity % as recorded on Finnigan MS at 70eV; [b] Significant isotopic contribution from ion type of lower mass; NA = not applicable

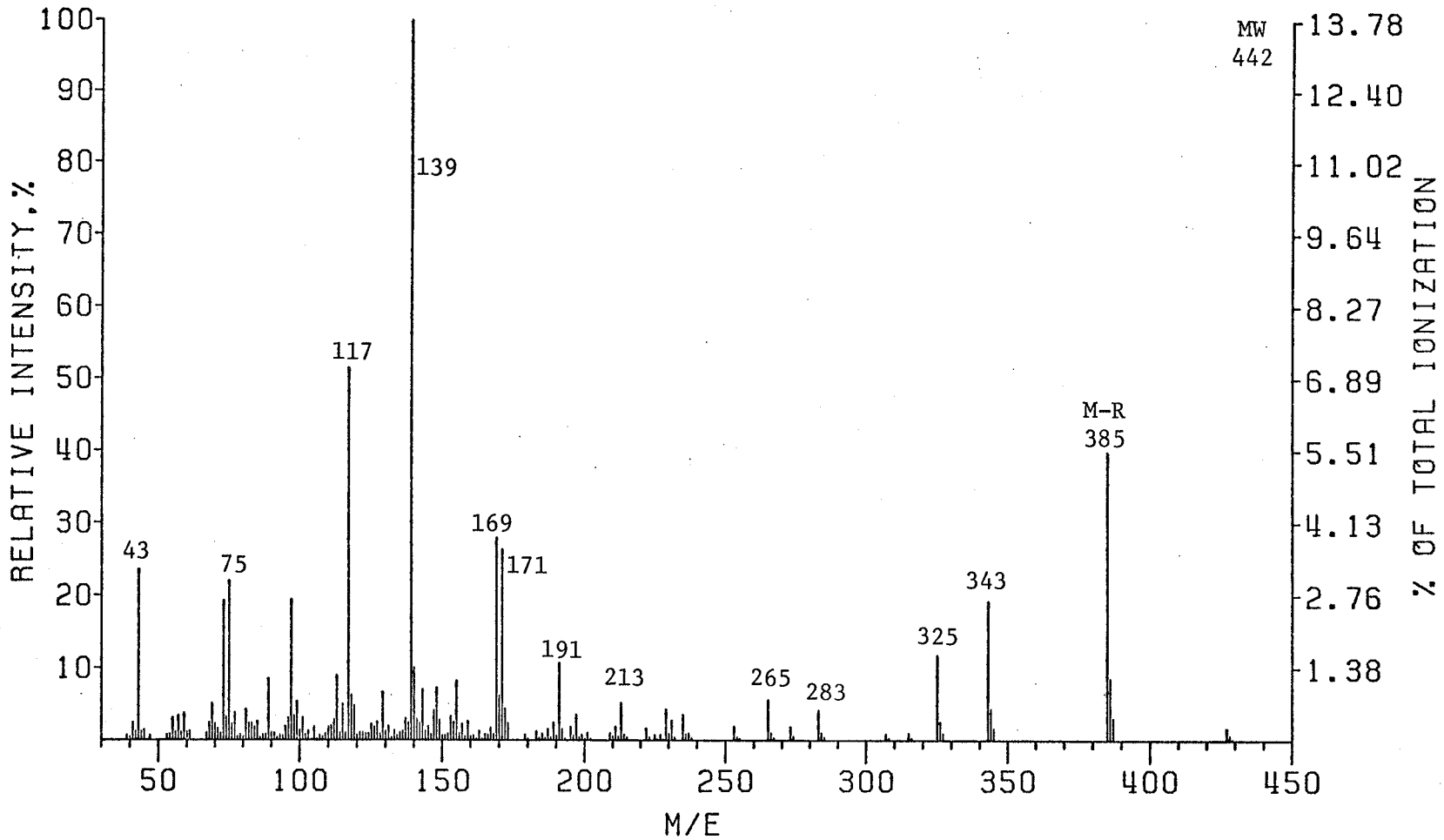


Figure 78. Mass spectrum of 2',3'bis-O-Ac-5'-O-TBDMSi-uridine (VIbgg) recorded on Finnigan MS at 70 eV.

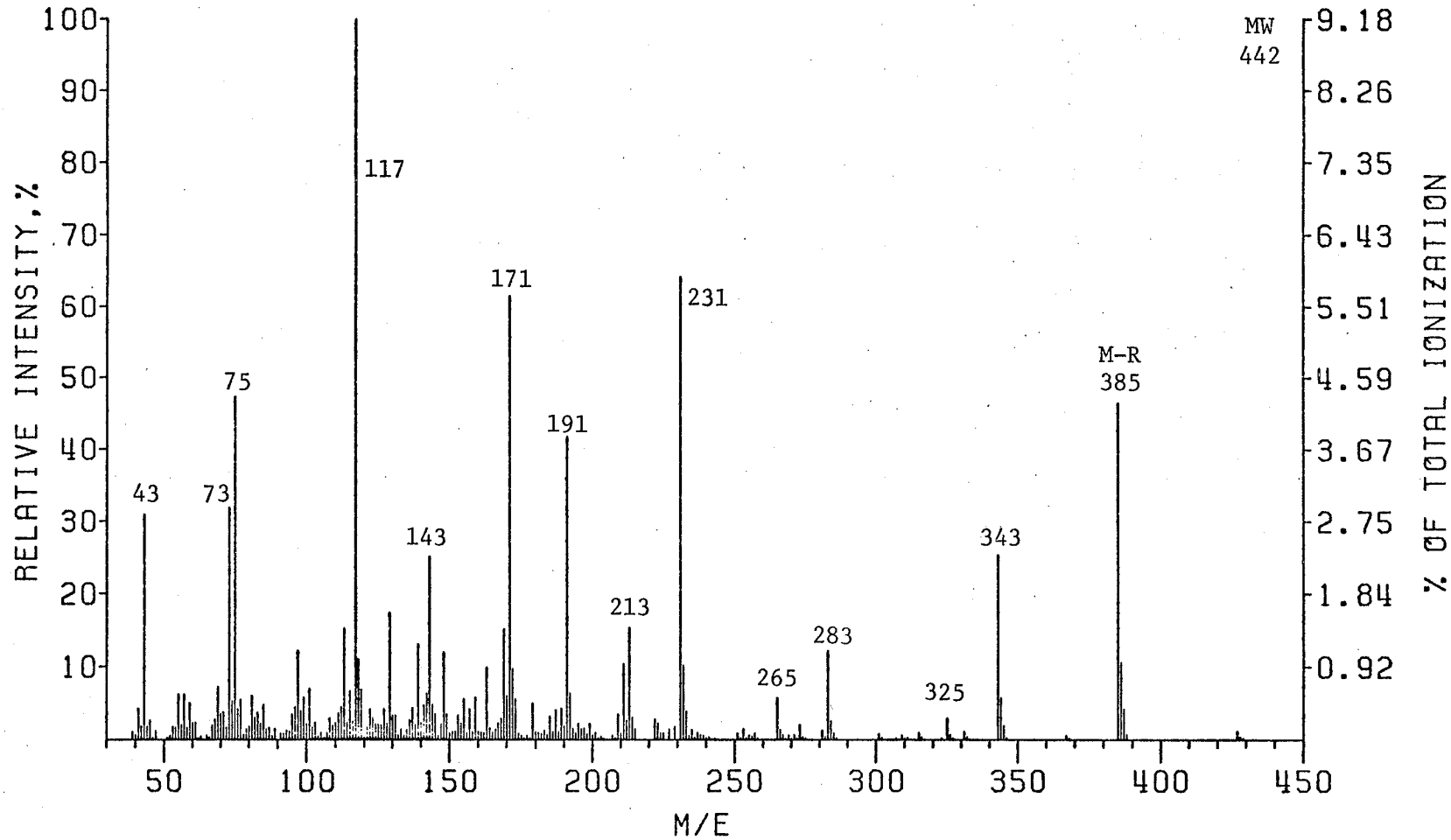


Figure 79. Mass spectrum of 2',5'bis-O-Ac-3'-O-TBDMSi-uridine (VIgbg) recorded on Finnigan MS at 70 eV.

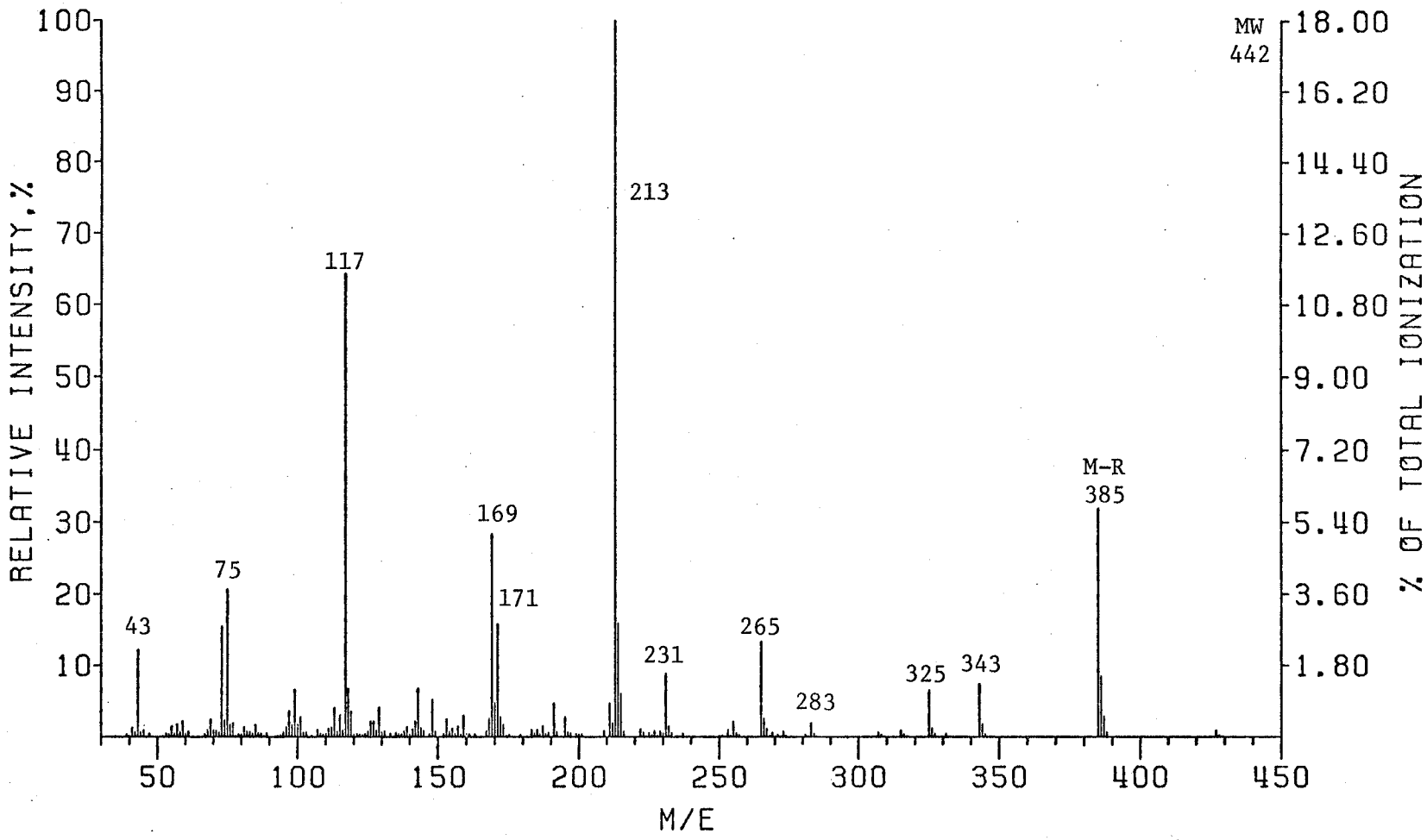


Figure 80. Mass spectrum of 3',5'bis-O-Ac-2'-O-TBDMSi-uridine (VIggb) recorded on Finnigan MS at 70 eV.

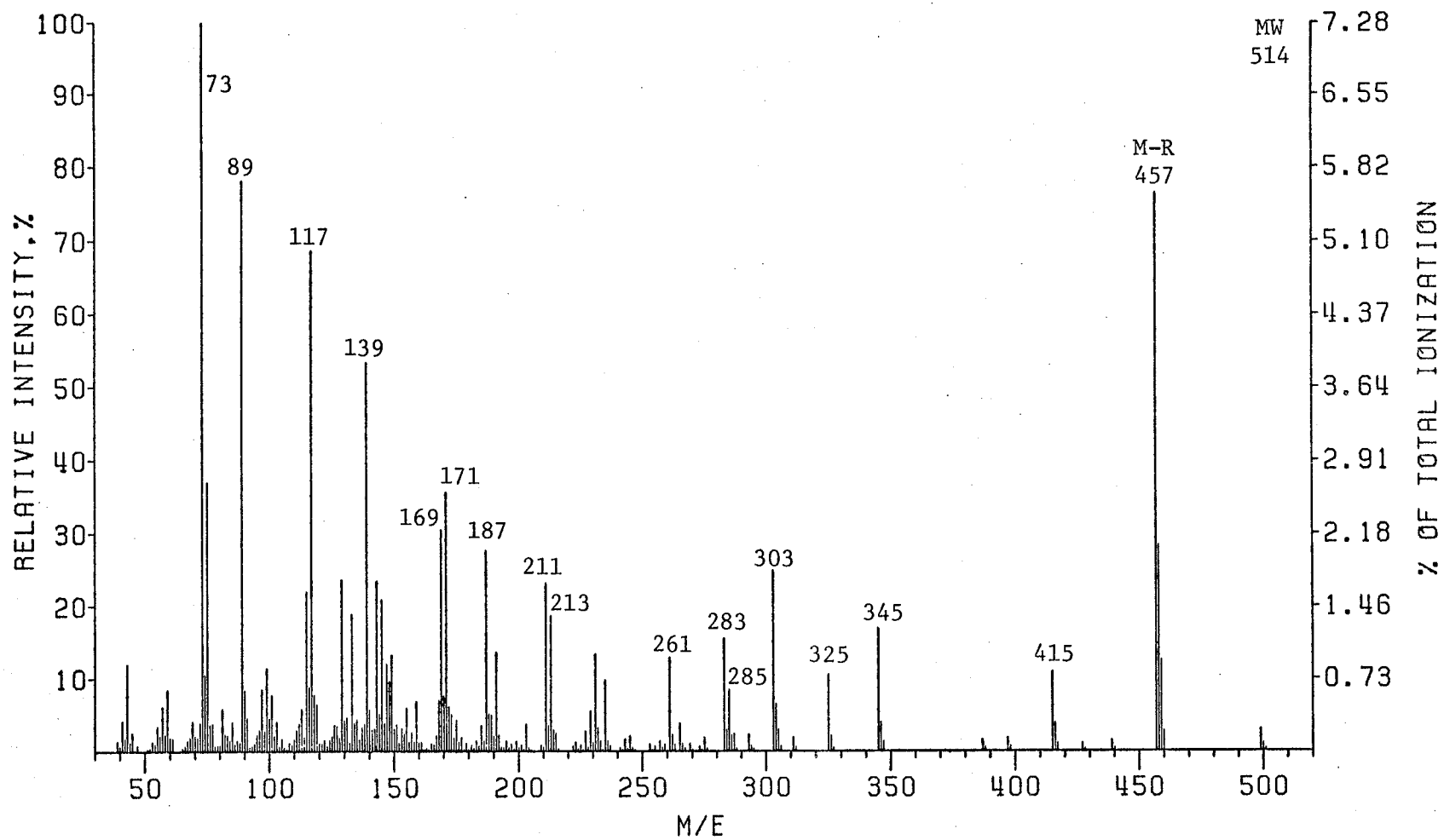


Figure 81. Mass spectrum of 2'-O-Ac-3',5'bis-O-TBDMSi-uridine (VIbbg) recorded on Finnigan MS at 70 eV.

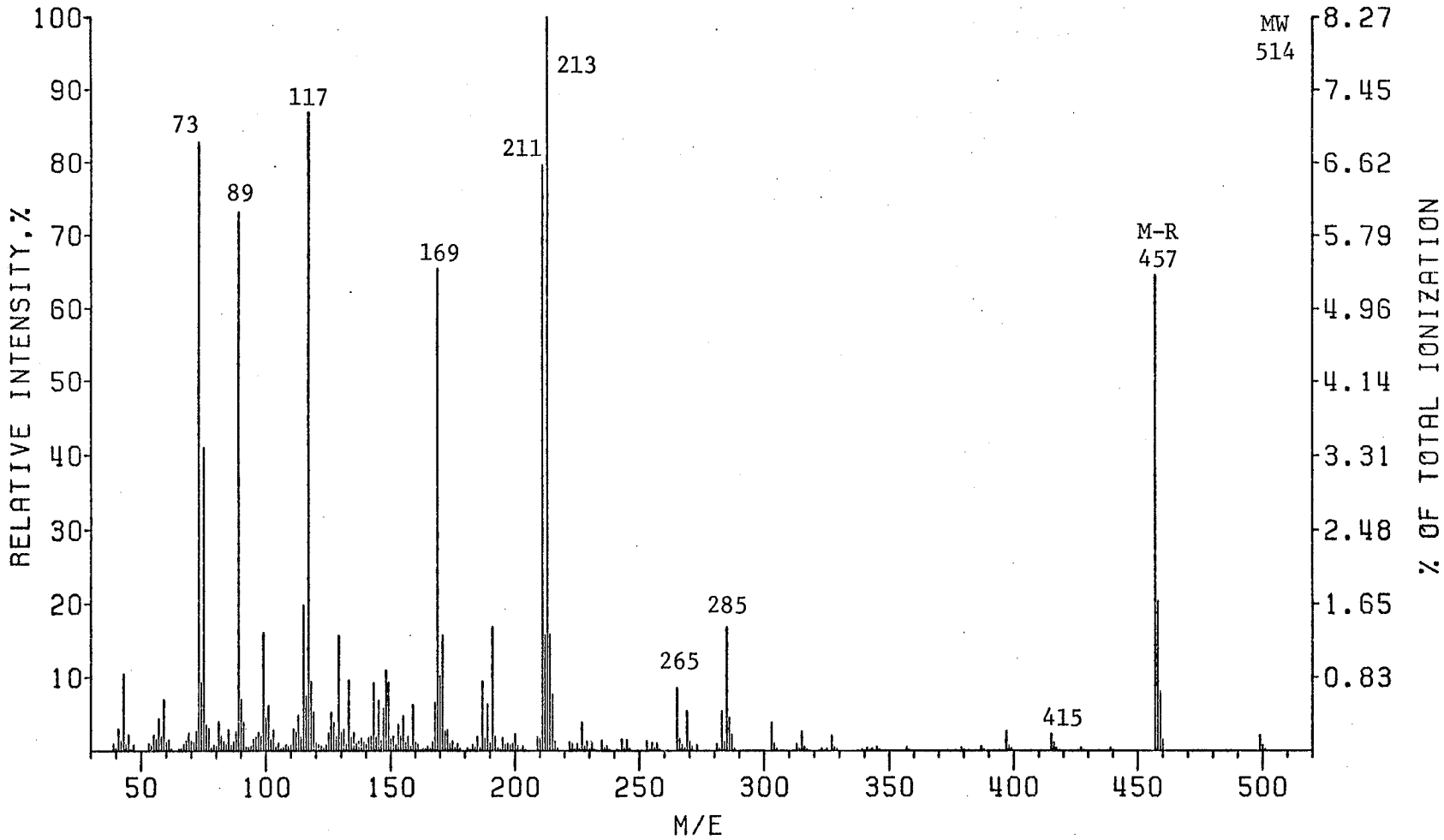


Figure 82. Mass spectrum of 3'-O-Ac-2',5'bis-O-TBDMSi-uridine (VIbgb) recorded on Finnigan MS at 70 eV.

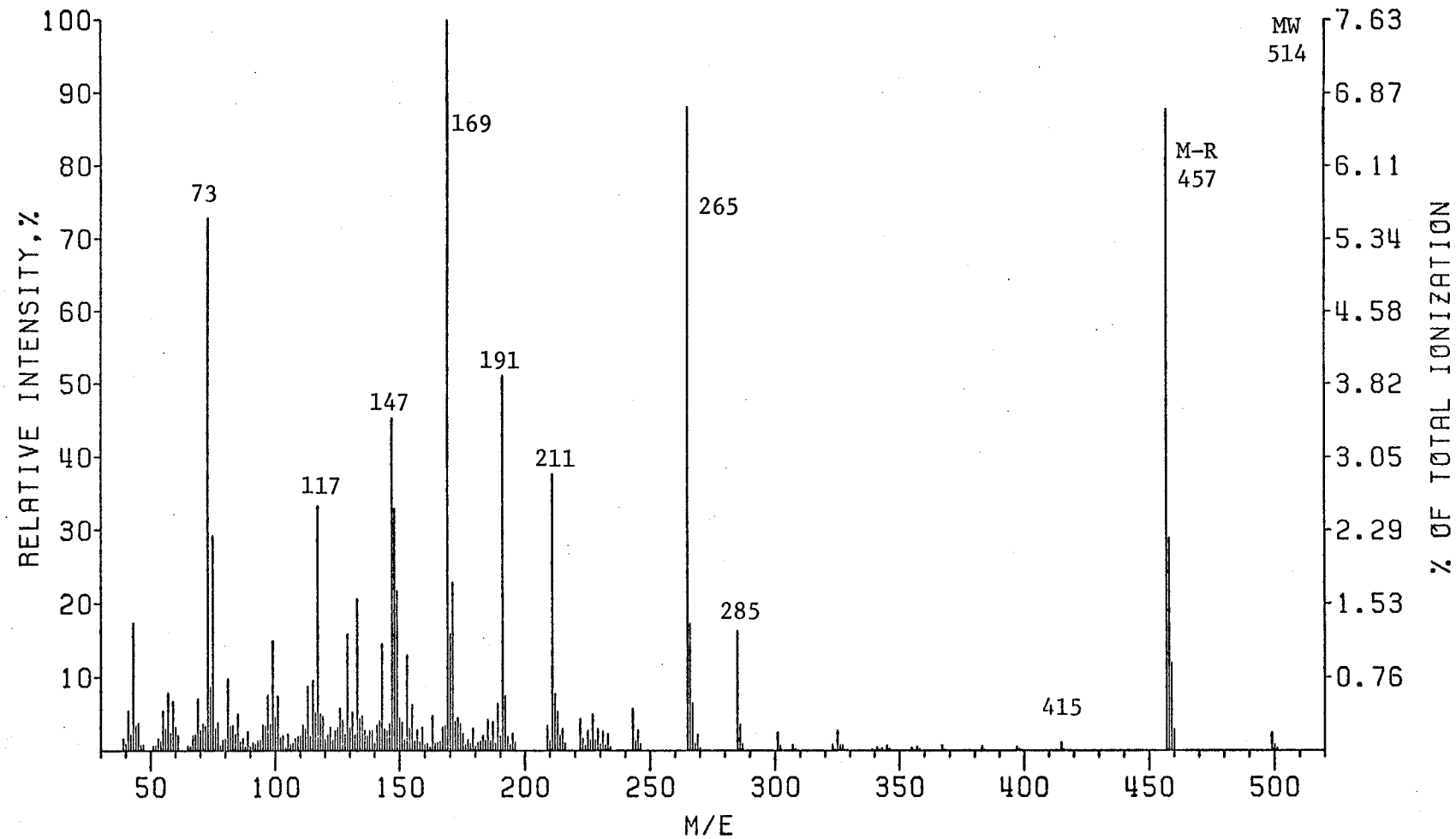


Figure 83. Mass spectrum of 5'-O-Ac-2',3'bis-O-TBDMSi-uridine (VIgbb) recorded on Finnigan MS at 70 eV.

TABLE XVIII. Important fragment ions in the spectra of mixed Ac/TBDMSi derivatives of uridine.[a]

Assignment	m/e	bis-Ac-mono-TBDMSi VIbgg	VIgbg	VIggb	m/e	mono-Ac-bis-TBDMSi VIbbg	VIbgb	VIgbb
M	442	0	0	0	514	0	0	0
M-R	385	40.	46.	32.	457	76.	65.	88.
M-R-CH_2CO	343	19.	25.	7.4	415	11.	2.3	1.1
M-R-AcOH	325	12.	2.9	6.5	397	1.7	2.6	0.6
M-R-AcOH-CH_2O	283	4.2	12.	2.0	NA			
M-R-RX_2SiOH	NA				325	11.	0.4	2.7
M-R-RX_2SiOH-CH_2CO	NA				283	16.	5.3	0
M-R-2AcOH	265	5.6	5.7	13.	NA			
M-R-AcOH-RX_2SiOH	NA				265	3.8	8.5	88.
M-R-BH	273	1.9	2.0	0.8	345	17.	0.5	0.7
M-R-BH-CH_2CO	231	2.8	64.	8.8	303	25.	3.8	0
M-R-BH-AcOH	213	5.2	15.	100.	285	8.4	17.	16.
M-R-BH-AcOH-CH_2CO	171	26.	62.	16.	NA			
M-R-BH-RX_2SiOH	NA				213	19.	100.	5.4[b]
M-R-BH-RX_2SiOH-CH_2CO	NA				171	36.	16.	23.
(M-R-J):								
M-R-BC_2H_2OAc	189	2.5	4.3	0.6	261	13.	0	0
M-R-$BC_2H_2ORX_2Si$	NA				189	5.0[b]	6.3	6.4
$C_5H_4O_2RX_2Si$ and ($J-R_w$)	211	1.9	10.4	4.7	211	23.	80.	38.
B+C_5H_4O(80)	191	11.	42.	4.7	191	14.	17.	51.
B+X_2Si+H_2O	187	1.6	4.1	1.5	187	28.	9.5	3.9
B+X_2Si	169	28.	15.	28.	169	31.	65.	100.
$CH_3X_2SiOSiX_2$	NA				147	12.	5.8	45.
$C_5H_4O_2Ac$	139	100.	13.	1.4	139	53.	1.3	2.8
X_2SiOAc	117	51.	100.	64.	117	69.	87.	33.
HX_2SiOCH_2	89	8.5	1.5	0.5	89	78.	73.	2.7
X_2SiOH	75	22.	47.	21.	75	37.	41.	29.
CH_3X_2Si	73	19.	32.	16.	73	100.	83.	73.
CH_3CO	43	24.	31.	12.	43	12.	10.	17.
$\%\Sigma_{40}$		13.8	9.18	18.0		7.28	8.28	7.63

For footnotes see TABLE XVII.

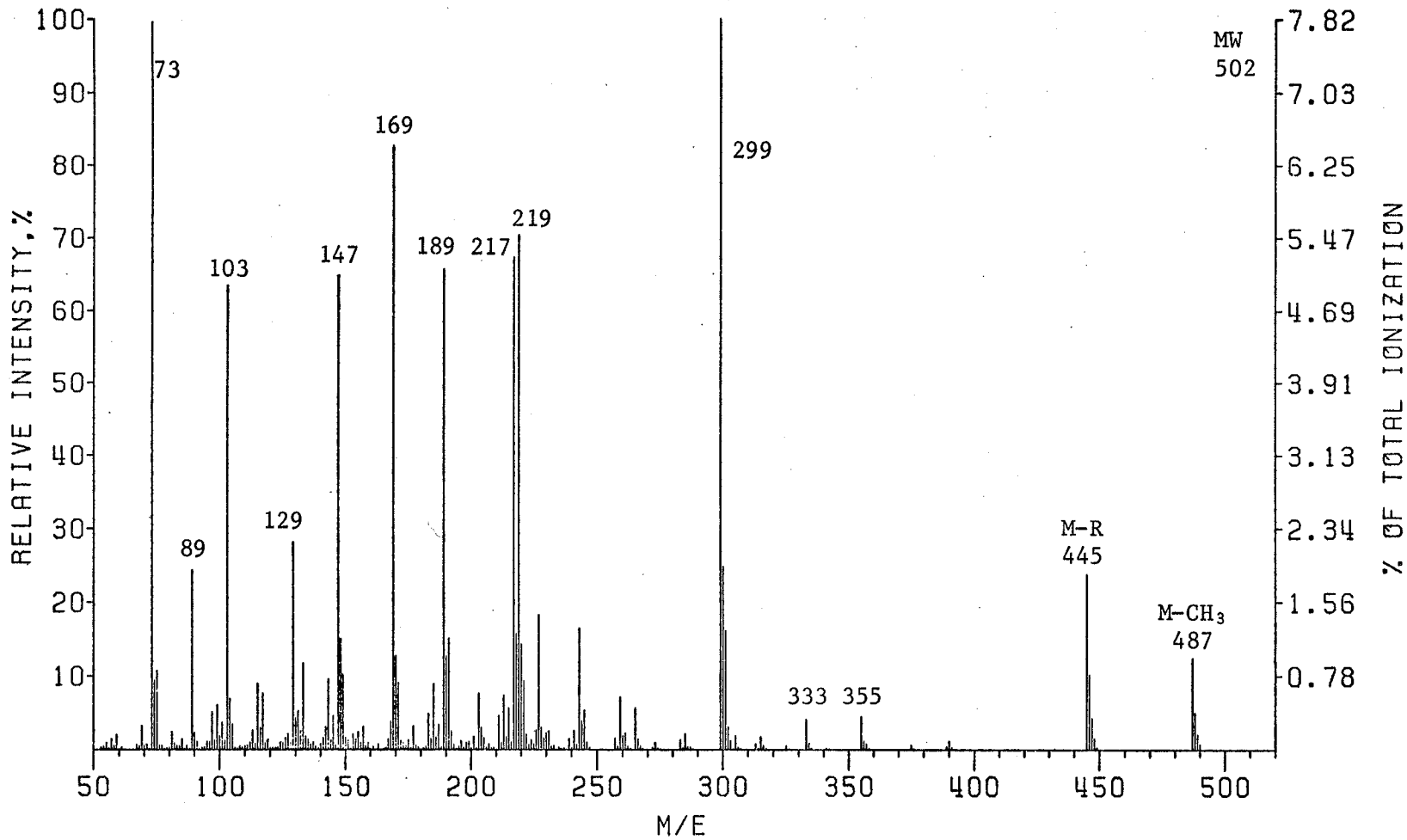


Figure 84. Mass spectrum of 2',3'bis-O-TMSi-5'-O-TBDMSi-uridine (VIbee) recorded on Finnigan MS at 70 eV.

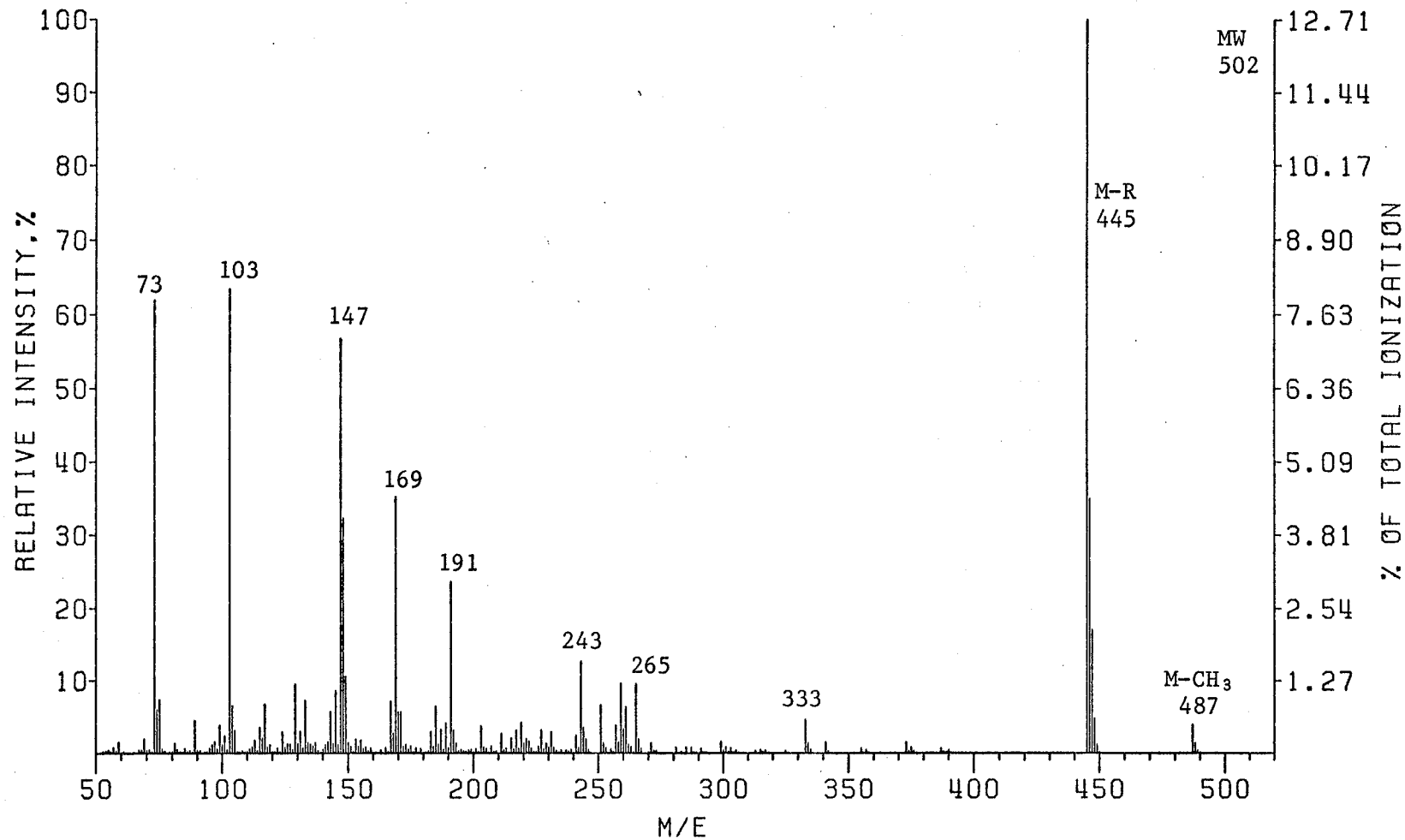


Figure 85. Mass spectrum of 2',5'bis-O-TMSi-3'-O-TBDMSi-uridine (VIebe) recorded on Finnigan MS at 70 eV.

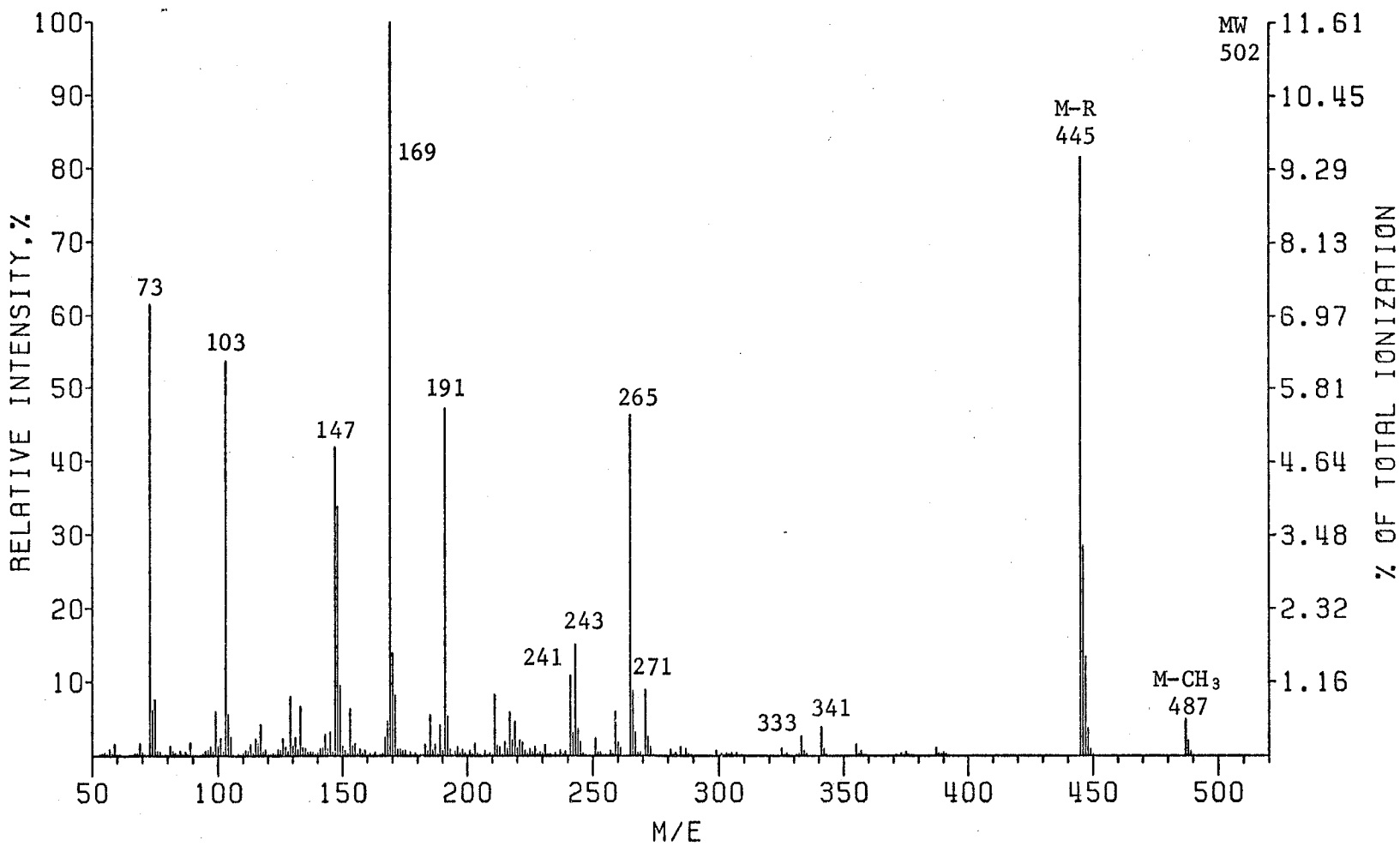


Figure 86. Mass spectrum of 3',5'bis-O-TMSi-2'-O-TBDMSi-uridine (VIeeb) recorded on Finnigan MS at 70 eV.

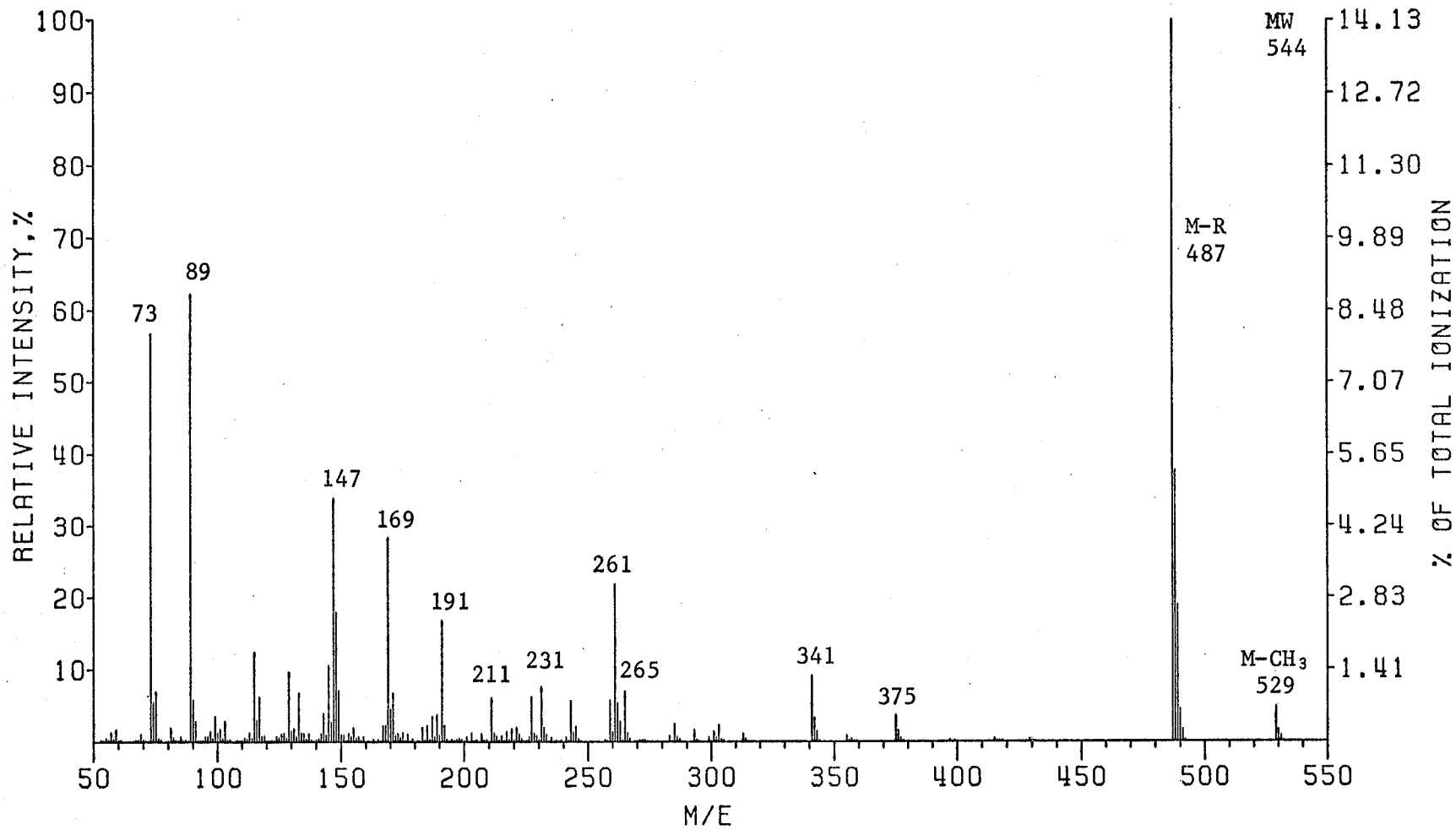


Figure 87. Mass spectrum of 2'-O-TMSi-3',5'bis-O-TBDMSi-uridine (VIbbe) recorded on Finnigan MS at 70 eV.

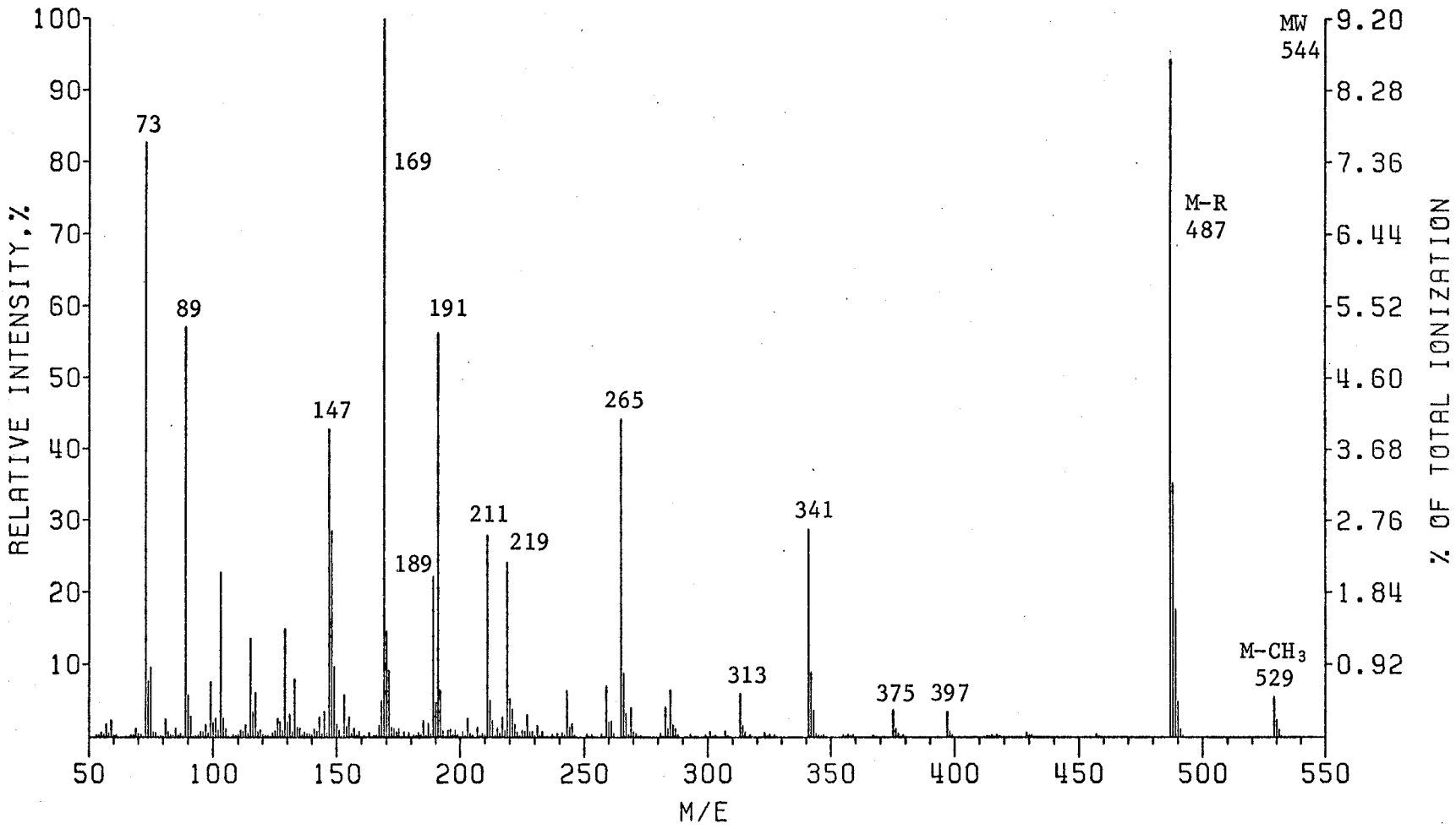


Figure 88. Mass spectrum of 3'-O-TMSi-2',5'bis-O-TBDMSi-uridine (VIbeb) recorded on Finnigan MS at 70 eV.

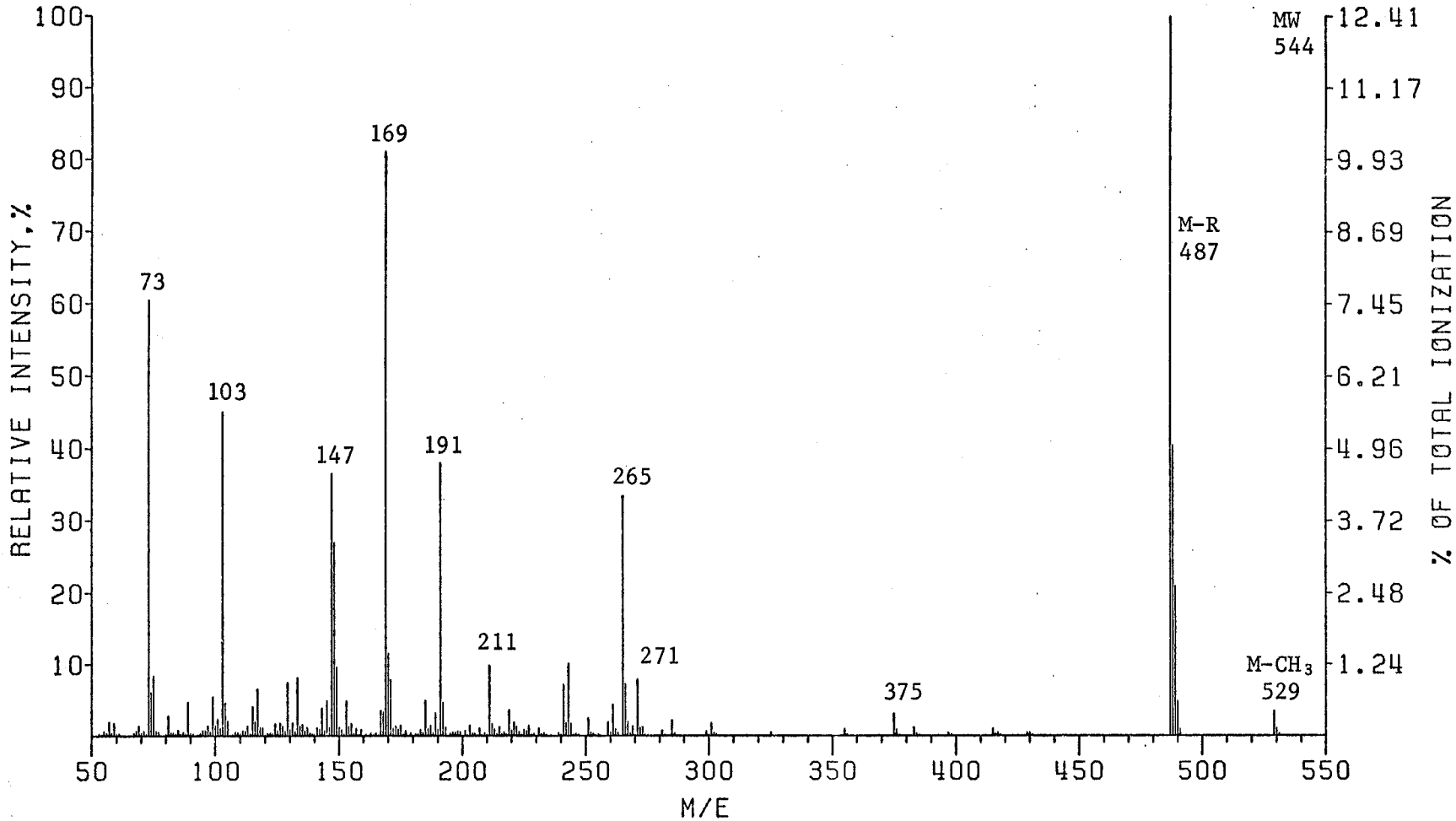


Figure 89. Mass spectrum of 5'-O-TMSi-2',3'bis-O-TBDMSi-uridine (VIebb) recorded on Finnigan MS at 70 eV.

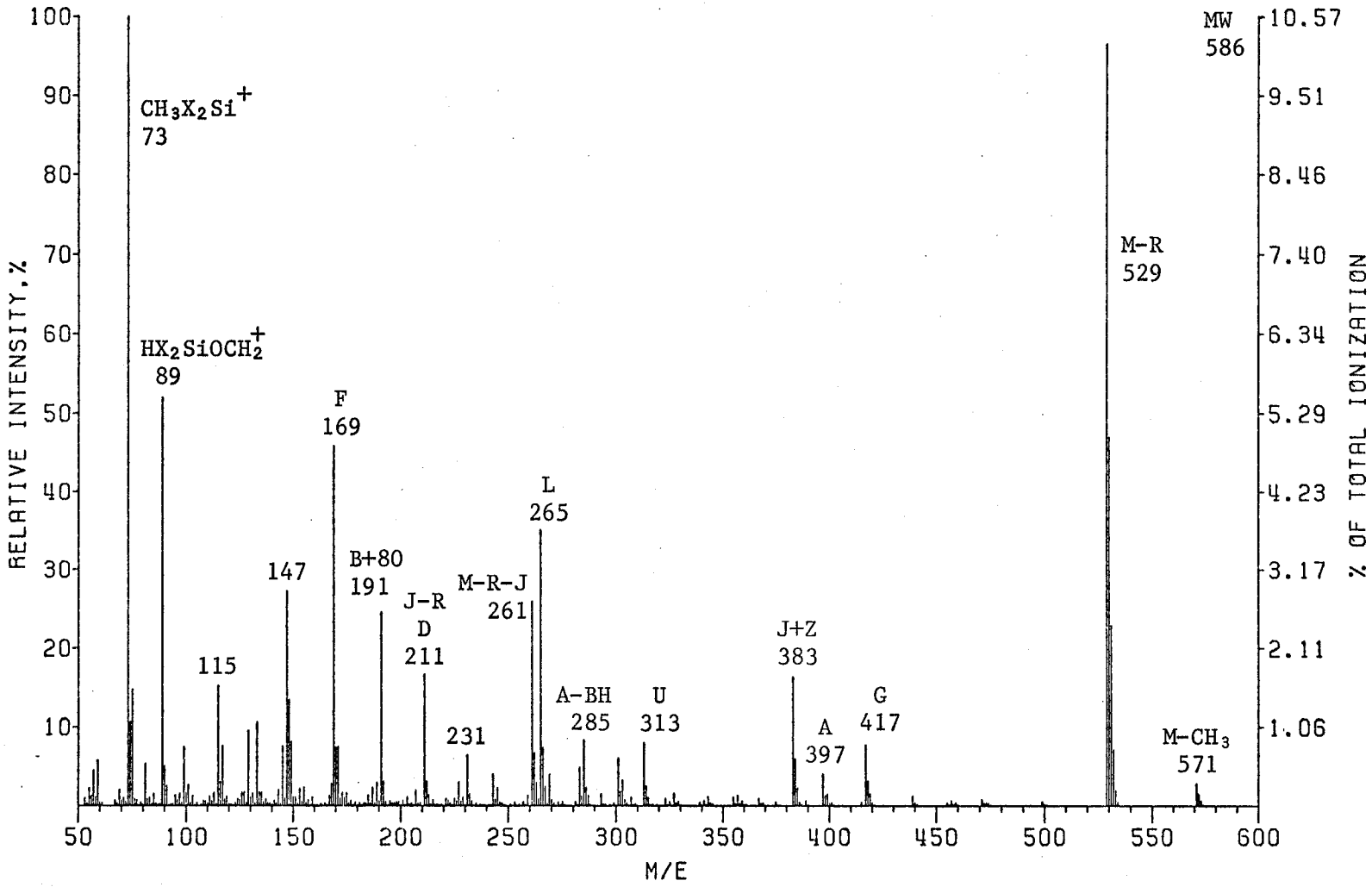


Figure 90. Mass spectrum of 2',3',5'tris-O-TBDMSi-uridine (VIbbb) recorded on Hitachi MS at 50 eV.

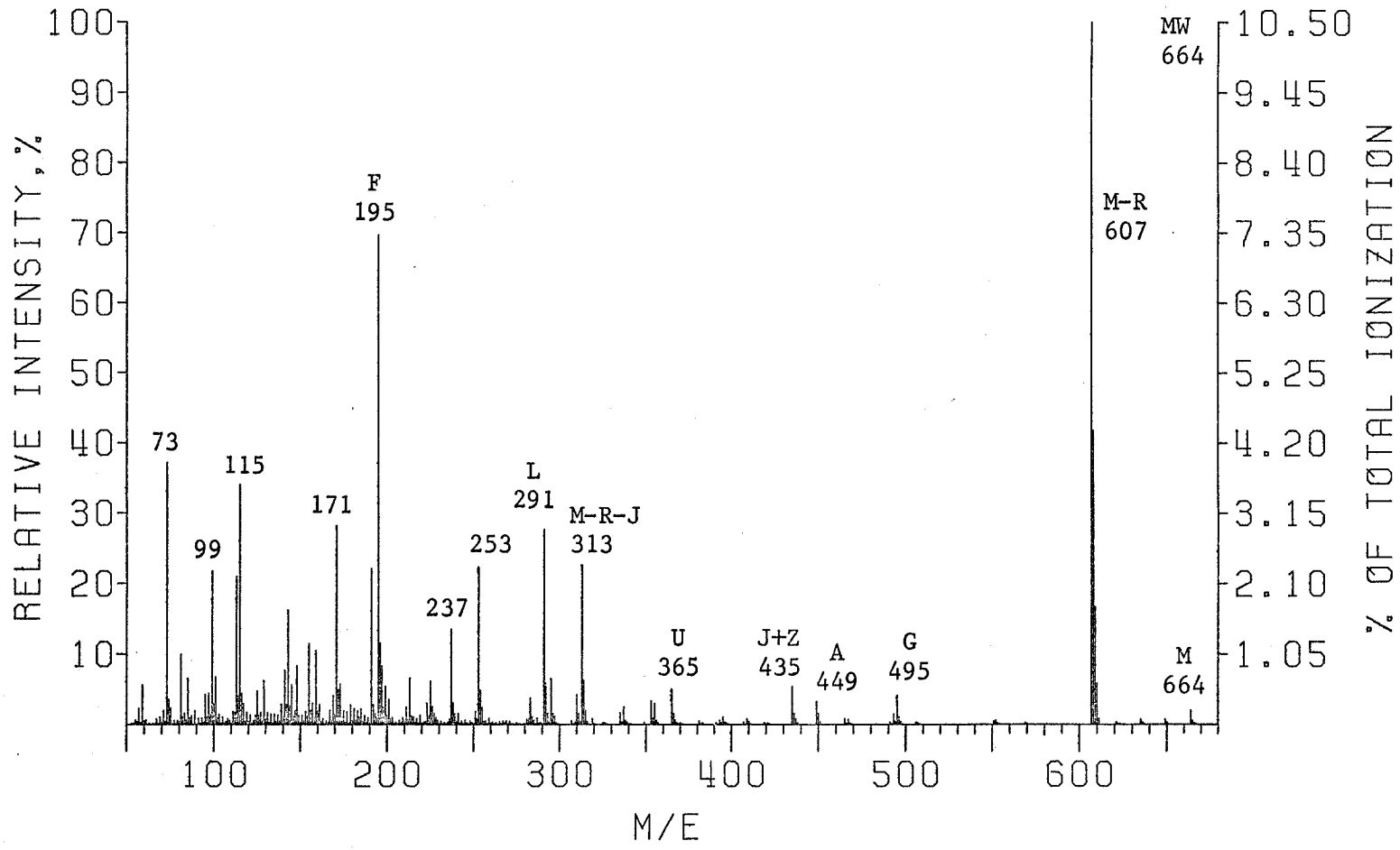


Figure 91. Mass spectrum of 2',3',5'tris-O-TMTBSi-uridine (VIccc) recorded on Finnigan MS at 70 eV.

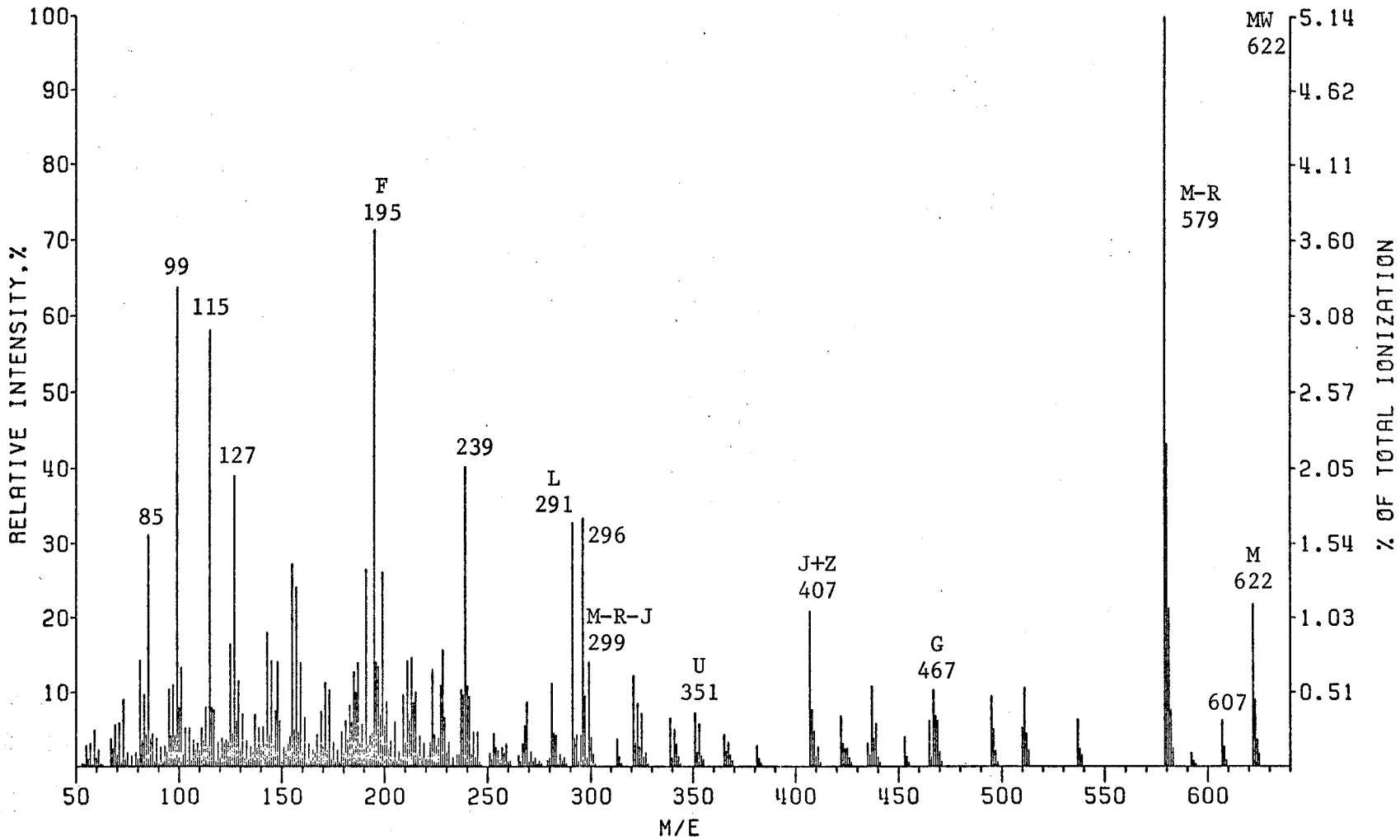


Figure 92. Mass spectrum of 2',3',5'tris-O-TMIPSi-uridine (VIddd) recorded on Finnigan MS at 70 eV.

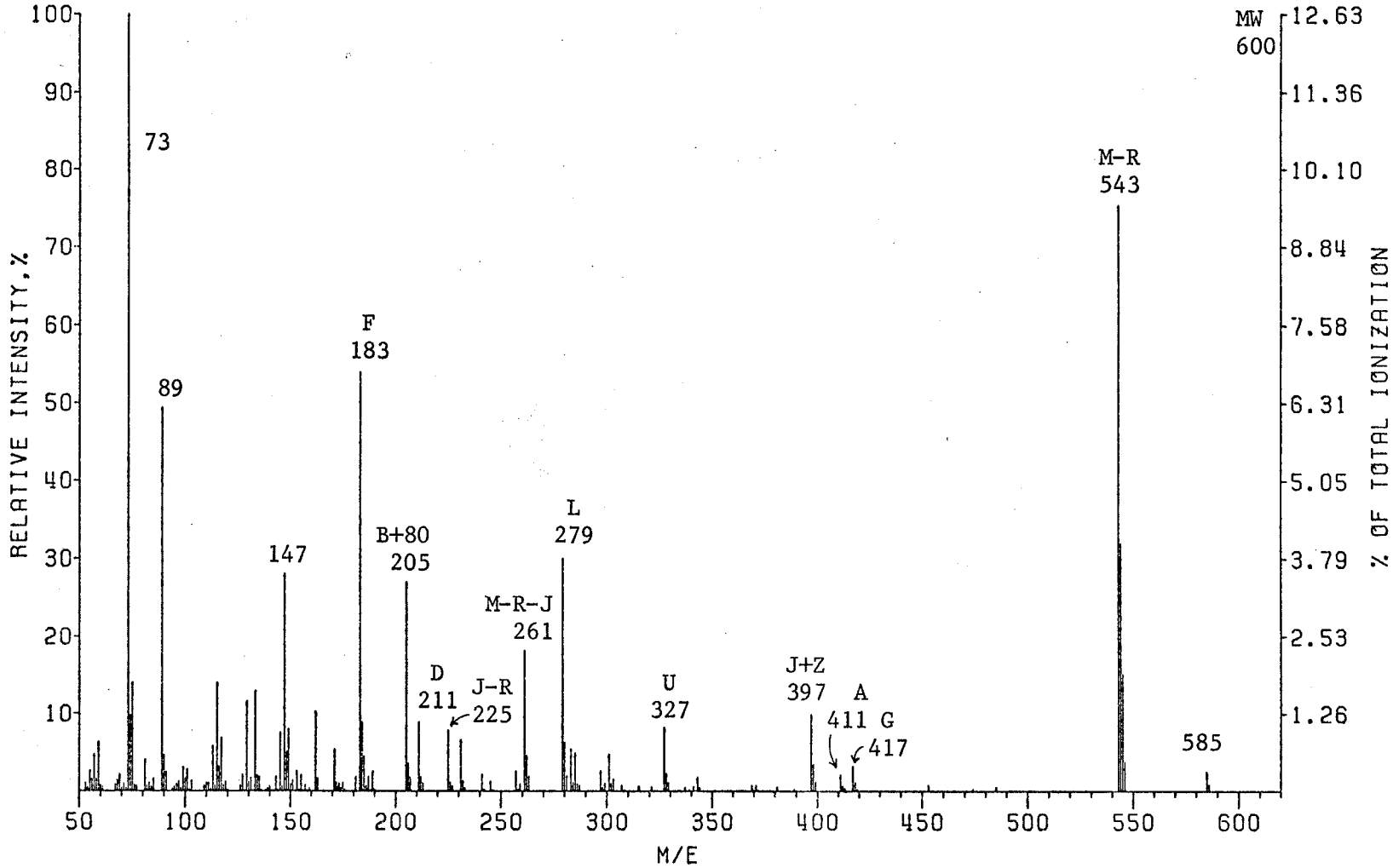


Figure 93. Mass spectrum of 2',3',5'tris-O-TBDMSi-5-methyluridine (VIIbbb) recorded on Finnigan MS at 70 eV.

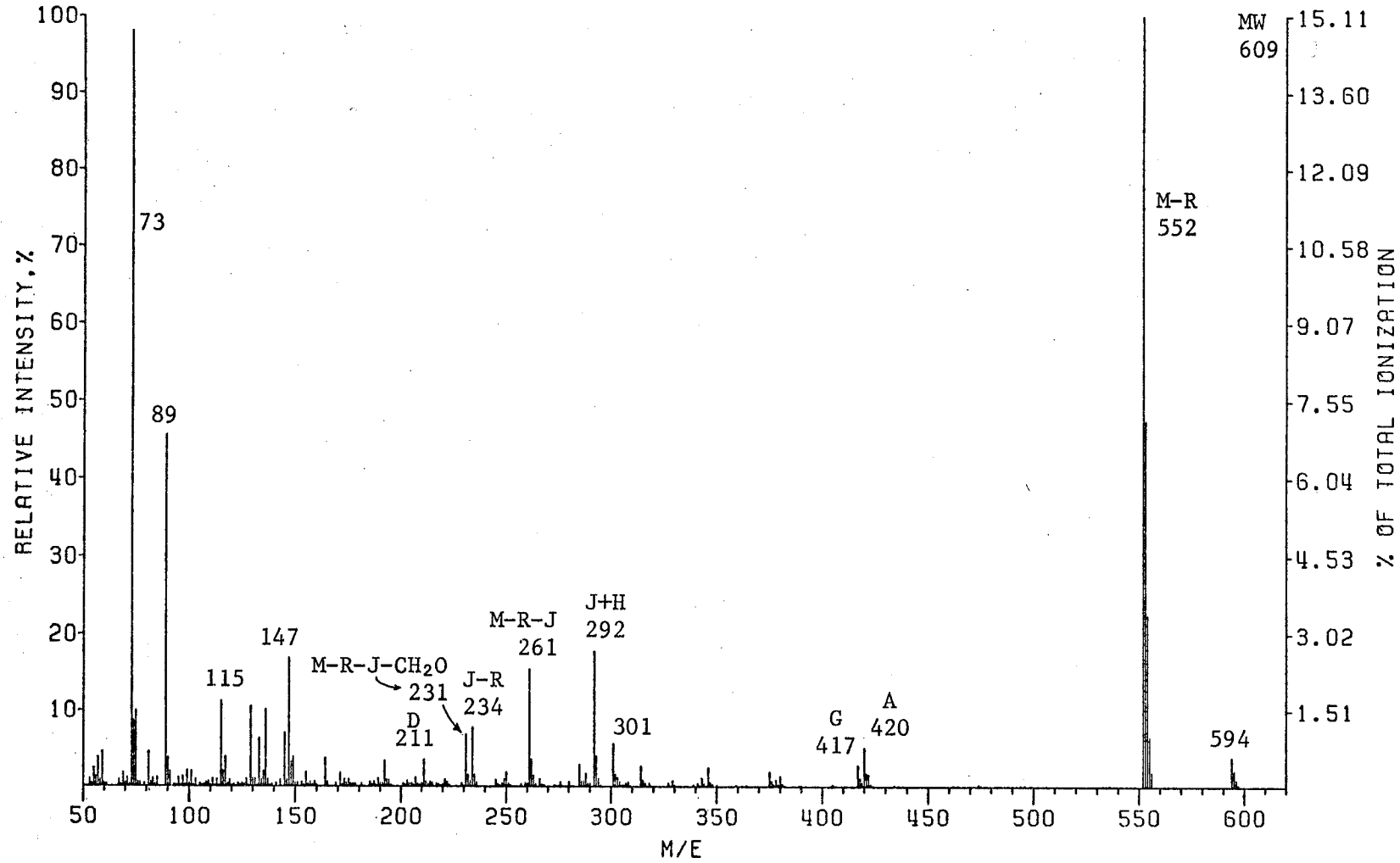


Figure 94. Mass spectrum of 2',3',5'tris-O-TBDMSi-adenosine (VIIIbbba) recorded on Hitachi MS at 50 eV.

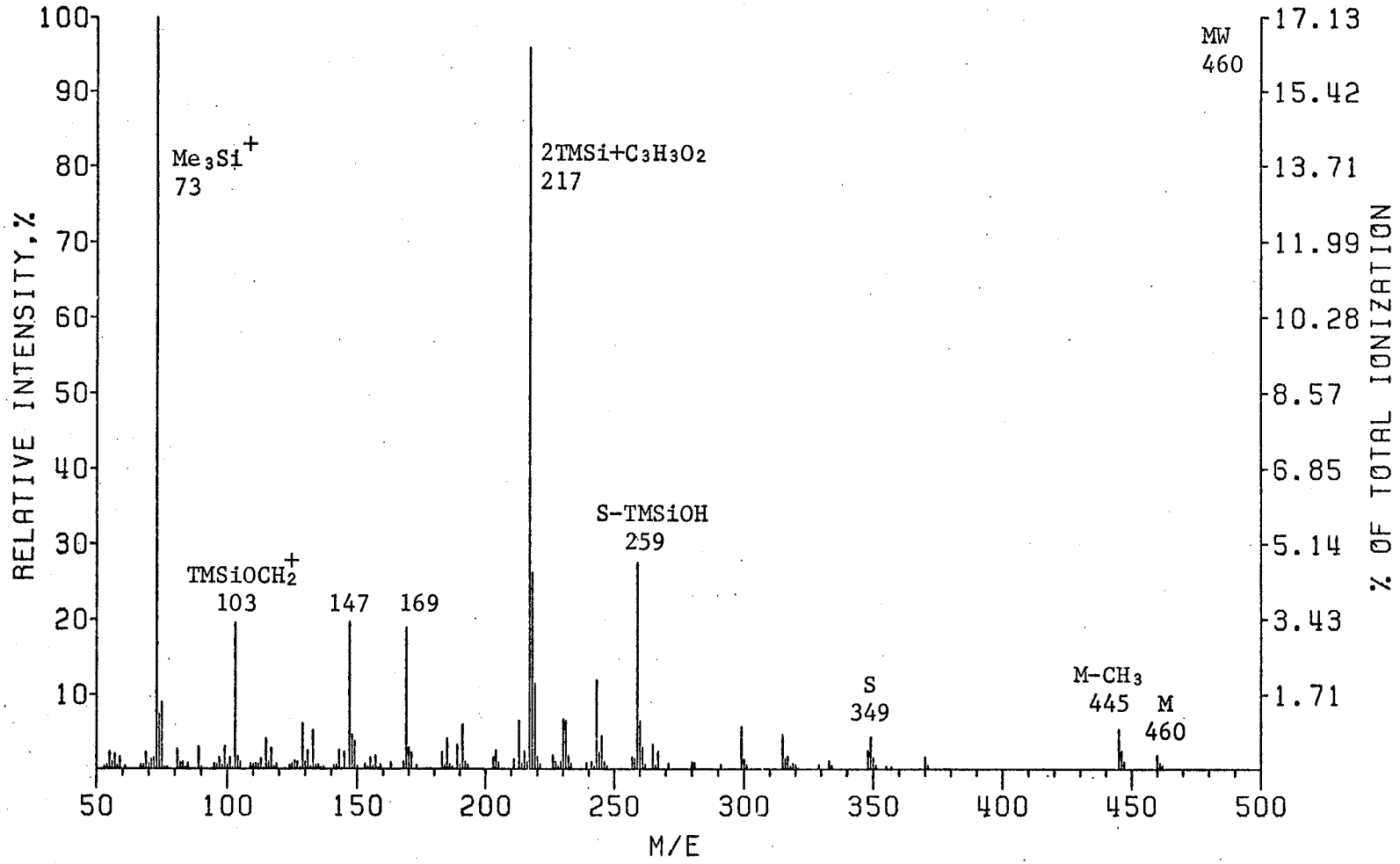


Figure 95. Mass spectrum of 2',3',5'tris-O-TMSi-uridine (VIeee) recorded on Hitachi MS at 50 eV.

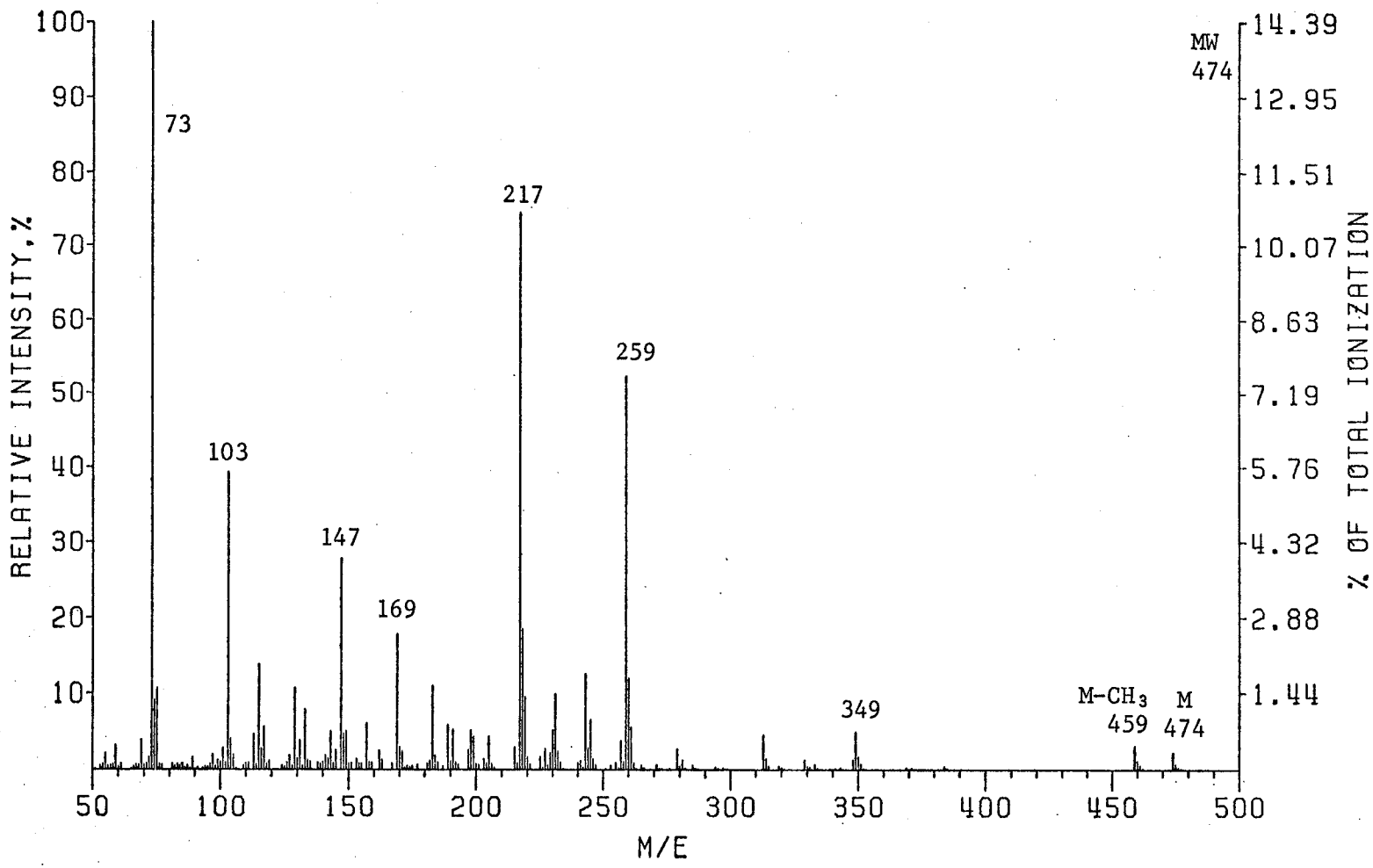


Figure 96. Mass spectrum of 2',3',5'tris-O-TMSi-5-methyluridine (VIIeee) recorded on Finnigan MS at 70 eV.

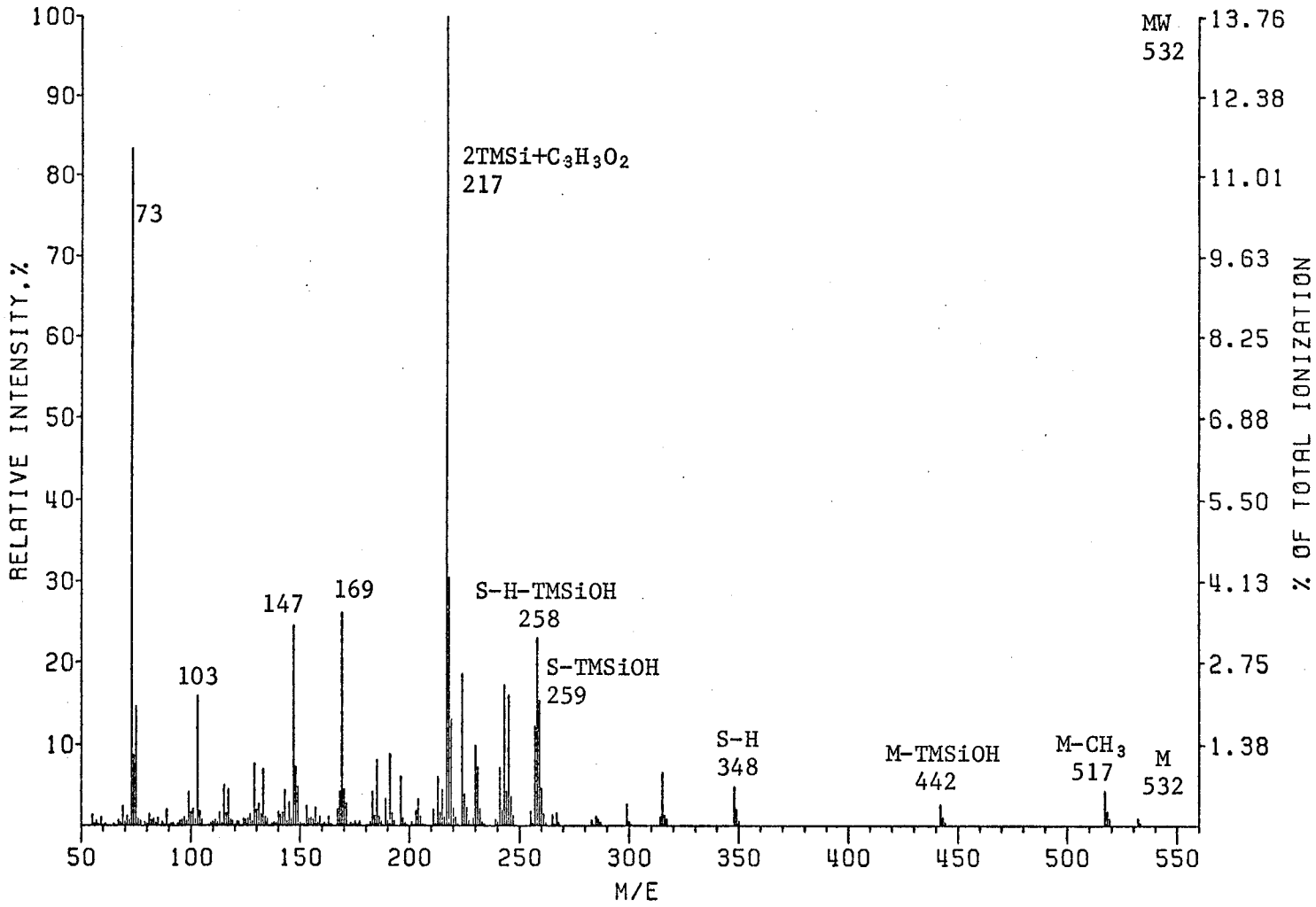


Figure 97. Mass spectrum of 2',3',5',4-tetrakis-O-TMSi-uridine recorded on Finnigan MS at 70 eV.

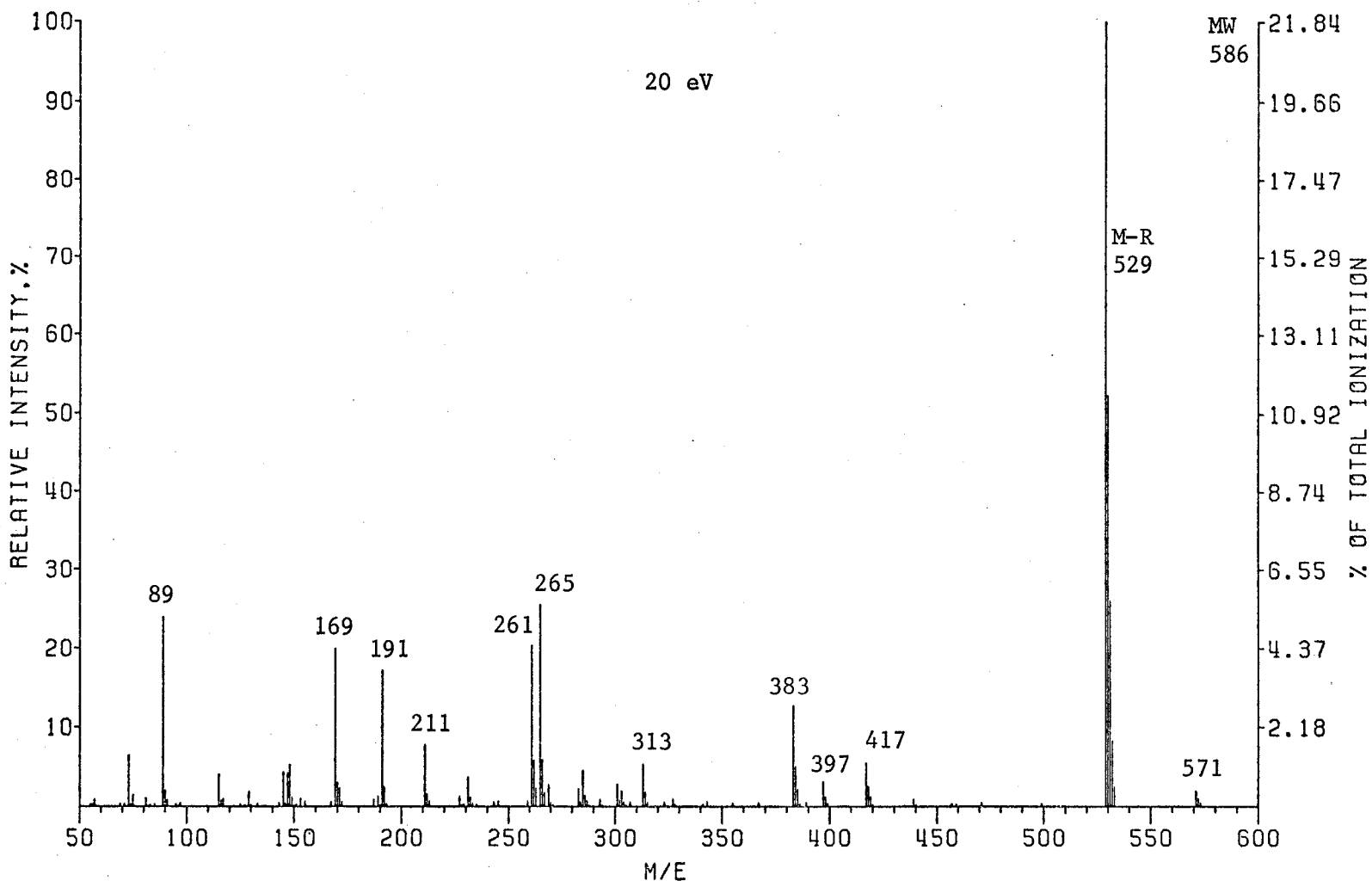


Figure 98. Mass spectrum of 2',3',5'tris-O-TBDMSi-uridine (VIbbb) recorded on Hitachi MS at 20 eV.

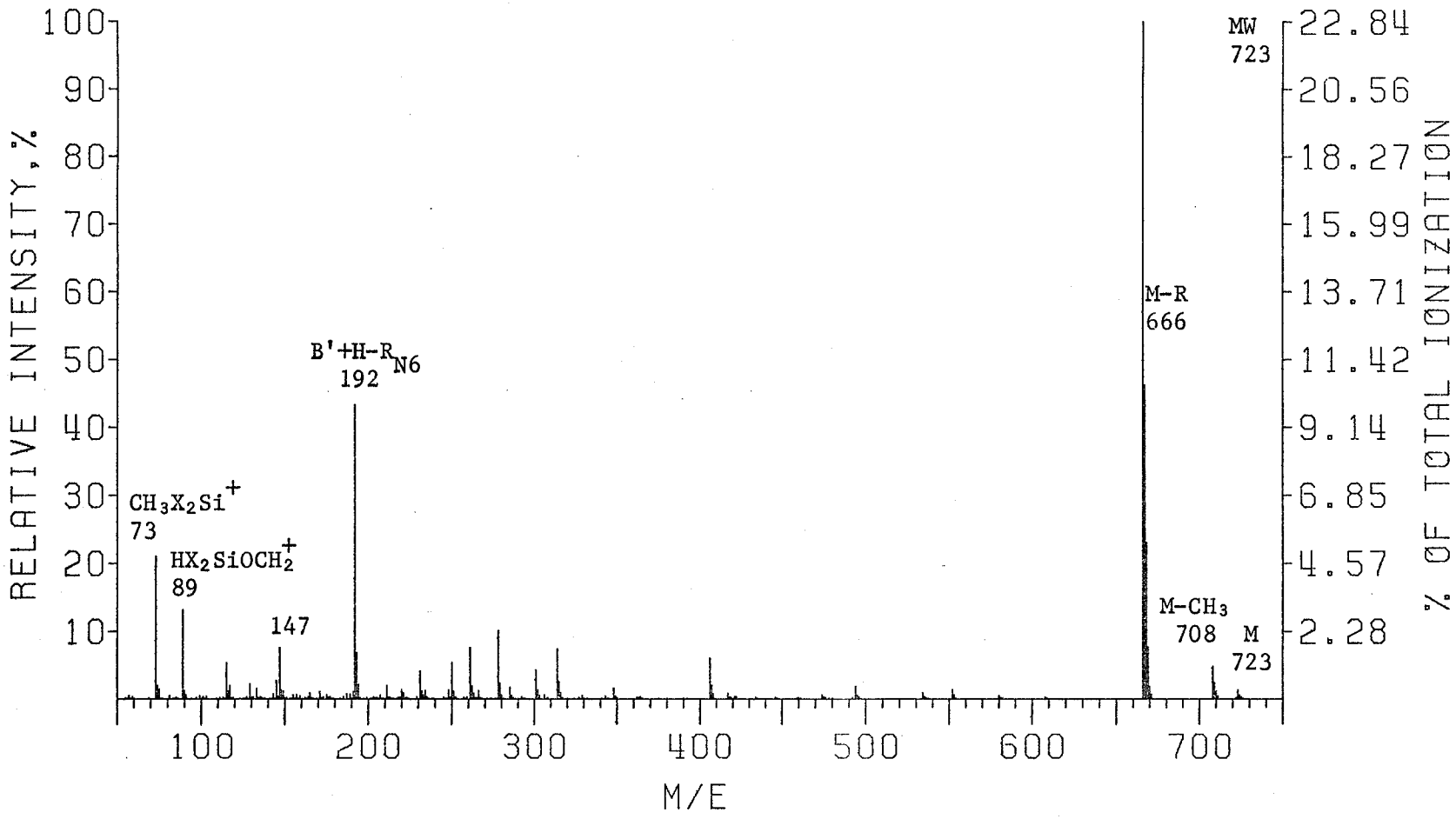


Figure 99. Mass spectrum of 2',3',5'tris-O-TBDMSi-N6-TBDMSi-adenosine (VIIIbbbb) recorded on Finnigan MS at 70 eV.

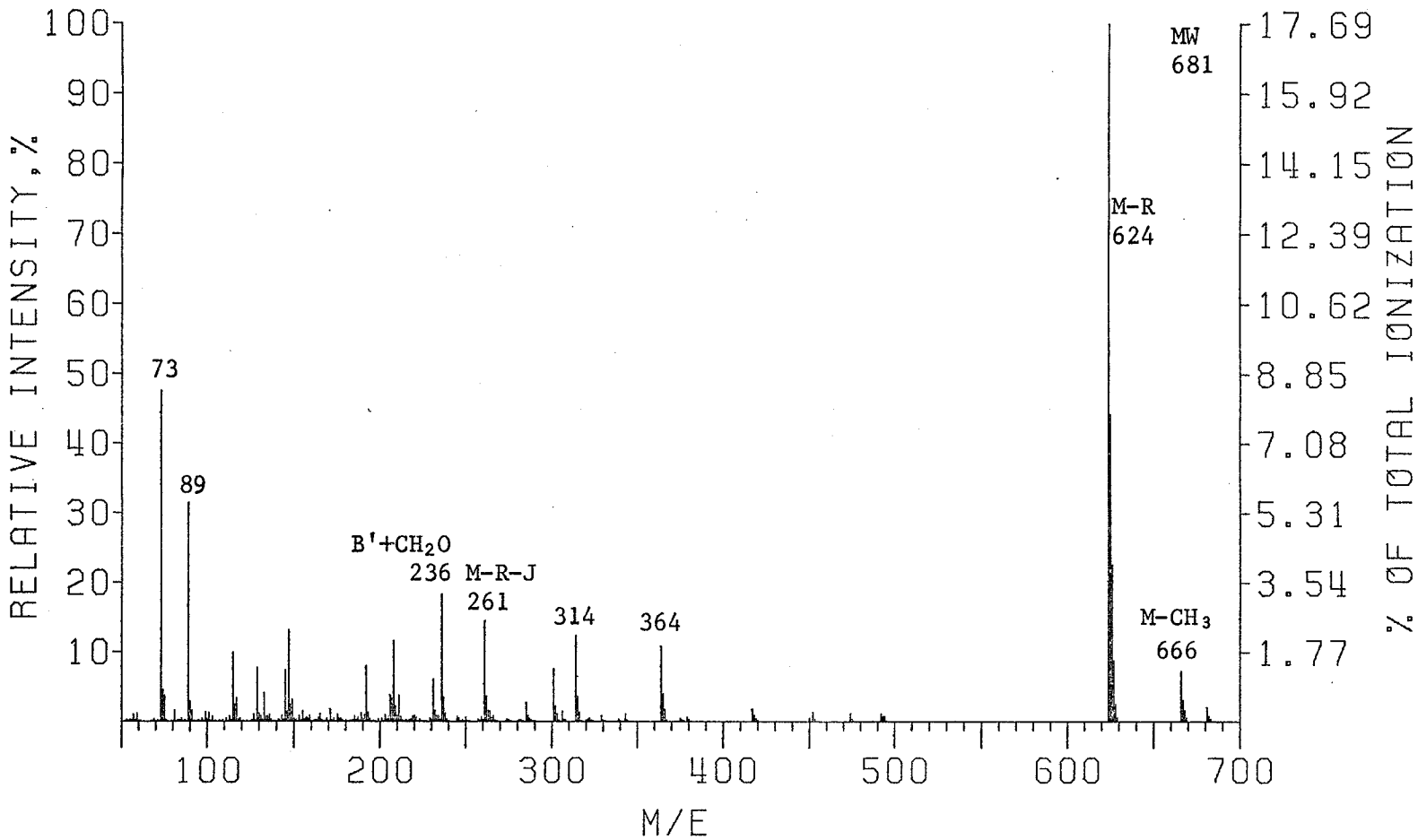


Figure 100. Mass spectrum of 2',3',5'tris-O-TBDMSi-N6-TMSi-adenosine (VIIIbbbe) recorded on Finnigan MS at 70 eV.

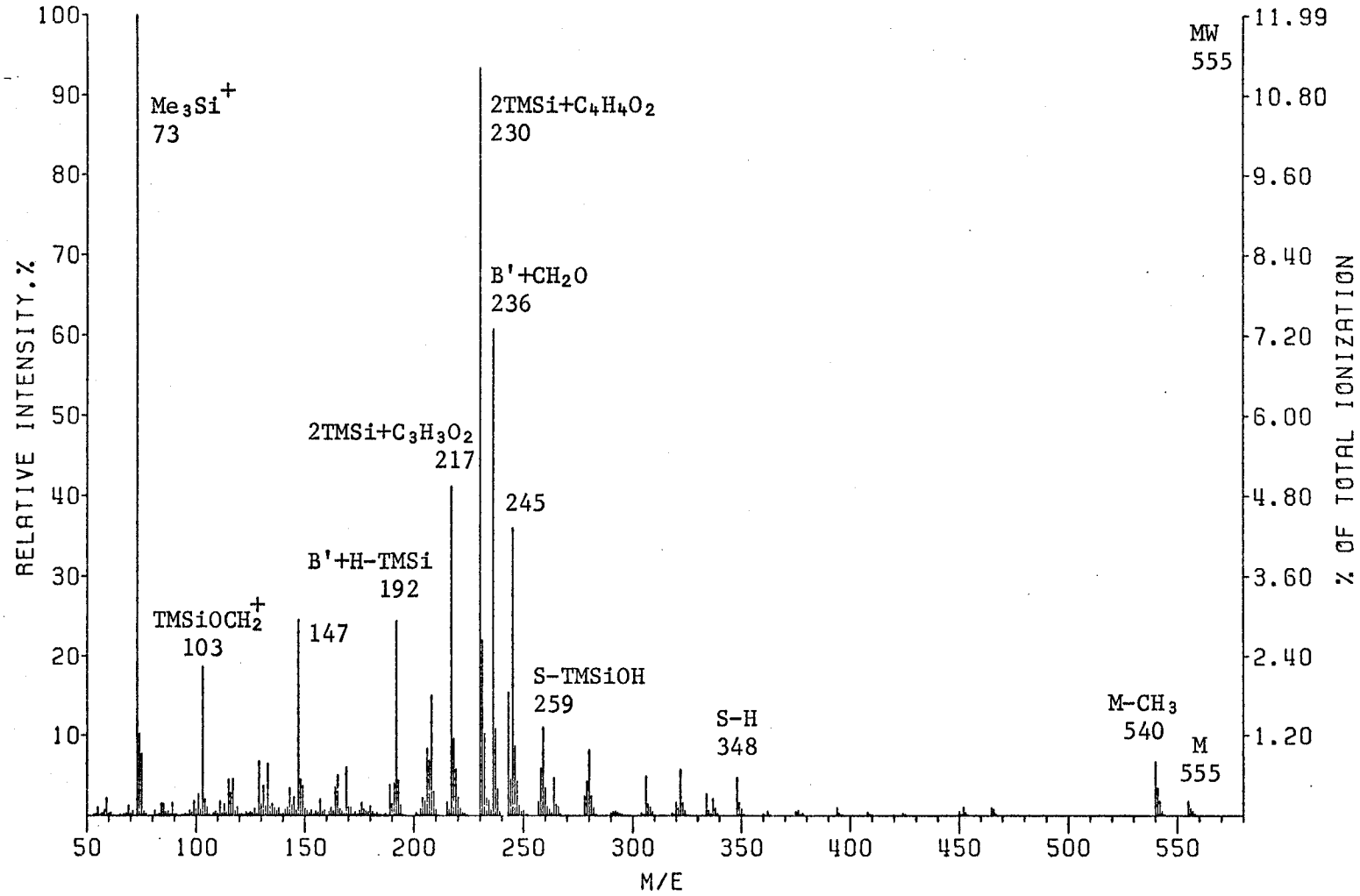


Figure 101. Mass spectrum of 2',3',5'tris-O-TMSi-N6-TMSi-adenosine (VIIIeeee) recorded on Finnigan MS at 70 eV.

TABLE XIXa. Mass spectral data for 2',3',5'tris-O-TBDMSi-uridine (VIbbb).

Line #	Assignment[e]	Elemental Composition C-H-Si-N-O	Exact Mass Calculated	Exact Mass Measured	m/e	Rel.[f] Int.%
1.	M	[27-54-3-2-6]	-	-	586	0
2.	$M-CH_3$	[26-51-3-2-6]	-	-	571	2.7
3.	M-R	23-45-3-2-6	529.259	529.254	529	97.
4.	[M-R-BH] or G	19-41-3-0-4	417.231	417.231	417	7.6
5.	$[M-R-RX_2SiOH]$ or A	17-29-2-2-5	397.162	397.161	397	3.9
6.	$[J+RX_2Si]$ or [J+Z]	18-35-2-2-3	383.219	383.220	383	16.
7.	$[B+CHO+RX_2Si+X_2Si]$ or U	13-25-2-2-3	313.140	313.140	313	8.0
8.	$M-CH_3-J$	14-31-2-0-3	303.181	303.182	303	3.2
9.	$2RX_2Si+C_3H_3O_2(71)$	15-33-2-0-2	301.202	301.203	301	5.9
10.	$[M-R-RX_2SiOH-BH]$ or [A-BH]	13-25-2-0-3	285.134	285.134	285	8.3
11.	$B+RX_2Si+X_2Si-H$	12-23-2-2-2	283.130	283.130	283	4.8
12.	J+H	12-21-1-2-3	269.132	269.133	269	4.0
13.	$[M-R-2RX_2SiOH]$ or L	11-13-1-2-4	265.064	265.063	265	35.
14.	M-R-J	11-25-2-0-3	261.134	261.135	261	26.
15.	$M-R-J-CH_2O$	10-23-2-0-2	231.124	231.124	231	6.4
16.	$B+H+RX_2Si$	10-19-1-2-2	227.122	227.124	227	2.9
17.	$[RX_2Si+C_5H_4O_2]$ or D	11-19-1-0-2	211.115	211.115[b]		
18.	B+RXSi	9-15-1-2-2	211.090	211.093[c]	211	17.
19.	$J-R_w$	8-11-1-2-3	211.054	211.055[b]		
20.	$B+C_5H_4O(80)$	9- 7-0-2-3	191.046	191.046	191	25.
21.	$RX_2SiOSiX_2$	8-21-2-0-1	189.113	189.115	189	2.9
22.	$[B+X_2Si]$ or F	6- 9-1-2-2	169.043	169.043	169	46.
23.	$CH_3X_2SiOSiX_2$	5-15-2-0-1	147.066	147.066	147	27.
24.	RX_2SiOCH_2	7-17-1-0-1	145.105	145.105[b]	145	7.5
25.	$X_2Si+C_3H_3O_3(87)$	5- 9-1-0-3	145.032	145.033[b]		
26.	$HX_2SiOSiX_2$	4-13-2-0-1	133.051	133.051	133	11.
27.	$X_2Si+C_3H_3O_2(71)$	5- 9-1-0-2	129.037	129.038	129	9.6

continued...

TABLE XIXa. continued

Line #	Assignment[e]	Elemental Composition C-H-Si-N-O	Exact Mass Calculated	Exact Mass Measured	m/e	Rel.[f] Int.%
28.	$X_2Si+C_2H_3O_2(59)$	4- 9-1-0-2	117.038	117.037	117	7.6
29.	RX_2Si	6-15-1-0-0	115.094	115.094[a]		
30.		5-11-1-0-1	115.058	115.058[c]	115	15.
31.		4- 7-1-0-2	115.022	115.019[c]		
32.	B+2H	4- 5-0-2-2	113.035	113.037	113	1.6
33.	$X_2Si+C_2H_3O(43)$	4- 9-1-0-1	101.042	101.043	101	2.6
34.		5-11-1-0-0	99.063	99.064[b]	99	7.5
35.	$X_2Si+C_2HO(41)$	4- 7-1-0-1	99.027	99.027[a]		
36.	HX_2SiOCH_2	3- 9-1-0-1	89.042	89.042	89	52.
37.	$C_5H_5O(81)$	5- 5-0-0-1	81.034	81.034	81	5.3
38.	X_2SiOH	-	-	-	75	15.
39.	CH_3X_2Si	-	-	-	73	100.
40.	HX_2Si	-	-	-	59	5.6

Base Peak as $\%\Sigma_{50}$ = 10.57

[a-c] Relative contribution to integer m/e abundance: a = major, b = medium, c = minor.

[e] Positive ions; sign left out for simplicity.

[f] Measured on Hitachi instrument at 50 eV.

TABLE XIXb. Correlation of m/e values of principal fragment ions in the mass spectra of tris-O-SCTASi derivatives of ribonucleosides.

Line #	Assignment	Compound: VIbbb	VIccc	VIddd	VIIbbb	VIIIbbba
1.	M	586[c]	664	622	600[c]	609[w]
2.	$M-CH_3$	571	649	607	585	594
3.	M-R	529	607	579	543	552
4.	G	417	495	467	417	417
5.	A	397	449	435[w]	411	420
6.	J+Z	383	435	407	397	406[w]
7.	U	313	365	351	327	336[c]
9.	$2RX_2Si+C_3H_3O_2(71)$	301	353	339	301	301
10.	A-BH	285	337	323	285	285
11.	$B+RX_2Si+X_2Si-H$	283	335	321	297	307[w]
12.	J+H	269	295	281	283	292
13.	L	265	291	291	279	288[w]
14.	M-R-J	261	313	299	261	261
15.	$M-R-J-CH_2O$	231	283	269	231	231
16.	$B+H+RX_2Si$	227	253[s]	239[s]	241	250
17.	D	211[a]	237[a]	223	211	211
19.	$J-R_w$	211[a]	237[a]	237	225	234
20.	$B+C_5H_4O(80)$	191	191	191	205	214[c]
21.	$RX_2SiOSiX_2$	189	241	227	189	189
22.	F	169	195	195	183	192[w]
23.	$CH_3X_2SiOSiX_2$	147	199	199	147	147
24.	RX_2SiOCH_2	145[a]	171[a]	157	145[a]	145[a]
25.	$X_2Si+C_3H_3O_3(87)$	145[a]	171[a]	171	145[a]	145[a]
26.	$HX_2SiOSiX_2$	133	185[w]	185	133	133
27.	$X_2Si+C_3H_3O_2(71)$	129	155	155	129	129
28.	$X_2Si+C_2H_3O_2(59)$	117	143	143	117	117

continued...

TABLE XIXb. continued

Line #	Assignment	Compound: VIbbb	VIccc	VIddd	VIIbbb	VIIIbbba
29.	RX_2Si	115	141	127	115	115
32.	B+2H	113	113[a]	113	127	136[s]
36.	HX_2SiOCH_2	89	115	115	89	89
37.	C_5H_5O	81	81	81	81	81
38.	X_2SiOH	75	101	101	75	75
39.	CH_3X_2Si	73	99[a]	99[a]	73	73
40.	HX_2Si	59	85	85	59	59

[a] Overlap of different ion types.

[b] Major isotopic contribution.

[c] Not present or not detectable.

[w] Weak compared to VIbbb.

[s] Stronger than for VIbbb.

TABLE XIXc. Mass spectral data for mixed TMSi/TBDMSi derivatives of uridine (VI)[a].

#	Ion Type[d]	m/e	VIbee	VIebe	VIeeb	m/e	VIbbe	VIbeb	VIebb	[Assignment][e]
	Compound #:		VIbee	VIebe	VIeeb		VIbbe	VIbeb	VIebb	
	O-Substituents Y(5'):		TBDMSi	TMSi	TMSi		TBDMSi	TBDMSi	TMSi	
	Z(3'):		TMSi	TBDMSi	TMSi		TBDMSi	TMSi	TBDMSi	
	W(2'):	m/e	TMSi	TMSi	TBDMSi	m/e	TMSi	TBDMSi	TBDMSi	
1.	M	502	0	0	0	544	0	0	0	
2.	$M-CH_3$	487	12.	4.0	5.0	529	4.8	5.4	3.5	
3.	M-R	445	24.	100.	82.	487	100.	94.	100.	
4.	M-R-BH	333	4.0	4.7	2.7	375	3.6	3.7	3.0	[G]
5a.	M-R-TMSiOH	355	4.4	0.7	1.5	397	0.3	3.4	0.4	$[M-R_y-ZOH]$
5b.	$M-R-RX_2SiOH$	-	-	-	-	355	0.9	0.4	0.9	
6a.	$BC_2H_2O+2TMSi$	299	100.	1.6	0.7	-	-	-	-	
6b.	$BC_2H_2O+TMSi+RX_2Si$	341	0.3	1.5	3.8	341	9.1	29.	0	[J+Z]
6c.	$BC_2H_2O+2RX_2Si$	-	-	-	-	383	0	0	1.1	
7a.	$B+CHO+X_2Si+TMSi$	271	0	1.4	9.0	271	0.3	0.5	7.9	$[B+CHO+X_2Si_W+Y]$
7b.	$B+CHO+X_2Si+RX_2Si$	-	-	-	-	313	1.0	6.0	0	
9a.	$M-BC_2H_3O_2TMSi$	259	6.9	9.6	6.0	301	1.3	0.8	1.8	
9b.	$M-BC_2H_3O_2RX_2Si$	217	67.	3.2	5.9	259	5.7	7.0	1.9	
10a.	M-R-TMSiOH-BH	243	16.	13.	15.	285	2.4	6.3	2.2	
10b.	$M-R-RX_2SiOH-BH$	-	-	-	-	243	5.6	6.3	10.	
12a.	$B+C_2H_3O+TMSi$	227[b]	18.	3.1	1.2	227[b]	6.3	3.0	1.4	[J+H]
12b.	$B+C_2H_3O+RX_2Si$	269	0	0	0.5	269	0.2	4.0	1.3	
13a.	M-R-2TMSiOH	265	5.6	9.6	46.	-	-	-	-	$[M-R_W-YOH-ZOH]$
13b.	$M-R-TMSiOH-RX_2SiOH$	-	-	-	-	265	6.9	44.	33.	
14a.	$M-R-BC_2H_2OTMSi$	219	70.	4.2	4.6	261	22.	2.2	4.4	$[M-R_y-J]$
14b.	$M-R-BC_2H_2ORX_2Si$	-	-	-	-	219	1.8	24.	3.6	

continued...

TABLE XIXc. continued

#	Ion Type[d]	m/e	VIbee TBDMSi TMSi TMSi	VIebe TMSi TBDMSi TMSi	VIeeb TMSi TMSi TBDMSi	m/e	VIbbe TBDMSi TBDMSi TMSi	VIbeb TBDMSi TMSi TBDMSi	VIebb TMSi TBDMSi TBDMSi	[Assignment][e]
15a.	M-R-BC_2H_2OTMSi-CH_2O	189	66.	4.1	4.2	231	7.7	1.6	1.1	[M-R_y-J-CH_2O]
15b.	M-R-$BC_2H_2ORX_2Si$-CH_2O	-	-	-	-	189[b]	3.7	22.	3.2	
16a.	B+H+TMSi	185	8.9	6.5	5.6	185	2.2	2.3	5.0	
16b.	B+H+RX_2Si	227[b]	18.	3.1	1.2	227[b]	6.3	3.0	1.4	
17a.	TMSi+$C_5H_4O_2$(96)	169[b]	83.	35.	100.	169[b]	28.	100.	81.	[D]
17b.	RX_2Si+$C_5H_4O_2$(96)	211[b]	4.6	2.7	8.3	211[b]	6.0	28.	9.8	
19.	B+X_2Si+C_2H_2O	211[b]	4.6	2.7	8.3	211[b]	6.0	28.	9.8	[J-R_w]
20.	B+C_5H_4O(80)	191	15.[c]	24.	47.	191	17.	56.	38.	
21a.	$RX_2SiOSiX_2$	-	-	-	-	189[b]	3.7	22.	3.2	
21b.	(+23) TMSi$OSiX_2$ or $CH_3X_2SiOSiX_2$	147	65.	57.	42.	147	34.	43.	37.	
22.	B+X_2Si	169[b]	83.	35.	100.	169[b]	28.	100.	81.	[B+X_2Si_w]>[B+X_2Si_y] >>[B+X_2Si_z]
24a.	RX_2SiOCH_2	145[b]	4.7	8.6	3.2	145[b]	11.	3.5	5.0	
24b.	TMS$iOCH_2$ or $CH_3X_2SiOCH_2$	103	63.	63.	54.	103	2.8	23.	45.	
25.	X_2Si+$C_3H_3O_3$(87)	145[b]	4.7	8.6	3.2	145[b]	11.	3.5	5.0	
26.	$HX_2SiOSiX_2$ or $H(CH_3)_2SiOSiX_2$	133	12.	7.3	6.7	133	6.7	8.0	8.1	
27.	X_2Si+$C_3H_3O_2$(71)	129	28.	9.4	8.0	129	9.6	15.	7.5	
28.	X_2Si+$C_2H_3O_2$(59)	117	7.7	6.7	4.2	117	6.2	6.1	6.6	

continued...

TABLE XIXc. continued

#	Ion Type[d]	m/e	VIbee TBDMSi TMSi TMSi	VIebe TMSi TBDMSi TMSi	VIeeb TMSi TMSi TBDMSi	m/e	VIbbe TBDMSi TBDMSi TMSi	VIbeb TBDMSi TMSi TBDMSi	VIebb TMSi TBDMSi TBDMSi	[Assignment][e]
29a.	RX_2Si	115	8.9	3.5	2.3	115	12.	13.	4.1	
29b.	TMSi									
39.	HX_2Si	73	99.6	62.	61.	73	57.	83.	60.	
36.	HX_2SiOCH_2 or $H(CH_3)_2SiOCH_2$	89	24.	4.4	1.8	89	62.	57.	4.7	$[HX_2Si_yOH]$
38.	X_2SiOH	75	11.	7.4	7.5	75	6.9	9.6	8.4	
	Base Peak as $\%\Sigma_{50}$		7.82	12.71	11.61		14.13	9.20	12.41	

[a] Results expressed as relative intensity % as measured on Finnigan instrument at 70 eV.

[b] Ion type overlap.

[c] Significant isotopic contribution from ion type of lower mass.

[d] RX_2Si = TBDMSi

[e] [Assignment] of major species as deduced from intensity data.

TABLE XX. Metastable transitions in the mass spectra of tris-O-TBDMSi derivatives of uridine and adenosine.[a]

Transition	Compound: VIbbb	VIIIbbba
[M-R] → [M-R-BH] or G	529 → 417 (328.7, 329s)	552 → 417 (315.0, 315w)
[M-R] → [M-R-RX_2SiOH] or A	529 → 397 (297.9, 298vw)	552 → 420 (319.6, 320m)
[M-R] → [M-R-2RX_2SiOH] or L	529 → 265 (132.8, 133m)	
L → F	265 → 169 (107.8, 108m)	
[M-R] → [J+Z]	529 → 383 (277.3, 277vw)	
[M-R] → [J+H]		552 → 292 (154.5, 155w)
[M-R-J] → [M-R-J-CH_2O]	261 → 231 (204.4, 204.5w)	261 → 231 (204.4, 204.5w)
[M-R-J] → RX_2SiOCH_2		261 → 145 (80.6, 80.5w)
U → $CH_3X_2SiOSiX_2$	313 → 147 (69.0, 69m)	
RX_2SiOCH_2 → HX_2SiOCH_2	145 → 89 (54.6, 54.7m)	145 → 89 (54.6, 54.7m)
RX_2Si → CH_3X_2Si	115 → 73 (46.3, 46.5m)	115 → 73 (46.3, 46.4m)

[a] Data given are: m_p → m_d (m*(calc), m*(obs), intensity)
vs = very strong, s = strong, m = medium, w = weak, vw = very weak

2. Discussion of Fragmentation Mechanisms

This section presents the proposed assignment of ion types, and mechanisms for their formation, for the SCTASi and mixed TMSi/SCTASi derivatives of ribonucleosides. As mentioned earlier, this series has not been studied in as much detail as the deoxynucleosides. Many of the interpretations should be considered tentative, until complete labelling studies have been performed. However, the assignments and mechanisms proposed are consistent with those made for the deoxy series, and a good deal of evidence is available: high resolution data on VIbbb, "homologous labelling" with different bases and SCTASi groups, mixed TMSi/TBDMSi derivatives, and metastable peak measurements. Tables XIXa to XIXc give detailed assignments for most of the ions observed. Scheme 45 illustrates the proposed genesis of important ions for the SCTASi derivatives of ribonucleosides. Many pathways are supported by metastable peaks, while others are given support by analogous ions observed for the deoxy series.

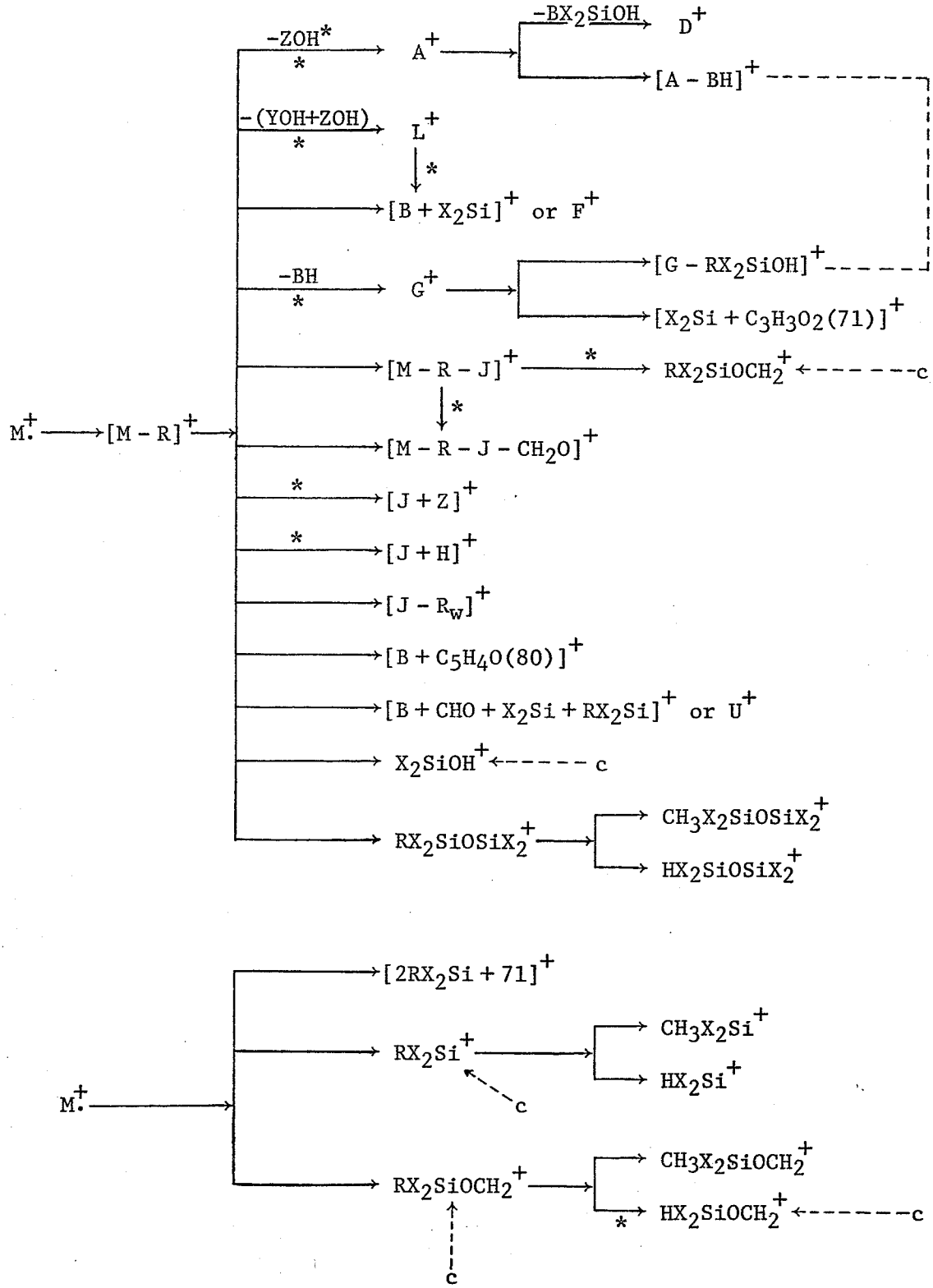
$M^{+\cdot}$ [XIX.1, VIbbb - m/e586]

The molecular ion is not detectable for TBDMSi derivatives of uridine and 5-methyluridine, but is observed in low abundance for tris-O-TBDMSi-adenosine and tris-O-TMTBSi-uridine and is quite intense for the TMIPSi derivative of uridine. The molecular weight is best determined from $[M-R]^+$.

$[M-CH_3]^+$ [XIX.2, VIbbb - m/e571]

As in the deoxy series, this ion is observed for TBDMSi and TMSi derivatives only and is generally quite weak. One exception to this is the mixed TMSi/TBDMSi derivative VIbee, where $[M-CH_3]^+$ is of medium intensity and $[M-R]^+$ is reduced in abundance compared to the other isomers. This may be due to the 2'- or 3'-oxygens of the cis-disilyloxy system

Scheme 45. Summary of the genesis of ions in the spectra of SCTASi derivatives of ribonucleosides. Other sources possible for some ions (c).



inducing loss of $CH_3\cdot$ from the Z- or W-TMSi groups, to produce a cyclic silyloxonium system (which may be quite stable).

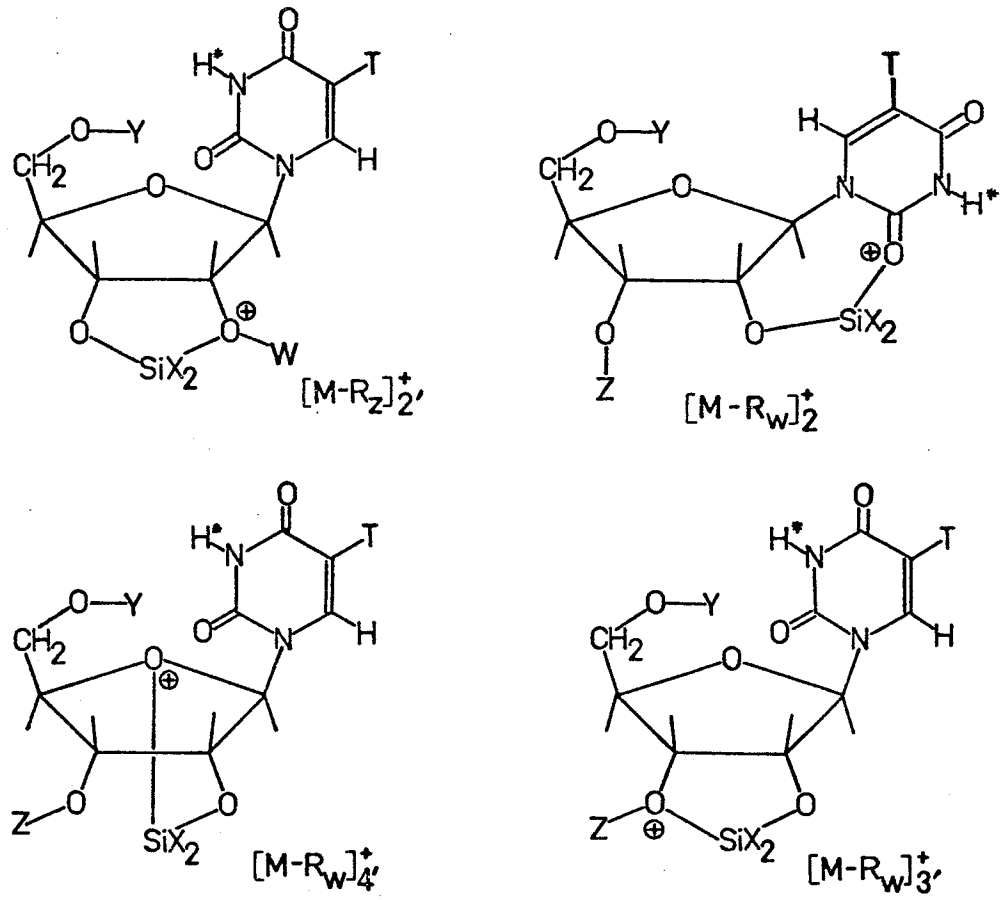
$\underline{[M-R]^+}$ [XIX.3, VIbbb - m/e529]

The $[M-R]^+$ ion is very intense for all ribonucleoside SCTASi derivatives, especially when compared to those of the deoxy series. The reason for the greater abundance is not known; it may be due to the formation of more stable ion structures (perhaps cyclic silyloxonium ions) and less fragmentation.

With the 2',3',5'tris-O-SCTASi derivatives, there is the possibility of producing three different siliconium ions by loss of R· from $M^{+\cdot}$: $[M-R_y]^+$, $[M-R_z]^+$, and $[M-R_w]^+$. Each of these may interact with electron dense centers within the molecule to yield cyclic silyloxonium ions and thereby direct fragmentation. The six already presented in Scheme 21 are still possible, while Scheme 46 illustrates four more, due to the presence of the 2'-silyloxy group, to make a total of ten possible precursors for fragment ions in the spectra. It is expected that the four additional cyclic ions should account for any ions that are unique to the ribose series. Examination of molecular models has revealed that other cyclic ions such as $[M-R_y]^+_{2'}$, $[M-R_w]^+_{5'}$, and $[M-R_w]^+_4$ are not sterically feasible. In the following interpretations, the mixed TMSi/TBDMSi derivatives have been invaluable for "isolating" each of the three siliconium ions and their daughter ions.

$\underline{A^+}$ [XIX.5, VIbbb - m/e397]

$[M-R-RX_2SiOH]^+$ is observed for ribonucleoside derivatives, and mixed TMSi/TBDMSi derivatives show a very strong dependence upon the presence of a 5'-siliconium ion and a specific elimination of ZOH. This fragmentation probably occurs by the same mechanism proposed for deoxy-



Scheme 46. Structures of cyclic silyloxonium ions specific for ribonucleoside derivatives. Scheme 21 illustrates six additional species which are possible for both deoxy- and ribo-nucleosides (where V = OW for the latter).

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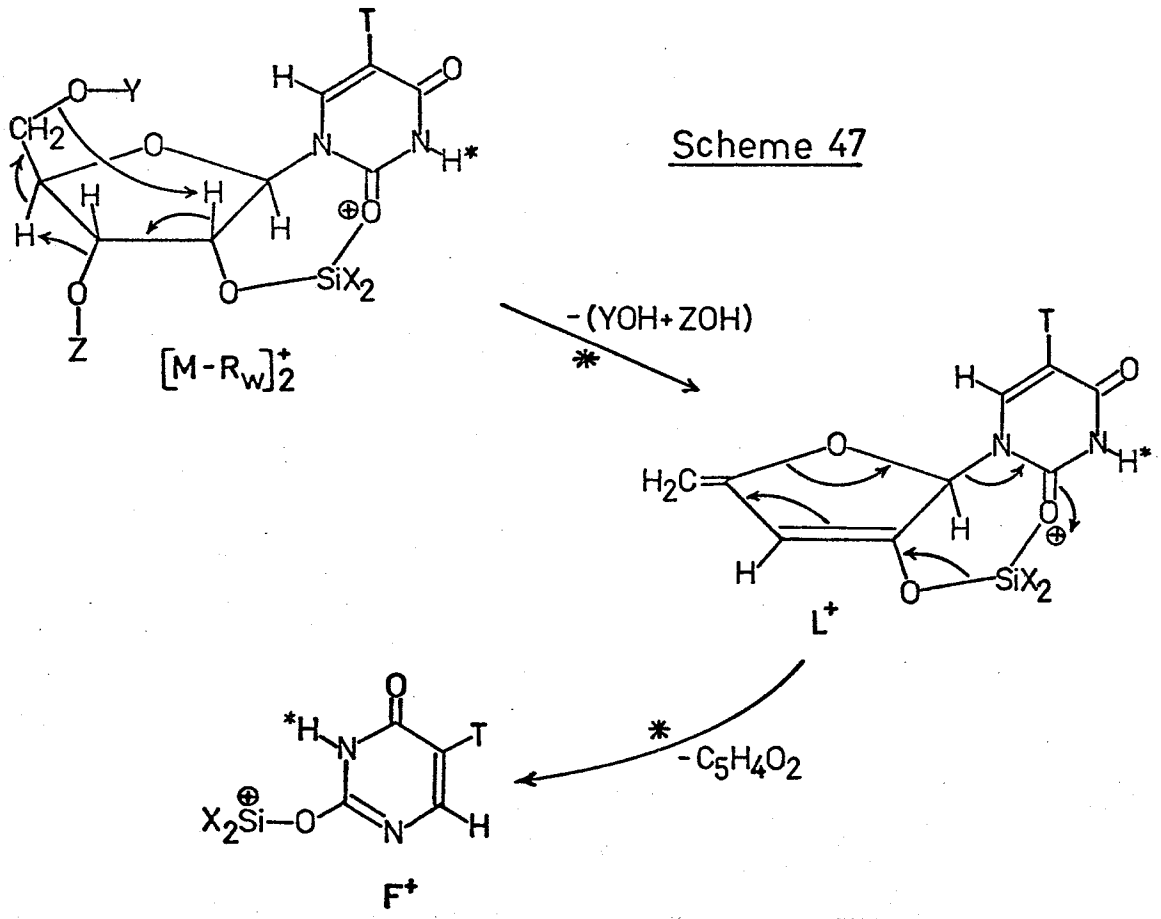
nucleosides (Scheme 22). Unfortunately, deuteration experiments have not yet been performed with this series of compounds for confirmation of labile base hydrogen involvement. Metastable peaks (Table XX) do confirm $[M-R]^+$ as precursor. In general, A^+ is of lower abundance for the derivatives of ribonucleosides versus deoxynucleosides. This may be due to the possibility that $[M-Ry]^+$ carries a much lower proportion of the total charge, or that other fragmentation pathways are preferred.

L^+ [XIX.13, VIbbb - m/e265]

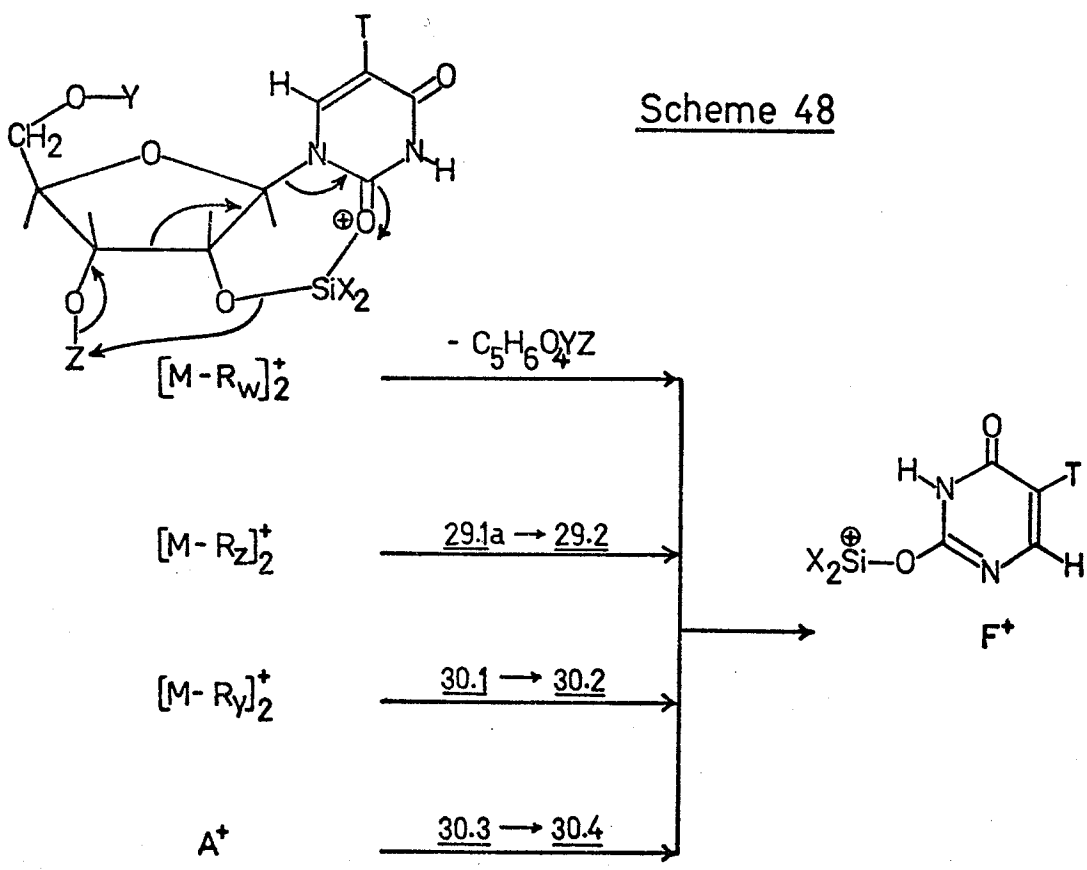
This fragment ion, of composition $[M - R - 2RX_2SiOH]^+$, is unique to the tris-O-silylated ribonucleosides It is quite abundant for derivatives of VI and VII, but weak for those of VIII. The ion may be produced in part by loss of RX_2SiOH from A^+, but a metastable peak for VIbbb indicates direct formation from $[M - R]^+$ by a concerted loss of two silanol groups. The mixed TMSi/TBDMSi derivatives' spectra suggest a strong dependence on the 2'-siliconium ion, $[M - R_w]^+$, and a preferred elimination of (YOH + ZOH). A mechanism that is consistent with this information is given in Scheme 47. This is probably the predominant mechanism, but certainly not the only one that is occurring as evidenced by a small amount of non-specificity.



$\underline{F^+}$ [XIX.22, VIbbb - m/e169]

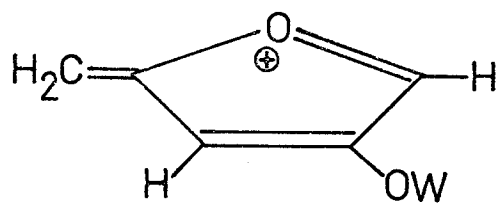
$[B+X_2Si]^+$ or F^+ is an intense ion for the SCTASi derivatives of VI and VII, although only of moderate intensity for those of VIII. A metastable peak signifies L^+ as a precursor, and Scheme 47 suggests a mechanism. The results with mixed derivatives show that it is predominantly $[B+X_2Si_w]^+$, but both $[B+X_2Si_y]^+$ and $[B+X_2Si_z]^+$ do occur. A number of sources are possible as shown in Scheme 48.

The related ion, E^+ or $[B+X_2Si+H_2O]^+$, is not observed to any extent for ribonucleoside derivatives. This is consistent with the proposed mechanism for deoxynucleosides in which V = H is required (Scheme 29).



$\underline{D^+}$ [XIX.17, VIbbb - m/e211]

An ion of composition $[RX_2Si + C_5H_4O_2(96)]^+$ is observed for tris-O-silylated ribonucleosides. In the spectra of VIbbb and VIccc it overlaps with $[J - R]^+$ at m/e211 and m/e237, respectively. It is proposed that this ion corresponds to $C_5H_4VO^+$, or D^+, which is observed at m/e81 (V = H) in deoxynucleoside spectra. The same mechanisms in Scheme 28 and 34, are proposed to produce an ion of this structure:



D^+

As expected, m/e81 ($C_5H_5O^+$) is of very low abundance for ribonucleoside derivatives.

$\underline{G^+}$ [XIX.4, VIbbb - m/e417]

$[M - R - BH]^+$ is a fragment ion of medium intensity for ribonucleoside derivatives. Metastable peaks signify $[M - R]^+$ as precursor for this ion, while mixed derivatives show no preference for formation from any particular siliconium ion. Mechanisms similar to those in Scheme 31 and 32 are proposed.

$[G\text{-}CH_2O]^+$ does not occur for fully protected ribonucleosides, consistent with the same observation for deoxynucleosides.

$\underline{[X_2Si + C_3H_3O_2(71)]^+}$ [XIX.27, VIbbb - m/e129]

This ion is observed in medium abundance and probably originates from G^+ as suggested for deoxynucleosides (Scheme 32).

$[M - R - BH - RX_2SiOH]^+$ [XIX.10, VIbbb - m/e285]

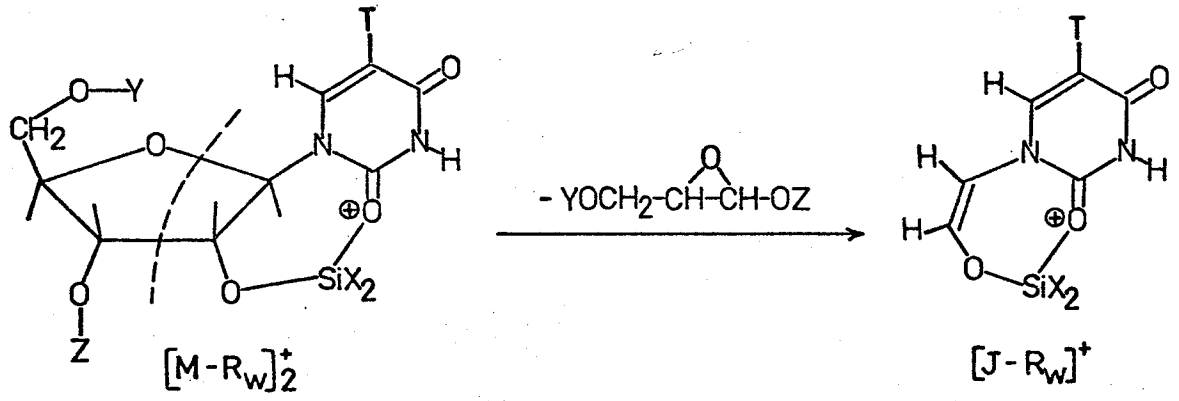
There is no metastable peak to indicate the origin of this ion. The fact that it is abundant for all the isomeric mixed TMSi/TBDMSi derivatives indicates non-specific origins. Both $[A - BH]^+$ and $[G - RX_2Si-OH]^+$ are possible assignments, and mechanisms similar to those presented in Schemes 27 and 32 should be applicable.

$[M - R - J]^+$ [XIX.14, VIbbb - m/e261]
$[M - R - J - CH_2O]^+$ [XIX.15, VIbbb - m/e231]

These ions were observed for the deoxy series, and are of comparable abundance for ribonucleoside derivatives. The intensity variations and mass shifts for mixed TMSi/TBDMSi derivatives confirm their composition and indicate a dependence upon a 5'-siliconium ion, while at the same time illustrating the value of these ions for identification of isomeric substituted derivatives. Metastable peaks confirm the decomposition of $[M - R - J]^+$ to $[M - R - J - CH_2O]^+$ and also to $RX_2SiOCH_2^+$. The same mechanisms as in Schemes 35 and 36 are proposed.

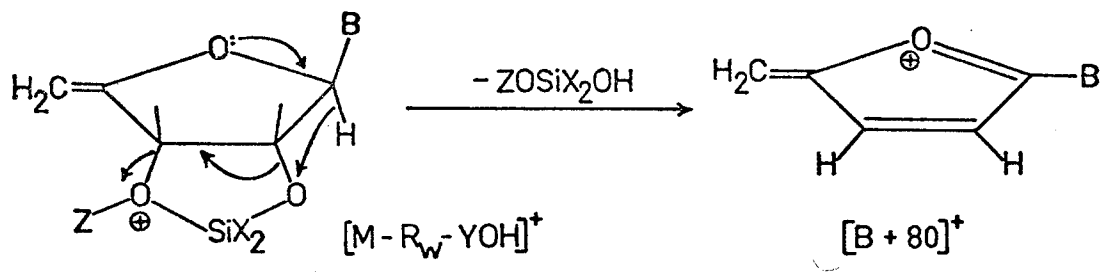
$[J - R_W]^+$ [XIX.19, VIbbb - m/e211]

This fragment ion is specific for ribonucleoside derivatives with a 2'-O-SCTASi function. For VIbbb and VIccc, it overlaps with ion D^+ at m/e211 and 237 respectively. The proposed mechanism is as follows:



$[B + C_5H_4O(80)]^+$ [XIX.20, VIbbb - m/e191]

This unusual ion has no analog in the spectra of deoxynucleosides and does not occur for VIIIbbba. It is very abundant for compounds with a 2'- or 3'-O-SCTASi group, especially the former. One possible precursor is $[M - R - RX_2SiOH]^+$, which could decompose by a mechanism such as that illustrated below for $[M - R_W - YOH]^+$:



$[J + H]^+$ [XIX.12, VIbbb - m/e269]

$[J + Z]^+$ [XIX.6, VIbbb - m/e383]

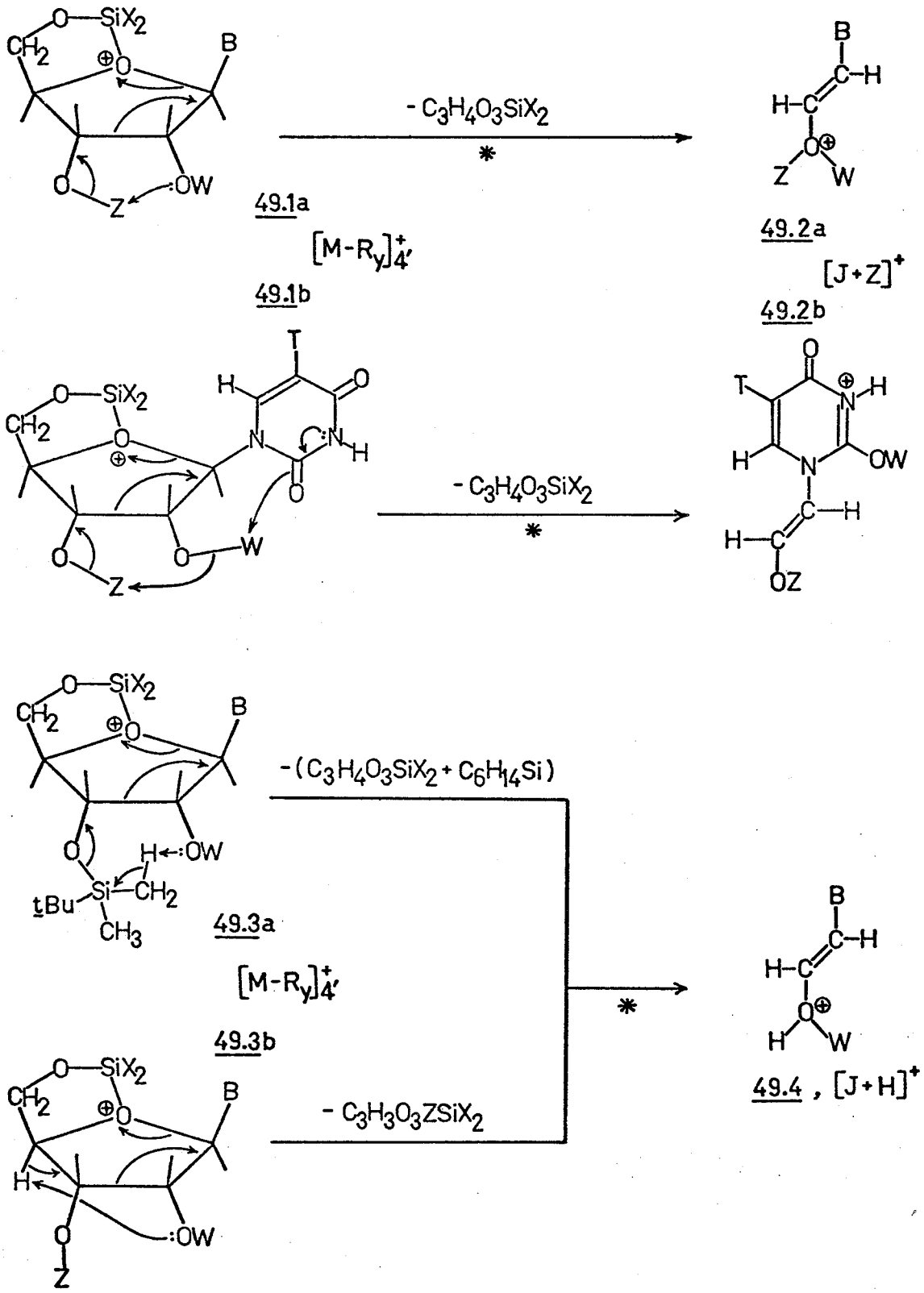
These abundant ions are useful for identifying isomeric substituted derivatives of ribonucleosides. Their counterparts in the deoxy series are much weaker. Metastable peaks indicate that $[M - R]^+$ is the precursor for both $[J + H]^+$ and $[J + Z]^+$. Mixed TMSi/TBDMSi derivatives were valuable in the assignment of these ion compositions and the proposed mechanisms. Peaks at m/e227 ($BC_2H_3OTMSi^+$) for VIbee and VIbbe, and at m/e269 ($BC_2H_3OTBDMSi^+$) for VIbeb and VIbbb, confirm a composition of $[J + H]^+$; the low abundance of this ion type for VIebe, VIeeb, and VIebb signifies the requirement of a 5'-siliconium ion. Similarly, ions at m/e299 ($[BC_2H_2OTMSi + TMSi]^+$) for VIbee, m/e341 ($[BC_2H_2OTMSi + TBDMSi]^+$) for VIbbe and VIbeb, and m/e383 ($[BC_2H_2OTBDMSi + TBDMSi]^+$) for VIbbb indicate that the two silyl groups involved are Z and W, and that $[M - R_y]^+$

is the precursor. It is interesting to note that the intensity of $[J+Z]^+$ appears to be inversely dependent on the steric bulk of W and Z, especially the latter. The ion is most abundant for compound VIbee, where Z = W = TMSi. This behavior is presumably due to a greater ease of silyl group migration with less steric crowding. (A weak $[J+Z]^+$ also occurs for VIeeb and VIebb; this is probably due to fragmentation direction by the weak $[M-CH_{3(y)}]^+$ ion.) Ions corresponding to $[J+H]^+$ and $[J+Z]^+$ are very weak for TMSi derivatives of nucleosides, but $[J+H]^+$ is observed in underivatized nucleosides (V = OH), although a different mechanism is required (15).

The mechanism proposed for $[J+Z]^+$ formation is given in Scheme 49. Migration of Z to $O_{2'}$ with fission of $C_{2'}$-$C_{3'}$ and $C_{1'}$-$O_{4'}$ bonds, all directed by the 5'-siliconium ion, explains the very specific nature of the ion. The rearrangement may be assisted by concurrent migration of W to the base to give a more stable ion structure 49.2b. The mechanisms proposed earlier for $[J+Z]^+$ and $[J+Y]^+$ for deoxynucleosides (Scheme 37) may be operating to a certain extent, but the low intensity for deoxynucleosides versus ribonucleosides indicates the importance of $O_{2'}$ in the mechanism.

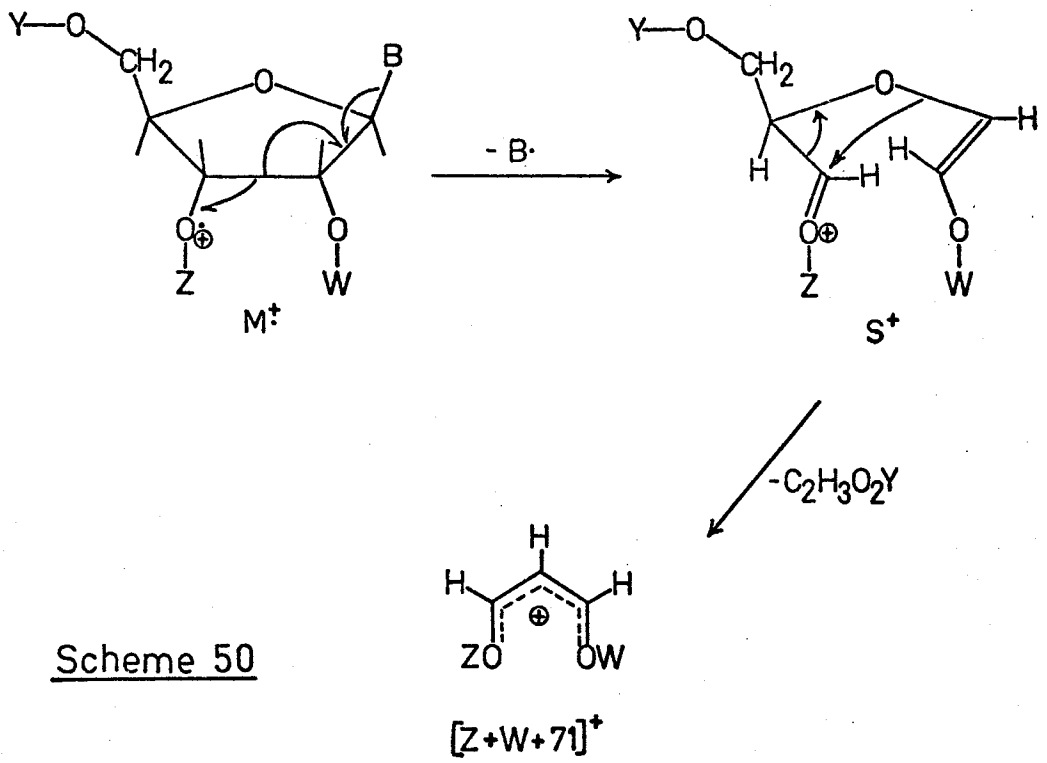
For $[J+H]^+$, the origin of the migrating hydrogen is not known. Any mechanism proposed should be consistent with the fact that the corresponding ion is very weak for SCTASi-derivatives of thymidine and deoxyuridine. Scheme 49 illustrates that $H_{4'}$ or a hydrogen from one of Z's alkyl substutuents are reasonable suggestions.

Scheme 49


- $C_3H_4O_3SiX_2$
*
49.1a
$[M-R_y]^{+\cdot}_4$
49.1b
49.2a
$[J+Z]^+$
49.2b
- $C_3H_4O_3SiX_2$
*
- $(C_3H_4O_3SiX_2 + C_6H_{14}Si)$
49.3a
$[M-R_y]^{+\cdot}_4$
49.3b
- $C_3H_3O_3ZSiX_2$
*
49.4 , $[J+H]^+$

$[2RX_2Si + C_3H_3O_2(71)]^+$ [XIX.9, VIbbb - m/e301]

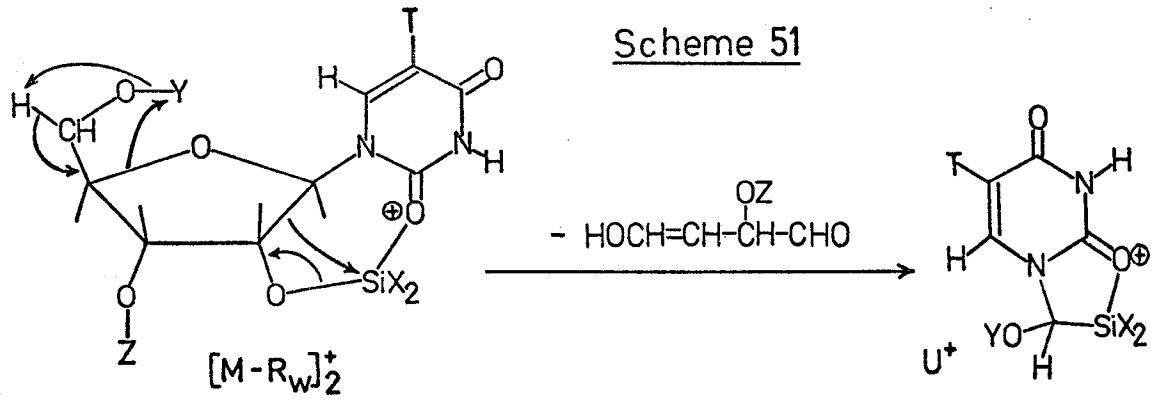
In the spectra of TMSi derivatives of ribonucleosides, m/e217 is a very abundant fragment (sometimes the base peak). In the literature (15), it has been identified as $C_3H_3(OTMSi)_2^+$. For the SCTASi derivatives of ribonucleosides, the analog of this ion, $(RX_2Si)_2C_3H_3O_2^+$ (m/e301 in VIbbb), is only of moderate abundance. Mixed derivatives reveal that both $[Y+Z+71]^+$ and $[Z+W+71]^+$ may be present, although the latter is very prominent for VIbee (Z = W = TMSi, m/e217). $[Y+Z+71]^+$ may be produced from either M^+ or S^+ by mechanisms described previously for deoxynucleosides (Scheme 44). The mechanism for $[Z+W+71]^+$ is not completely clear. Scheme 50 suggests one possibility. Whatever the precursor or mechanism of formation, the low abundance of these ions for tris-O-SCTASi derivatives is probably due to poor competition with formation of $[M-R]^+$.



Scheme 50

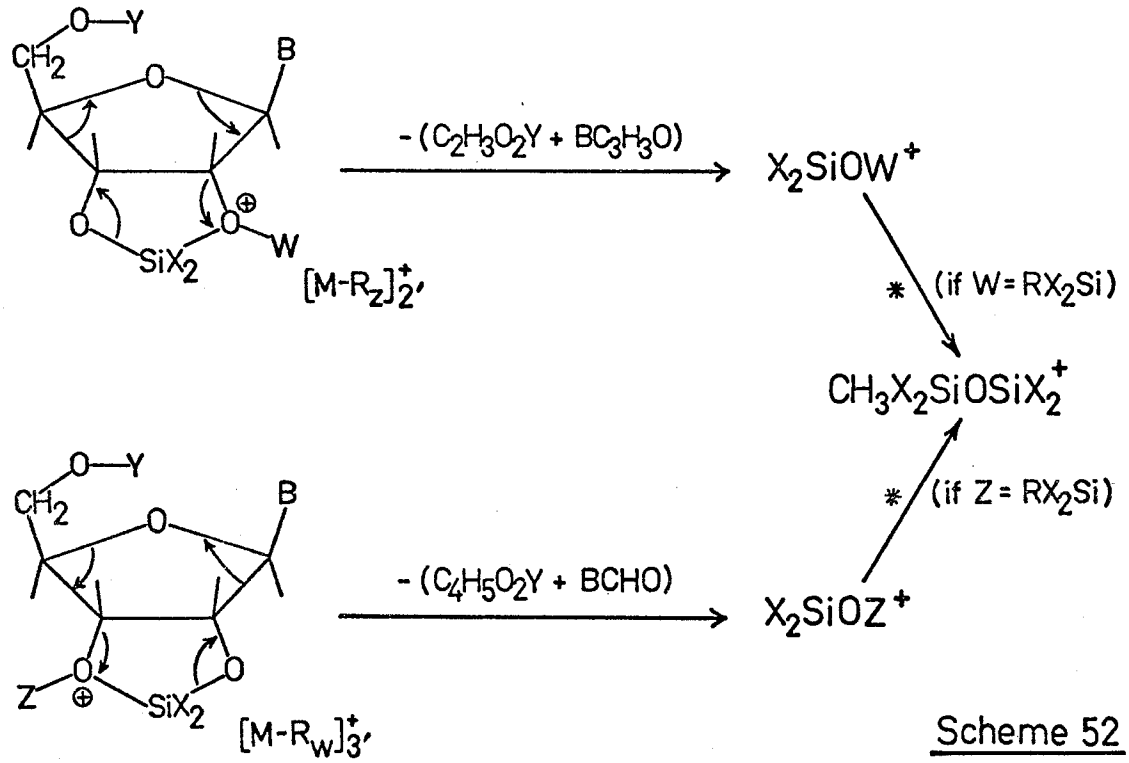
U^+ [XIX.7, VIbbb - m/e313]

This fragment ion is observed for tris-O-silyl derivatives of VI and VII with a 2'-O-SCTASi group. Mixed derivatives indicate the ion composition to be predominantly $[BCHO + X_2Si_w + Y]^+$, although there is a small contribution from $[BCHO + X_2Si_z + Y]^+$. There is no metastable peak to indicate a precursor. One possible mechanism is that illustrated in Scheme 51.



$RX_2SiOSiX_2^+$	[XIX.21, VIbbb - m/e189]
$CH_3X_2SiOSiX_2^+$	[XIX.23, VIbbb - m/e147]
$HX_2SiOSiX_2^+$	[XIX.26, VIbbb - m/e133]
X_2SiOH^+	[XIX.38, VIbbb - m/e75]

These ions are present for ribonucleoside derivatives as they were for deoxynucleosides. $CH_3X_2SiOSiX_2^+$, the rearrangement product of the first ion, is more intense for ribonucleosides. This is probably due to the facile interaction of the 2'- and 3'-O-silyloxy groups (Scheme 52) leading to X_2SiOW^+ and X_2SiOZ^+ which subsequently rearrange to CH_3X_2SiO-SiX_2^+. X_2SiOH^+ is quite weak for the fully silylated derivatives as discussed previously for the deoxy series.



Scheme 52

$YOCH_2^+$	[XIX.24, VIbbb - m/e145]
$CH_3X_2SiOCH_2^+$	[- , VIbbb - m/e103]
$HX_2SiOCH_2^+$	[XIX.36, VIbbb - m/e89]

The first two ions are not as abundant for ribonucleoside derivatives as they were for the deoxy series. This may be due to the low abundance of K^+, an important precursor for $YOCH_2^+$ with the deoxynucleosides. The related ion $HX_2SiOCH_2^+$ is still quite intense. Although metastable peaks indicate formation from $YOCH_2^+$, other sources are probably more important.

RX_2Si^+	[XIX.29, VIbbb - m/e115]
$CH_3X_2Si^+$	[XIX.39, VIbbb - m/e73]
HX_2Si^+	[XIX.40, VIbbb - m/e59]

These are prominent for ribonucleoside derivatives as they were for the deoxy series.

Other ions

Fragment ions corresponding to S^+ and its daughter ions, K^+, $[K - CH_2O]^+$, and $[RX_2Si + 72]^+$, that were observed for SCTASi derivatives of deoxynucleosides, are very weak or completely absent for ribonucleosides.

$[B + H]^+$, $[B + 2H]^+$, and $[B + H + RX_2Si]^+$ are present for the ribonucleoside derivatives but in low abundance (except for VIIIbbba, where $[B + 2H]^+$ is of moderate intensity).

D. Summary

Although many underivatized nucleosides yield acceptable mass spectra, volatile derivatives are generally preferred for electron-impact MS, and are essential for GC/MS. Trimethylsilylation is the most valuable derivatization procedure available. However, research into new types of derivatives is important since it may be possible to improve mass spectral fragmentation behavior and derive more structural information. The use of two complementary types of derivatives also allows more confident "fingerprint" identification.

The SCTASi derivatives have excellent potential for mass spectrometry. In addition to having characteristic spectra that provide detailed structural information on modifications in both the base and sugar moieties, they possess good chromatographic properties, are easily prepared, and are stable for isolation. In addition, mixed derivatives that are valuable for determining fragmentation mechanisms are relatively easy to prepare.

Hopefully, the spectral interpretations proposed in this chapter should be valuable for future applications of SCTASi derivatization to MS, as well as the use of MS in characterizing isomeric substituted nucleosides of synthetic (and biological) interest. Of course there remains a great deal of work to be done in these areas, particularly with the cytosine and guanine nucleosides.

V. Applications of Sterically Crowded Trialkylsilylation in Gas Phase Analytical Chemistry

A. Introduction

Sterically crowded trialkylsilyl ether derivatives have the potential of being excellent analytical derivatives for the chromatography and mass spectrometry of a wide variety of hydroxylated compounds. A number of features of analytical value are apparent from previous experience with synthetic applications and the study of nucleoside derivatives with gas phase analytical techniques:

(a) Their hydrolytic stability allows easier handling, more quantitative TLC, and isolation of stable, pure standards for reference or for quantitative work.

(b) Most SCTASi derivatives have sufficient volatility for GC analysis. Also, their increased steric bulk and longer retention times (relative to TMSi derivatives) could give better separation of isomers and mixtures, and an homologous series of derivatives could be useful for structural inferences by GC.

(c) The mass spectra of SCTASi derivatives have an abundant $[M-R]^+$ fragment ion useful for determining molecular weights and for GC/MS-SIR analyses. Fragmentations are directed by the SCTASi group, often leading to structurally and stereochemically informative fragment ions. An homologous series of derivatives is useful for labelling, and mixed derivatization schemes allow a detailed elucidation of fragmentation mechanisms. (See discussion on pages 115 - 117.)

Therefore, this chapter re-examines, from an analytical viewpoint, some of the results reported in Chapters III and IV for nucleosides, and

in addition, reports the results for the derivatization, chromatography and mass spectrometry of another class of compounds--steroids.

Steroids were selected for several reasons, namely: (a) gas phase analysis is accepted as the most powerful analytical method in this area; (b) silyl derivatives are commonly used for the TLC, GC, and MS of steroids; (c) a large number of compounds are available, with a variety of different types of hydroxyl functions; and (d) the separation and characterization of certain steroid isomers presents an interesting challenge for SCTASi derivatization. Scheme 53 outlines the series of steroid derivatives examined in Sections B1 to B4 of this chapter. The complete list of individual derivatives appears in Table XXII.

Details and evaluations of reagent systems, with respect to the silylation of various functions, are presented in the next section, followed by discussions of the chromatographic and mass spectral behavior of various derivatives. Finally, the application of SCTASi derivatization to the analysis of a mixture of steroid metabolites is reported in Section B5.

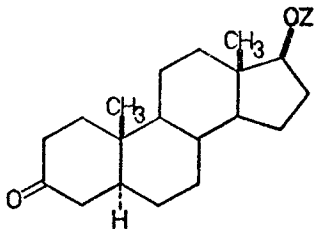
The results in this chapter demonstrate the dramatic effect of the SCTASi group on stability during TLC, separations by GC, and mass spectral fragmentations, and illustrate the potential of sterically crowded trialkylsilylation for the gas phase analytical chemistry of biologically important compounds in general.

Scheme 53

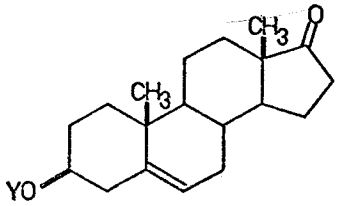
Substituents Y,Z,W: a = H
b = TBDMSi
c = TMTBSi
d = TMIPSi
e = TMSi

#	Parent Compound
XIy	5-cholesten-3β-ol (cholesterol)
XIIy	5-cholesten-3α-ol (epicholesterol)
XIIIy	3α-hydroxy-5α-androstan-17-one (androsterone)
XIVy	3β-hydroxy-5α-androstan-17-one (epiandrosterone)

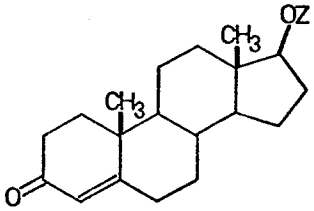
Scheme 53 (continued)



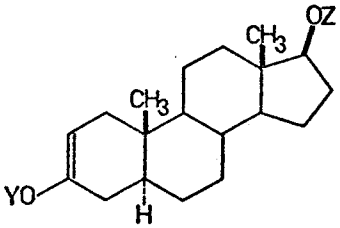
XVz 17β-hydroxy-5α-androstan-3-one (androstanolone)



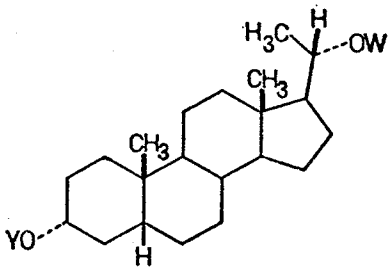
XVIy 3β-hydroxy-5-androsten-17-one (dehydroepiandrosterone)



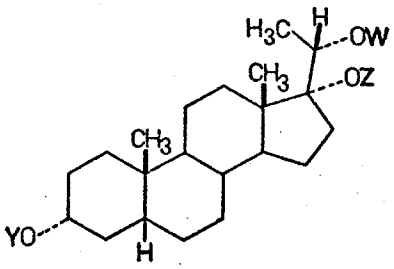
XVIIz 17β-hydroxy-4-androsten-3-one (testosterone)



XVIIIyz 5α-androst-2-en-3,17β-diol (enol form of XV)



XIXyw 5β-pregnane-3α,20α-diol



XXyzw 5β-pregnane-3α,17α,20α-triol

B. Results and Discussion

1. Derivatizations

The organic chemistry of derivatization is one of the most important considerations in the development of new gas phase analytical procedures. The following are some of the features desired for an analytical derivatization method:

(a) It should be relatively simple and suitable for micro-scale derivatizations.

(b) The reaction should be fast and produce a single product in quantitative yield, preferably under mild conditions.

(c) The solvent should readily dissolve substrates and retain products in solution.

(d) The reaction mixture should be suitable for direct analysis.

(e) There should be no side-reactions between substrate and reagent or solvent.

(f) The reagent and the side-products of the reaction should not interfere with the analysis.

(g) The derivatives should be stable under experimental conditions.

The first concern with SCTASi derivatization is that with the extensive steric crowding in the silyl groups, it may be difficult to achieve fast, quantitative silylation. However, it has been demonstrated by Corey and Venkateswarlu (63) that a variety of alcohols can be converted to TBDMSi ethers in high yield under mild conditions by using the reagent TBDMSiCl with imidazole (Im) as a catalyst and dimethylformamide (DMF) as a solvent. This approach has since been extended to other sterically crowded silyl groups for the synthesis of nucleoside derivatives (66,67,69).

TABLE XXI. SCTASi Reagent Systems*

Ax	1M SCTASiCl + 2M Im + DMF
Bx	1M SCTASiCl + 2M Im + PYR
Cx	1M SCTASiCl + PYR
D	1M TBDMSiIm + PYR
E	1M $TBDMSi_2Ac$ + PYR

* subscript "x" indicates the type of chlorosilane: b = TBDMSiCl, c = TMTBSiCl, d = TMIPSiCl

For this study, with analytical applications in mind, a number of new reagent systems for SCTASi derivatization have been investigated. These are listed in Table XXI and their reactions with various functions are discussed below.

a) Sterically Accessible Hydroxyls

Reagent A (b to d) gave very fast silylations of all primary and sterically accessible secondary hydroxyl functions studied. These include the 3α, 3β, 4α, 6α, 17(2°, α andβ), and 20α hydroxyls of steroids and the 2', 3', and 5' hydroxyls of nucleosides. Even with the very sterically crowded TMTBSi group, most reactions were suitable for analysis within an hour at room temperature and yields were quantitative (i.e., >99.9%) as shown by GC and TLC analyses. Sterically hindered hydroxyls may present a problem and are discussed below. As a precaution, and for convenience, reactions were usually left overnight. If there is any doubt about the reactivity of certain hydroxyls, heating is advisable. Despite its good reactivity, Reagent A is not ideal for all analytical work. The low polarity of the silyl ethers of some steroids (e.g., cholesterol) resulted in the precipitation of the derivatives from the

DMF solvent. Although this is useful in the synthesis and isolation of pure reference standards, it is unfavorable for direct analysis of the reaction. Addition of dichloromethane to the reaction mixture (with or without prior removal of DMF) usually resulted in complete dissolution of the products.

Reagent B (b to d) was found to be the best reagent system for analytical work and was used for the preparation of most derivatives discussed in this thesis. Reactions were found to be as fast and quantitative as for Reagent A. In addition, pyridine is a better solvent for both substrates and derivatives studied, allowing direct analysis of the reaction mixture by GC. Occasionally, a crystalline precipitate was observed but isolation and analysis revealed no substrate or derivative. Therefore, it was assumed to be the hydrochloride of either imidazole or pyridine.

Reagent C (b to d) is a much less reactive system due to the lack of imidazole as a catalyst. Although primary hydroxyls were silylated fairly readily by all chlorosilanes, secondary hydroxyls required overnight reaction with TMIPSiCl and TBDMSiCl, and overnight heating at 80° with TMTBSiCl. However, this reagent may have some advantages since, as discussed below, enol ether formation was not observed.

As analytical applications of SCTASi derivatives grow in number, particularly for multifunctional compounds, there will be a need for the development of new silylating reagents. They will probably be based on silyl donors analogous to those already developed for trimethylsilylation (23). Recently, Kutschinski (109) has prepared a series of such reagents for TBDMSi derivatization and investigated their reactivity with various functional groups. Prior to the publication of these studies, we had

made a preliminary investigation of two new reagents: N-tert-butyldimethylsilyl imidazole (TBDMSiIm) and N,O-bis-tert-butyldimethylsilyl acetamide ($TBDMSi_2Ac$) (66). Our results agree with those of Kutschinski (109). TBDMSiIm is presumably an intermediate in the reactions of Reagents Ab and Bb, and in fact we have observed its presence in these reagents by GC/MS. However, the fast silylations displayed by these reagents are probably due to catalysis by HCl or TBDMSiCl. This is indicated by the fact that when pure TBDMSiIm was prepared, it was a very weak silyl donor in either pyridine (Reagent D) or dimethylformamide (see results in Figure 19, Chapter III). This is a useful property for synthesis where selective partial silylations can be easily controlled, but one that is unsuitable for analytical work. However, the addition of TBDMSiCl (0.05M to 0.5M) to Reagent D was observed to dramatically increase the reactivity. Similar results were observed with $TBDMSi_2Ac$. The possible advantages for these reagents include the absence of HCl (and subsequent pyridine and imidazole hydrochloride salt formation) released during silylations by chlorosilane-based reagents, and as discussed below, the selective silylation of hydroxyls in the presence of amines. Thus, with suitable conditions, these reagents could prove analytically useful, although none of the alternative reagents investigated thus far can challenge the potency of Reagents A and B.

b) Sterically Hindered Hydroxyls

Certain secondary and tertiary hydroxyls are sterically hindered and could present problems in the application of SCTASi derivatives. Such problems have been encountered even with trimethylsilylation of such functions as the secondary 11β or tertiary 17α hydroxyls of steroids. Rather forcing conditions are required in order to achieve trimethyl-

silylation of such hydroxyls (176,246,247). For example, treatment of 5β-pregnane-3α,17α,20α-triol (XX) with Tri-Sil Z at room temperature led primarily to the 3α,20α-bis-TMSi ether derivative (XXeae); complete silylation to the tris-TMSi derivative (XXeee) was achieved by reaction with pure TMSiIm (which acts as both solvent and reagent) at 80° overnight. With the TBDMSi and TMTBSi reagents (Ab,Ac,Bb,Bc), no detectable 17α-OH silylation was observed, even after 90 hours at 80°. The only product produced was the 3α,20α-bis-SCTASi ether derivative (XXbab or XXcac). Thus, with the present reagents, SCTASi ethers of very sterically hindered hydroxyls are not readily produced. However, this behavior may be used to advantage since, with SCTASi derivatization, one can be fairly well assured that such hydroxyls will not be silylated; with trimethylsilylation there is always the danger of multiple derivatives being formed unless forcing conditions are used to ensure complete silylation.

Another steric influence on silylations using SCTASi groups may occur for polyhydroxy compounds with adjacent hydroxyls. The first silyl group that is attached may hinder the next incoming silyl group, resulting in slow formation of the completely derivatized compound. It was anticipated that such a situation might exist with ribonucleosides. Figure 102 gives the results of an experiment in which the silylation of uridine (VI) in Reagent Ab at room temperature was monitored by GC. Analysis revealed that within 10 min, the reaction had proceeded to 92 mole% of the bis-TBDMSi derivatives (mostly VIbba + VIbab) and 8 mole% of the tris-TBDMSi derivative (VIbbb). Complete conversion to VIbbb required about 5 to 6 hours at room temperature. Another experiment showed that Reagent Bb gives almost identical rates. Heating at 80° for one hour achieved quantitative silylation. The TMTBSi reagents (Ac and

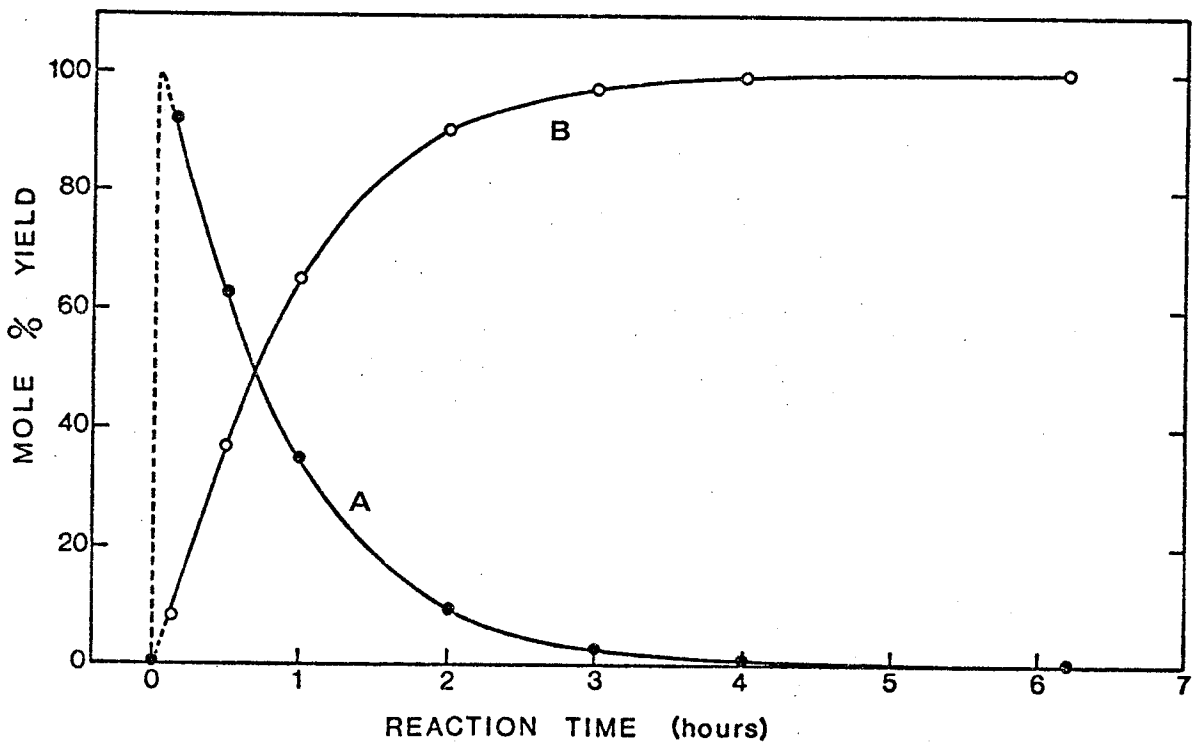


Figure 102. The derivatization of uridine (0.05M) with Reagent Ab at room temperature (22°). GC analysis was performed by method 2 (see Experimental): aliquots were removed at timed intervals and treated with TMSiIm to quench the reaction and to prevent injector port reaction errors. As illustrated in Fig. 30 (Chapter III), this allows for the determination of VI, mono-TBDMSi-VI, bis-TBDMSi-VI, and VIbbb as the mixed TMSi/TBDMSi derivatives. Curves A and B give the mole % yield measured for (VIbba + VIbab + VIabb) and VIbbb, respectively. No trace of unreacted uridine or mono-TBDMSi derivatives could be detected at 10 minutes reaction time.

Bc), on the other hand, required heating at 80° overnight to effect complete derivatization. Thus, there is a steric effect in the silylation of the ribonucleoside 2',3'-cis-diol system which can present problems for analytical work if adequate conditions are not employed.

c) Partial and Mixed Derivatizations; Silyl Group Exchange and Migration

Partial derivatization, by treatment of an unknown compound with less reagent than required for all reactive groups (148,149) or by selective silyl reagents (150), has been suggested as a structurally informative technique. Similarly, mixed derivatizations by treatment of such partial derivatives with different silyl groups (53,248), or by treatment of compounds with mixed reagents (36) can be useful for characterization of compounds and elucidation of mass spectral fragmentations.

Since SCTASi reagents have excellent selectivity under controlled conditions, they have considerable potential for such applications. Chapter IV has already demonstrated the utility of partial and mixed derivatives for the elucidation of fragmentation pathways of silylated nucleosides. This section reports some of the results obtained with steroids.

An illustration of the selectivity of SCTASi reagents is given by the TBDMSi derivatization of 5β-pregnane-3α,20α-diol (XIX). A reaction of this steroid with 1.2 equivalents of Reagent Bb gave the partial silyl derivatives, XIXba (3-O-TBDMSi-XIX) and XIXab (20-O-TBDMSi-XIX), in a 15:1 mole ratio (in addition to some unreacted steroid and 3,20bis-O-TBDMSi ether derivative, XIXbb). All products were separable by GC (see Table XXII and Figure 103) and were identified by MS. The equatorial 3-hydroxyl is much more reactive than the 20-hydroxyl, although both are secondary. (Hosoda et al.(75,77) have also reported such selectivity in

the TBDMSi derivatization of other steroids.) Treatment of this partial derivative mixture with Tri-Sil Z gave the mixed TMSi/TBDMSi derivatives, XIXbe and XIXeb, which were separable by GC (see Figure 103). These proved useful in the interpretation of the mass spectra of SCTASi derivatives of XIX (see Section B4).

One concern in mixed derivatization schemes is the possibility of silyl group exchange, especially under the forcing conditions required for the silylation of sterically hindered hydroxyls. To investigate this, TMSi and TBDMSi derivatives of various steroids were subjected to different conditions and possible catalysts. TMSi derivatives of cholesterol (XIe), androsterone (XIIIe), and epiandrosterone (XIVe) were examined first. Although they were quite stable as isolated solids and in pyridine solution (even with heating), slow decomposition to the underivatized steroid was observed when in a solution of 2M imidazole in pyridine. This was accelerated by the addition of imidazole hydrochloride (Im·HCl). When they were dissolved in Reagent Bb, complete exchange of TMSi for TBDMSi (to give the TBDMSi derivatives) occurred within one hour at room temperature. Thus, the TMSi group is quite labile under these catalytic conditions. All TBDMSi steroid derivatives (XIb,XIIIb,XIVb, and XIXbb), on the other hand, were stable to a variety of conditions such as: (a) neat TMSiIm at 90° over 2 days, or (b) a mixture of 50% TMSiIm and 1M imidazole (Im) in pyridine at 90° over 2 days. However, when Im·HCl was added to reaction (a), slow exchange of equatorial 3-silyloxy functions occurred (in compounds XIVb and XIXbb). This also arose when an entire TBDMSi derivatization reaction mixture (i.e., steroid + TBDMSiCl + Im + PYR (or DMF)) was evaporated to dryness and then the residue treated directly with neat TMSiIm (50% conversion of XIXbb to XIXeb after 50

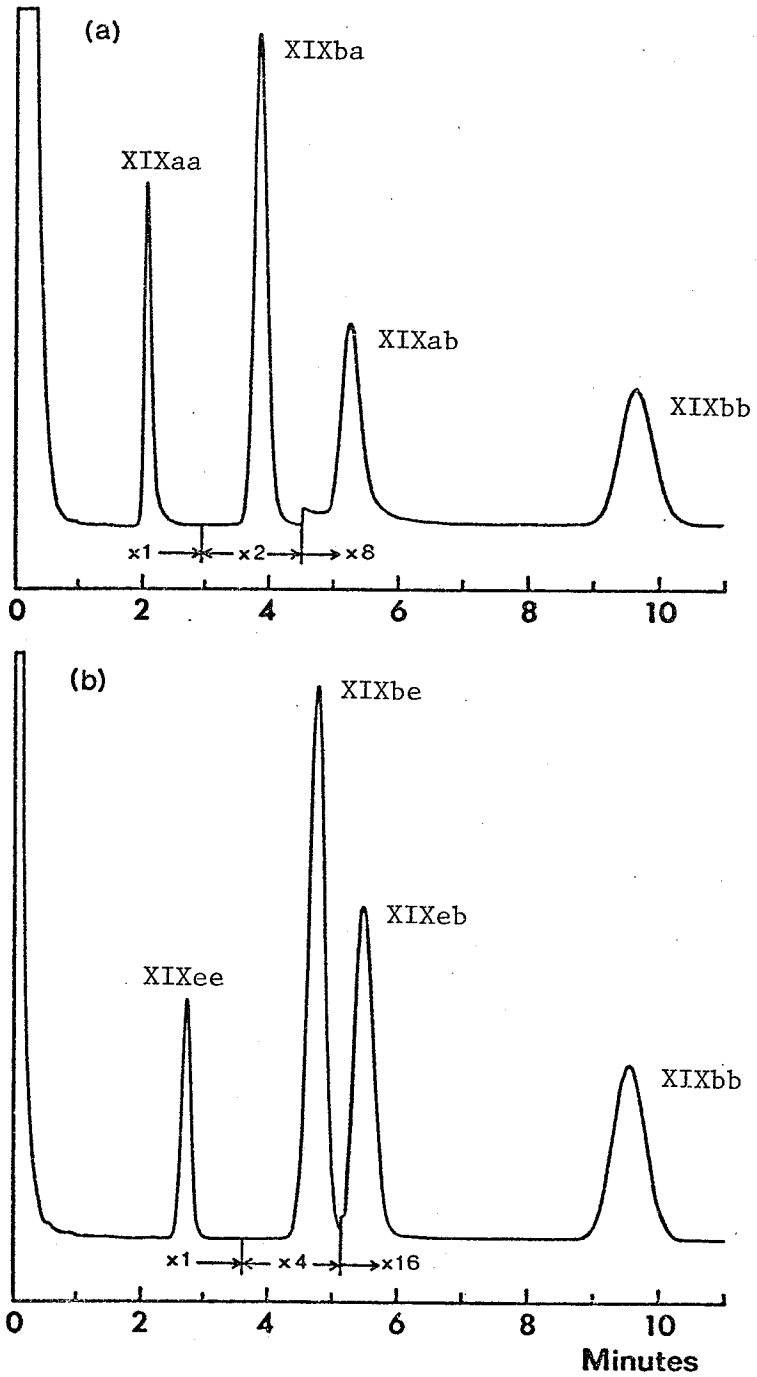
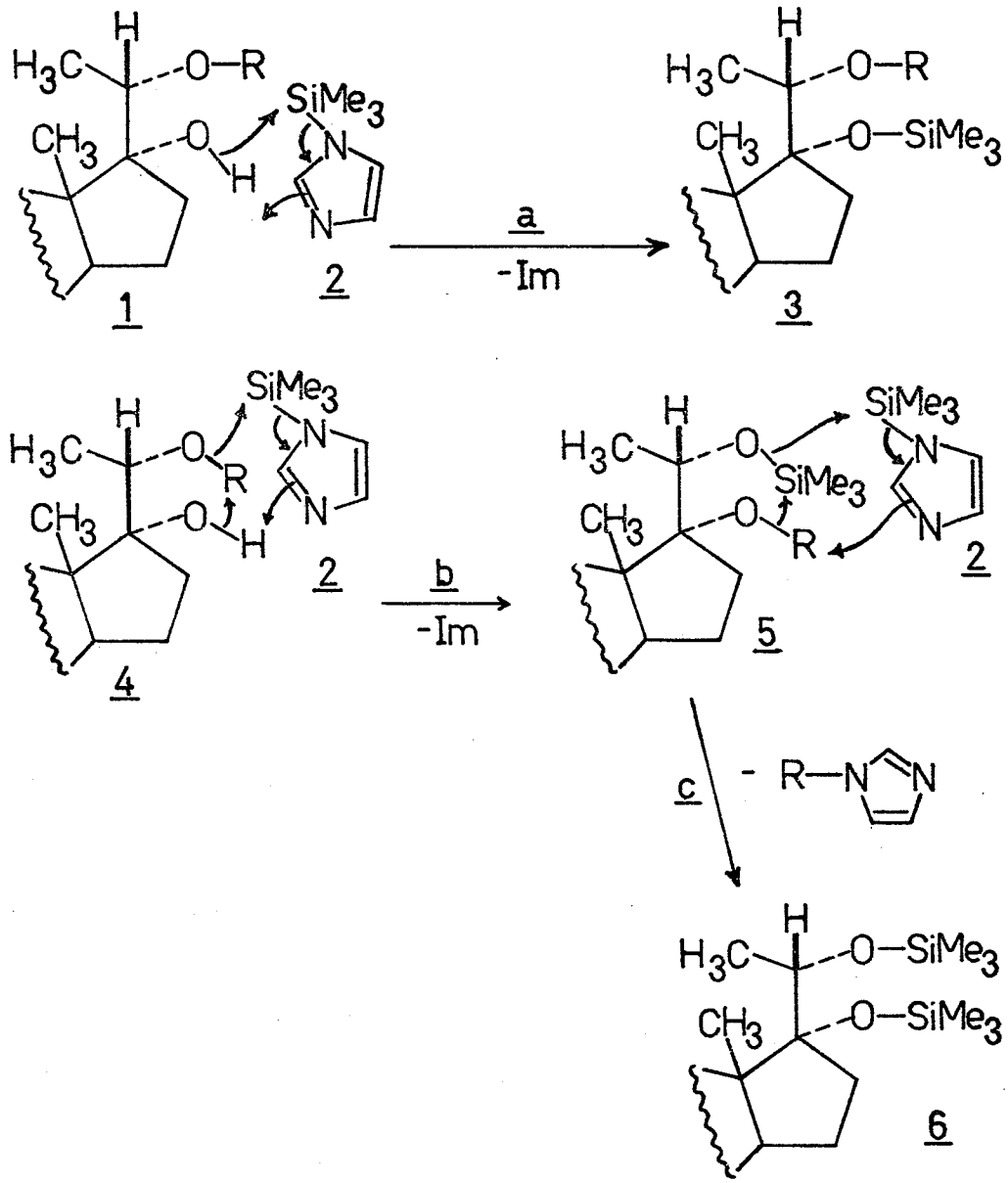


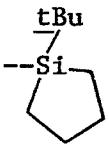
Figure 103. Gas chromatograms of (a) partial TBDMSi derivatization reaction of 5β-pregnane-3α,20α-diol XIXaa, and (b) further TMSi derivatization of (a) to give mixed TMSi/TBDMSi derivatives. Conditions: column A (10% OV-1, 1m x 2mm-ID), 260°, 30 ml/min N_2 carrier.

hours at 90°). Im·HCl is present in the TBDMSi reagent system and catalyzes the exchange. Axial 3-silyloxy and 20-silyloxy groups were not affected by any of these conditions. Thus, derivatives should first be isolated from Im·HCl if further derivatization is to be performed under forcing conditions.

With these results in mind, the trimethylsilylation of the partial 3,20bis-O-SCTASi derivatives of 5β-pregnane-3α,17α,20α-triol (XXbab and XXcac) was studied. There were two reasons for this experiment. Firstly, complete derivatization of all functions is desired for quantitative analysis by GC (see Section B3). Secondly, it was of interest to determine if SCTASi groups migrate between adjacent hydroxyls (17- and 20-OH's), as has been observed for TMSi by Vouros (248) in the study of mixed TMSi/dTMSi derivatization of 17α,20-dihydroxy steroids. In that case, silylation of the 20-O-dTMSi derivative by TMSiIm yielded the 17-O-dTMSi-20-O-TMSi derivative, quantitatively, indicating that rearrangement b, as illustrated in Scheme 54 (R = dTMSi), had occurred.

In the first experiment with SCTASi derivatives, XXbab (produced in the reaction of XX with Reagent Bb) was isolated by Sephadex LH-20 column chromatography to remove Im·HCl from the reaction mixture (in order to avoid catalyzed exchange of the equatorial 3-silyloxy group), and was treated with neat TMSiIm. However, after 50 hours at 90°, there was still 40% unreacted XXbab, indicating the strong steric influence that the 20-O-TBDMSi group has upon the attacking TMSi reagent. The products consisted of the 3,20bis-O-TBDMSi-17-O-TMSi (XXbeb) and 3,17bis-O-TBDMSi-20-O-TMSi (XXbbe) derivatives in a 5:1 mole ratio. These are due to reactions a and b of Scheme 54 (R = TBDMSi), respectively. Thus, migration of the TBDMSi group does occur, but not as readily as does



Scheme 54. R = $--Si(CD_3)_3$; $--Si(CH_3)_2-tBu$; or 

TMSi. This may be due to the increased steric bulk hindering rearrangement to a 3°-OH. It is obvious, however, that these conditions are not suitable for analytical work.

The trimethylsilylation of XXbab was also attempted in the presence of Im·HCl. The TBDMSi reaction mixture containing XXbab (i.e., XX + Reagent Bb) was evaporated and treated directly with neat TMSiIm. In this case, the reaction proceeded more readily, with the Im·HCl acting as a catalyst for trimethylsilylation. After 12 hours at 90°, 70% had been converted to the 3,20bis-O-TBDMSi-17-O-TMSi derivative (XXbeb). No migration of the TBDMSi group was observed. However, the remaining 30% of the reaction products was the 3,17bis-O-TMSi-20-O-TBDMSi derivative, XXeeb, due to the exchange of the 3-silyloxy group.

When the trimethylsilylation of the 3,20bis-O-TMTBSi derivative, XXcac, was attempted under similar conditions (with Im·HCl present), the results were quite different from the TBDMSi experiment. After 12 hours at 90°, all hydroxyls had been silylated and there were three products: 30% XXcec (3,20bis-O-TMTBSi-17-O-TMSi), 30% XXcce (3,17bis-O-TMTBSi-20-O-TMSi), and 40% XXcee (3-O-TMTBSi-17,20bis-O-TMSi). In this reaction, no exchange of the equatorial 3-silyloxy group occurred, likely due to the increased hydrolytic stability of the TMTBSi ether; but, in addition to silylation of the 17-OH to give XXcec (reaction a, Scheme 54), the TMTBSi group migrated from the 17-OH to the 20-OH to give XXcce (reaction b). The third product, XXcee, was possibly formed by reaction c in Scheme 54. It is unlikely that XXcee was formed by exchange of the 20-TMTBSi group of XXcec, since the more labile 3-TMTBSi was not even exchanged.

Thus, the TMTBSi group appears to have a greater aptitude for

migration than TBDMSi, despite its greater steric bulk. This agrees with an observation of the behavior of the partial SCTASi derivatives of uridine (VI); migration of a SCTASi group between the 2' and 3' hydroxyls can occur under certain conditions (e.g., see Chapter III, section B2). In these cases, TMTBSi migrates far more readily than TBDMSi. The reason for this is not known at this time.

Although these reactions are interesting, they are not suitable for complete derivatization of the pregnanetriol-type system for quantitative work. Moreover, they indicate that due to the lability and migratory ability of silyl groups, care should be taken in the application of mixed derivatization schemes to MS fragmentation elucidation.

d) Enol Silyl Ether Formation

A possible source of error and confusion in the GC analysis of steroids is the formation of enol silyl ethers from certain enolizable ketones. Enol TMSi ethers of 3-, 17-, and 20-ketone functions have been observed and their yields depend upon reagents used and reaction conditions (249). It is now a common procedure to protect such ketones as methoxime (MO) derivatives prior to silylations in order to prevent multiple product formation (168,249). However, it has been suggested that enol TMSi ethers of some ketosteroids could be analytically useful derivatives (250).

In this study, enol SCTASi ether formation was observed for two ketohydroxysteroids: 17β-hydroxy-5α-androstan-3-one (XV, androstanolone) and 17β-hydroxy-4-androsten-3-one (XVII, testosterone). The chromatographic data for both the TMSi and TBDMSi enol ether derivatives are given in Table XXII. No such derivatives were observed for the 17-ketosteroids studied. It should be noted that enol ether formation is

easier to recognize by GC for the SCTASi derivatives than for TMSi derivatives due to larger retention increments for the addition of each silyl group (see Figure 106, Section B3). In some cases, the multiple TMSi products are not separable on a nonpolar column such as OV-1.

The yields of enol TBDMSi ethers of androstanolone were measured for the different reagent systems. Reagent Bb gave the greatest rate of formation--about 5% per day at room temperature. This can be compared with about 1% per day for Tri-Sil Z (TMSiIm in pyridine). The DMF-based Reagent Ab gave only 2% per day at room temperature. It has been reported that DMF reduces enol TMSi ether formation (168). Reagent Cb did not give any detectable enol ether formation even after reaction for 2 days at 80°. However, the overall low reactivity of this reagent detracts from the advantage of avoiding enol ether formation.

For testosterone, enol TBDMSi ether formation using Reagent Ab was very slow--about 3% in 12 days. Tri-Sil Z gave a similar rate for enol TMSi ether formation. For both TMSi and TBDMSi derivatizations, two isomeric enol silyl ethers were observed, along with a number of small peaks. Figure 104 illustrates the results for the TBDMSi reaction. Chambaz et al. (168) determined that the enol TMSi ether products had 2,4- and 3,5-diene structures. The enol TBDMSi ethers' identities were tentatively assigned by analogy according to relative retention times. The smaller peaks in Figure 104 are probably oxysilylation products as proposed for the TMSi reaction (168).

Other reagent systems for SCTASi derivatization may be found which are more selective for hydroxyls versus ketones, or alternatively more efficient for enol ether formation. However, a better approach, in general, would be to use MO-SCTASi derivatives.

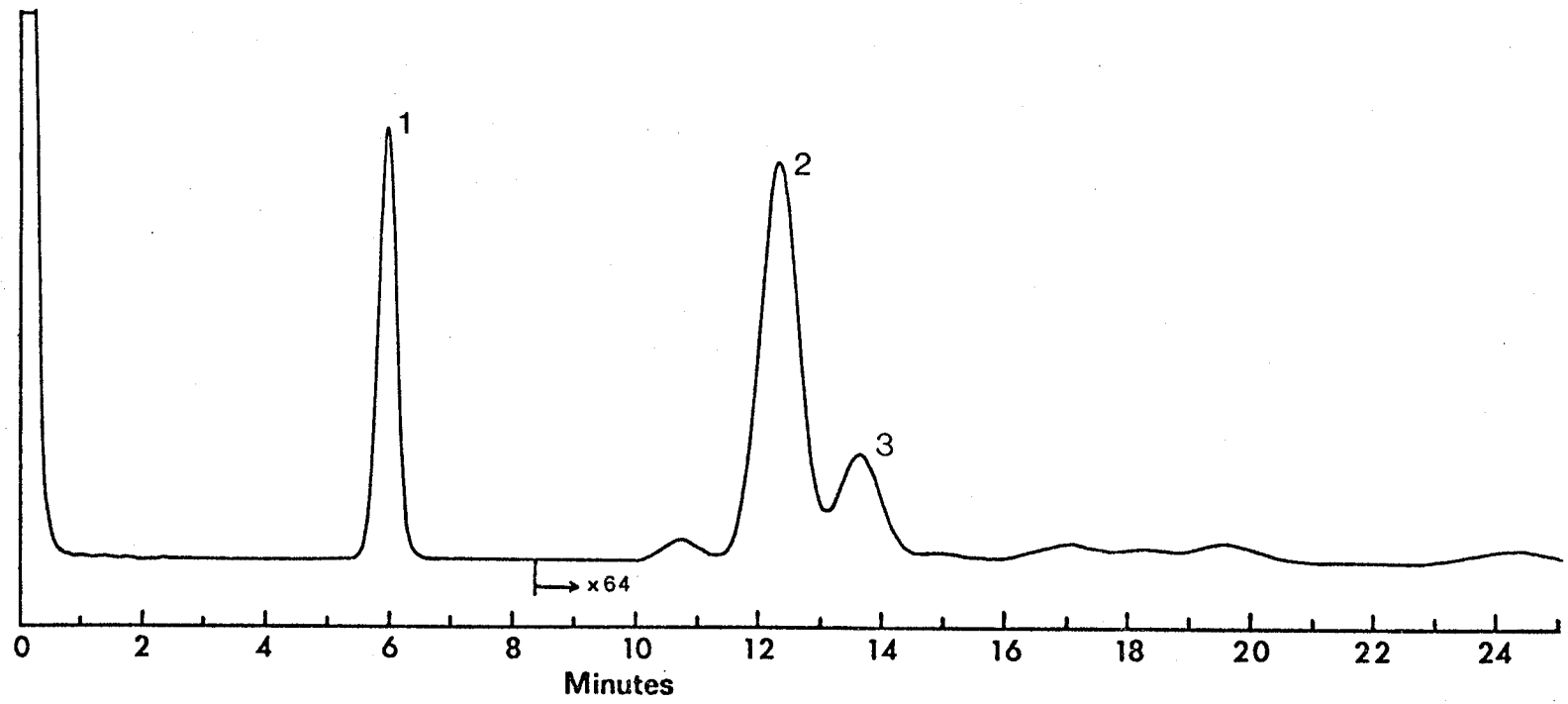


Figure 104. Gas chromatogram of TBDMSi derivatives of testosterone, produced by reaction at room temperature for 12 days with Reagent Ab. Peak identities are: 1 = TBDMSi-testosterone (XVIIb); and tentatively: 2 = 3,17βbis-TBDMSiO-androst-2,4-diene, XXIbb, 3 = 3,17βbis-TBDMSiO-androst-3,5-diene, XXIIbb. The remaining small peaks are probably oxysilylation products. Conditions: column A (10% OV-1, 1m x 2mm-ID), 250°, 30 ml/min N_2 carrier.

For some nucleosides, it is possible to form enol TMSi ethers from the base carbonyls if forcing conditions are used (128). With the SCTASi reagents and conditions employed in this study, no base silylation of either thymidine or uridine was observed.

e) Silylation of Amines

An additional reason for investigating other types of reagents is that they may have different selectivities in their reactions with different types of functional groups. For example, a reagent that is selective for hydroxyls in the presence of amines would be useful (TMSiIm possesses such a property (251)), as would a reagent that is equally efficient in silylating both. Reagents A and B (b to d) appear to be midway between these extremes; in the silylation of adenosine and deoxyadenosine, the base amino group is not silylated quantitatively, even with heating. Reagent D (1M TBDMSiIm/PYR), on the other hand, was found to be selective for hydroxyls, not giving any detectable N-TBDMSi derivatives. However, its overall low reactivity is a limitation for analytical work. As pointed out later, N-SCTASi derivatives may be analytically useful and therefore, research is continuing with new reagent systems.

f) Isolation and Purification of Derivatives

One of the advantages of SCTASi ethers is their far greater stability over TMSi ethers towards hydrolysis, permitting easier handling and isolation of derivatives for use as standards. All the TBDMSi and TMTBSi ether derivatives that have been isolated are stable, crystalline compounds with sharp melting points (see Tables III and XXII). They are easy to purify by extractions and crystallizations, thin layer chromatography, and for steroids, vacuum sublimation. (The latter method has been used for steroid TMSi ethers (25).) The TMIPSi derivatives are not

quite as stable: 1°-O-TMIPSi groups are labile enough that some hydrolysis occurs during TLC isolations. However, the main application for this group in analytical work is for incorporation into mixed derivatives and the elucidation of mass spectral fragmentation pathways. As shown below, the TBDMSi and TMTBSi groups are, in general, the most useful ones for GC and TLC. It should be noted that halomethyldimethylsilyl (37,40) and pentafluorophenyldimethylsilyl (46) derivatives have been observed to be more stable than TMSi derivatives, but it is not possible to comment on their stabilities relative to those of SCTASi ethers.

Sephadex LH-20 column chromatography of steroid or nucleoside reaction mixtures proved to be a useful method for removal of imidazole and either DMF or PYR, and isolation of the SCTASi derivatives. This technique has been used by Kelly and Taylor (97) for the isolation of steroid TBDMSi ethers from Reagent Ab.

It should also be mentioned that SCTASi groups are readily removed to yield the original substrate by treatment of the derivative with tetra-n-butyl ammonium fluoride in THF (63). Selective desilylation can be achieved by slow, controlled hydrolysis with 80% aqueous acetic acid (67,69,83).

2. Thin Layer Chromatography

The TLC data for the various steroid derivatives studied are presented in Table XXII. The mobility of the steroid derivatives depends upon their polarity. Using cyclohexane/ethyl acetate (9:1,v/v) as development solvent, underivatized hydroxysteroids remain near the origin, ketosilyloxysteroids have medium mobility with R_f = 0.4 to 0.5, and silyl ethers of hydroxysteroids have R_f = 0.6 to 0.7. The more polar nucleoside derivatives require a more polar development solvent such as ether (see

Tables III and VIII for data). It is important to note that R_f values for silyl derivatives are independent of the type of silyl group used. Thus, all the advantages of the use of TMSi ethers in TLC work (25-30) are applicable to SCTASi ethers, i.e., greater mobility, less tailing and easier recovery than underivatized compounds, and compatibility with GC and MS methods. Most importantly, SCTASi derivatives offer the additional advantage of good stability under the mild hydrolytic conditions encountered during TLC.

It has been observed that TMSi ethers of hydroxysteroids (usually 2° hydroxyl functions) are quite stable under conditions of TLC development (26), but partial hydrolysis occurs upon elution and recovery (25). This results in the necessity to re-silylate material eluted from the silica gel prior to further analysis by GC. TMSi ethers of phenolic and 1° hydroxyl functions are far less stable during TLC development. In our own experiments, despite extensive care in drying TLC plates prior to use and preventing exposure of spotted material to atmospheric moisture, partial hydrolysis of such silyl ethers was observed during development, and extensive decomposition occurred during elution from the silica gel. For example, with 3',5'bis-O-TMSi-thymidine (Iee, $R_f^{ether} = 0.74$), the primary 5'-O-TMSi group was partially lost during spotting and development, resulting in streaking and an additional band ($R_f^{ether} = 0.52$) corresponding to the 3'-O-TMSi derivative. Elution of the 3',5'bis-O-TMSi derivative for isolation (even under dry conditions) resulted in extensive loss of both silyl groups.

SCTASi ethers, on the other hand, have been found to be very stable under TLC conditions. No decomposition has been observed for all the TBDMSi and TMTBSi derivatives studied so far. As mentioned above,

TMIPSi derivatives of 1° hydroxyls are slightly labile and some hydrolysis occurs during TLC isolations. Quantitative recoveries of all SCTASi ethers of steroids (2° hydroxyls only) have been achieved by preparative TLC. This makes the SCTASi ethers very suitable for coupled TLC/GC/MS and TLC/MS analyses. Thus, group separations of hydroxy- from ketohydroxy-steroids in a metabolite mixture have been achieved by TLC, followed directly by GC/MS without re-silylation (see Section B5).

It may be possible to extend TBDMSi and TMTBSi derivatization for TLC to other functions such as carboxyls and some amines. For example, the 3',5',N6-tris-TBDMSi-deoxyadenosine derivative, Vbbb, was found to be completely stable during preparative TLC development and elution (as determined by re-TLC, GC, and direct MS analyses). The 3',5',N6-tris-TMSi derivative, Veee, decomposed completely during development to the 3',5'bis-TMSi and 3'-TMSi derivatives. Of course, the N-TBDMSi group is far more labile than TBDMSi ethers: Vbbb gradually decomposes to Vbba if it is not stored under dry conditions. However, this N-TBDMSi derivative is considerably more stable than TMSi ethers of primary alcohols.

3. Gas Chromatography

The gas chromatography of the SCTASi derivatives of nucleosides has already been examined in detail in Chapter III. The fully protected derivatives of thymidine (I), deoxyuridine (II), deoxyadenosine (V), uridine (VI), 5-methyluridine (VII), and adenosine (VIII) appeared to behave well during GC with an OV-1 stationary phase. For example, it was demonstrated that 3',5'bis-O-TBDMSi-thymidine (Ibb) was suitable for quantitative analysis (Figure 18). Unfortunately, some of the more labile nucleosides could not be anlyzed by GC as O-SCTASi derivatives. Mixed derivatization schemes were suggested as possible solutions to these

problems, but have not as yet been investigated in detail.

At this stage in the application of SCTASi derivatives to analytical GC, a thorough understanding of the influence of the silyl groups upon chromatographic behavior will be very valuable in future work. This is best obtained by examining the derivatives of simpler compounds, such as steroids, which are easier to work with. Also, with steroids, there is available a great deal of information on TMSi derivatives for the purpose of comparison. Table XXII presents the GC retention indices for a variety of steroids and their TMSi, TBDMSi, TMIPSi, and TMTBSi derivatives, on both OV-1 and OV-17 stationary phases.

The first interesting aspect is the influence of the type of stationary phase and the qualitative information available from this. The magnitude of the difference between the retention indices on moderately polar (OV-17) and nonpolar (OV-1) columns, $\Delta I_{OV17-OV1}$, is a measure of the polarity of the molecule (10,101,139,153). When functions that contribute to the polarity are blocked with silyl groups, the ΔI value is reduced. For example, the underivatized ketohydroxysteroid, XVa, has a ΔI value of 410; trimethylsilylation of the hydroxyl (XVe) reduces it to 259; and further silylation of the ketone to the enol TMSi ether (compound XVIIIee) gives a value of 103. The underivatized hydroxysteroid, XIa, has ΔI = 277, which is reduced to 123 in the TMSi ether. Thus, retention data on two columns and derivatizations reveal a great deal about the functions present in the molecule.

The ΔI values are not constant over the series of silyl derivatives, but depend upon the silicon substituents. With cholesterol (XI), the derivatives have the following ΔI values: TBDMSi = 121, TMSi = 123, TMTBSi = 167, and TMIPSi = 184. This trend is accentuated with bis-silyl

TABLE XXII. Melting points and chromatographic data for steroid derivatives.

Parent Compound Name[a]	O-Substituents Y(3)	Z(17)	W(20)	Comp'd[a] No.	Melting Point (°C)	R_f TLC[b]	GC DATA[c] I_{OV-1} 230°	260°	280°	I_{OV-17} 230°	260°	280°	$\Delta I_{OV17-OV1}$[d]
Androsterone	H			XIIIa		<0.05	2498						
	TMSi			XIIIe		0.49	2485						
	TBDMSi			XIIIb		0.49	2702	2740					
	TMIPSi			XIIId		0.49	2976	3031					
	TMTBSi			XIIIc		0.49	2996	3055					
Epiandrosterone	H			XIVa		<0.05	2504			2900			396 (230°)
	TMSi			XIVe		0.43	2566			2823			257 (230°)
	TBDMSi			XIVb	155-156	0.43	2816	2870		3061	3124		254 (260°)
	TMIPSi			XIVd		0.43	3082	3143			3464		321 (260°)
	TMTBSi			XIVc		0.43	3113	3176			3480		304 (260°)
Androstanolone		H		XVa		<0.05	2536			2946			410 (230°)
		TMSi		XVe		0.44	2587			2846			259 (230°)
		TBDMSi		XVb	116-117	0.44	2856	2916		3103	3172		256 (260°)
		TMIPSi		XVd		0.44	3110	3177			3494		317 (260°)
		TMTBSi		XVc		0.44	3154	3222			3527		305 (260°)
Dehydroepiandrosterone	H			XVIa		<0.05	2482			2898			416 (230°)
	TMSi			XVIe		0.41	2555			2824			269 (230°)
	TBDMSi			XVIb	140-141	0.41	2803			3059			256 (230°)
Testosterone		H		XVIIa		<0.05	2602			3047			445 (230°)
		TMSi		XVIIe		0.30	2653			2934			281 (230°)
		TBDMSi		XVIIb		0.30	2920			3193			273 (230°)

continued...

Table XXII (continued)

	Y(3)	Z(17)	W(20)	#	m.p.	R_f	I_{OV-1} 230°	I_{OV-1} 260°	I_{OV-1} 280°	I_{OV-17} 230°	I_{OV-17} 260°	I_{OV-17} 280°	$\Delta I_{OV17-OV1}$ [d]
Cholesterol	H			XIa		0.13		3071	3118			3395	277 (280°)
	TMSi			XIe	120-121	0.67		3136	3166		3284	3289	123 (280°)
	TBDMSi			XIb	153-154	0.67		3395	3429		3529	3550	121 (280°)
	TMIPSi			XId		0.67			3717			3901	184 (280°)
	TMTBSi			XIc		0.67			3750			3917	167 (280°)
Epicholesterol	H			XIIa		0.13		3055	3107				
	TMSi			XIIe		0.65		3044	3074				
	TBDMSi			XIIb	101-102	0.65		3273	3303				
5α-androst-2-en-3,17β-diol (enol of XV)	TMSi	TMSi		XVIIIee		0.64	2653			2756			103 (230°)
	TBDMSi	TBDMSi		XVIIIbb		0.64	3165	3218		3255	3315		97 (260°)
Androst-2,4-dien-3,17β-diol[e] (enol 1 of XVII)	TMSi	TMSi		XXIee		0.61	f			2761			
	TBDMSi	TBDMSi		XXIbb		0.61	3197	3252		3312	3372		120 (260°)
Androst-3,5-dien-3,17β-diol[e] (enol 2 of XVII)	TMSi	TMSi		XXIIee		0.61	f			2810			
	TBDMSi	TBDMSi		XXIIbb		0.61	3241	3300		3383	3444		144 (260°)
5β-pregnane-3α,20α-diol	H		H	XIXaa		<0.05	2647	2701					
	TMSi		TMSi	XIXee		0.60	2783	2817		2854			71 (230°)
	TBDMSi		TBDMSi	XIXbb	105-106	0.60	3266	3304			3377		73 (260°)
	TMIPSi		TMIPSi	XIXdd		0.60			3886				
	TMTBSi		TMTBSi	XIXcc		0.60			3953				
	TBDMSi		H	XIXba		0.20	2908	2945					
	H		TBDMSi	XIXab		0.20	3011	3065					
	TBDMSi		TMSi	XIXbe		0.60	2998	3033					
	TMSi		TBDMSi	XIXeb		0.60	3053	3089					

continued...

TABLE XXII (continued)

	Y(3)	Z(17)	W(20)	#	R_f	I_{OV-1} 230°	I_{OV-1} 260°	I_{OV-1} 280°	I_{OV-17} 230°	I_{OV-17} 260°	I_{OV-17} 280°	$\Delta I_{OV17-OV1}$ [d]
5β-pregnane-3α,17α,20α-triol	TMSi	H	TMSi	XXeae	0.59	2920	2954		3027	3063		109 (260°)
	TMSi	TMSi	TMSi	XXeee	0.65	2811	2845		2838	2865		20 (260°)
	TBDMSi	H	TBDMSi	XXbab	0.59		3460			3548		88 (260°)
	TBDMSi	TMSi	TBDMSi	XXbeb	0.65		3349			3370		21 (260°)
	TBDMSi	TBDMSi	TMSi	XXbbe	0.65		3317					
	TMSi	TMSi	TBDMSi	XXeeb	0.65		3121			3151		30 (260°)
	TMTBSi	H	TMTBSi	XXcac	0.59		4109					
	TMTBSi	TMSi	TMTBSi	XXcec	0.65		3988					
	TMTBSi	TMTBSi	TMSi	XXcce	0.65		3896					
	TMTBSi	TMSi	TMSi	XXcee	0.65		3394					

[a] Common name of parent compound; see Scheme 53 for IUPAC names, structures, and numbering system.

[b] TLC development solvent: cyclohexane/ethyl acetate (9:1,v/v).

[c] GC data expressed as Kovats' retention indices.

[d] Temperature given in parentheses.

[e] Tentative identification.

[f] Not resolved from XVIIee; I_{OV-1} approximately the same but could not be determined accurately.

ethers of diols such as XXIV (see Table XXX, Section B5): TBDMSi = 64, TMSi = 80, TMTBSi = 162, and TMIPSi = 193. ΔI values consistently increase in the order: TBDMSi < TMSi < TMTBSi < TMIPSi. This order does not correlate with either the molecular weight, steric bulk of the group, or retention time. Evidently, the cyclo-tetramethylene ring increases the polarity of the silyloxy group, possibly by modification of the bond angles at silicon. The size of the R substituent (Me < iPr < tBu) would then account for the overall trend, since a bulkier R group would help to shield the oxygen from interaction with the liquid phase.

A more important aspect of the data in Table XXII is the effect of the type and number of silyl groups upon the retention indices. As demonstrated in Chapter III, the substitution of TMSi by SCTASi groups increases the retention index; the increments (δI) parallel the increasing mass of the silyl groups: TMSi = 73, TBDMSi = 115, TMIPSi = 127. and TMTBSi = 141 amu. The chromatogram in Figure 105 illustrates this effect for the different cholesterol derivatives. Retention increments are nearly the same on the two columns, except for the slightly increased retention of TMIPSi and TMTBSi ethers on OV-17. Table XXIII gives the average retention increments due to the variation of an hydroxyl substituent (including hydrogen for those steroids that can be chromatographed without derivatization). The δI values on OV-1 due to the replacement of one TMSi group by a SCTASi group are: TBDMSi = 260, TMIPSi = 530, and TMTBSi = 565. These are slightly higher than those determined previously for nucleoside derivatives (Table IV). The reason for this is not known at this time.

With the use of two types of derivatives and the measurement of retention increments, it is possible to determine the number of reactive

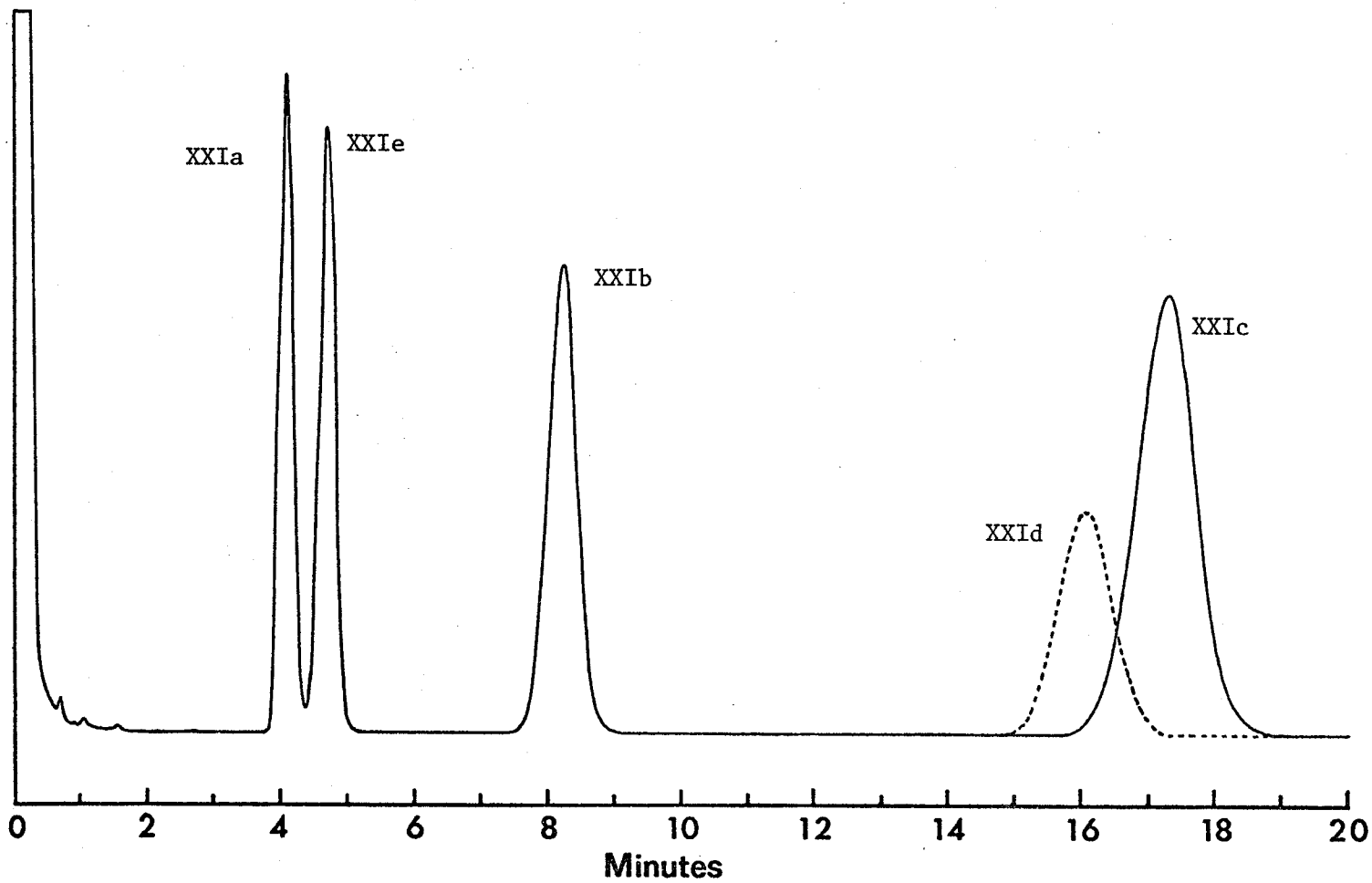


Figure 105. Gas chromatogram of a mixture of cholesterol (XXIa) and its TMSi (XXIe), TBDMSi (XXIb), and TMTBSi (XXIc) derivatives. The peak for the TMIPSi derivative (XXId) was traced from another chromatogram, as indicated by a dotted line. Conditions: column A (10% OV-1, 1m x 2mm-ID), 270°, 30 ml/min N_2 carrier.

TABLE XXIII. Average retention increments due to the variation of an hydroxyl substituent from K to J, on two different types of stationary phases, OV-1 and OV-17.

(i) δI_{J-K}^{OV-1}

K	J = H	TMSi	TBDMSi	TMIPSi	TMTBSi
H	0	60	320	590	625
TMSi	-60	0	260	530	565
TBDMSi	-320	-260	0	270	305
TMIPSi	-590	-530	-270	0	35
TMTBSi	-625	-565	-305	-35	0

(ii) δI_{J-K}^{OV-17}

K	J = H	TMSi	TBDMSi	TMIPSi	TMTBSi
H	0	-90	160	500	520
TMSi	+90	0	250	590	610
TBDMSi	-160	-250	0	240	260
TMIPSi	-500	-590	-240	0	20
TMTBSi	-520	-610	-260	-20	0

hydroxyls in the compound under study. Section B5 demonstrates this with an actual application. This concept has been suggested previously for chloromethyldimethylsilyl (CMDMSi) derivatives (31,36,42,43), which have an increment from TMSi close to that of TBDMSi, and for dimethylalkylsilyl (alkyl = Et, nPr) derivatives (51).

As mentioned in Chapter III, the retention indices of TMSi derivatives of hydroxylated compounds are not greatly unfluenced by the number of TMSiO substituents. This is very useful for achieving low retention times of polyhydroxy compounds, but can lead to problems with complex mixtures. Thus, mono-, di-, and tri-hydroxysteroids with similar skeletons may not be well separated. The use of SCTASi derivatives can allow better separations of some complex mixtures due to the differential retention increments which depend upon the number of SCTASi groups present. The application discussed in Section B5 demonstrates this quite well. This effect has also been shown for CMDMSi derivatives of steroids (42) and tri-n-alkylsilyl derivatives of cannabinol mixtures (49). The large retention indices of SCTASi derivatives may also be of value with very volatile compounds that do not separate well from solvents or reagents as their TMSi derivatives. However, the retention increment effect imposes serious limitations for the application of SCTASi derivatives to some polyhydroxy compounds. Due to large retention indices, excessively high column temperatures may be required to achieve reasonable retention times for some compounds.

Some of the most interesting findings came from a study of the effect of different silyl groups upon the GC separation of similar or isomeric compounds. The first compounds examined were the positionally isomeric steroids, epiandrosterone (XIV) and androstanolone (XV). Although

the underivatized steroids could be chromatographed and were easily separated, the TMSi derivatives had very close retention indices and were inseparable on either of the two columns used in this study. The SCTASi derivatives, on the other hand, afforded almost complete resolution, as illustrated in Figure 106. Also shown in this figure is the improved separation of XV and its enol silyl ether (XVIII) as TBDMSi vs. TMSi derivatives. The improved separation of positional isomers was used to advantage in the analysis of a steroid metabolite mixture (Section B5).

The separations of various epimers are also enhanced by changing from TMSi to SCTASi derivatives. Figure 107 illustrates the separation of androsterone (XIII) and epiandrosterone (XIV) as TMSi, TBDMSi, TMIPSi, and TMTBSi derivatives. The underivatized compounds are inseparable on most commonly used columns (see Figure 2, Chapter I).

In Figure 108, the separation factors for epimeric and positionally isomeric pairs are plotted for all silyl groups, which are arranged in order of increasing steric crowding around the silicon. The curves imply that, up to a point, separation of isomers increases with increasing steric crowding around silicon. Possibly, the bulkier (sterically) that the silyl group is, the more the steroids are forced into conformations which accentuate the differences between isomers. A steric effect on steroid epimer separations has also been observed for dimethylsilyl, trimethylsilyl, and chloromethyldimethylsilyl ether derivatives where similar proposals were advanced (31).

One other aspect of the GC analysis of steroids was studied--the suitability of SCTASi derivatives for quantitative work. The first requirement is that the reactions must yield a single product in 100% yield. It has already been shown that this is the case with primary and

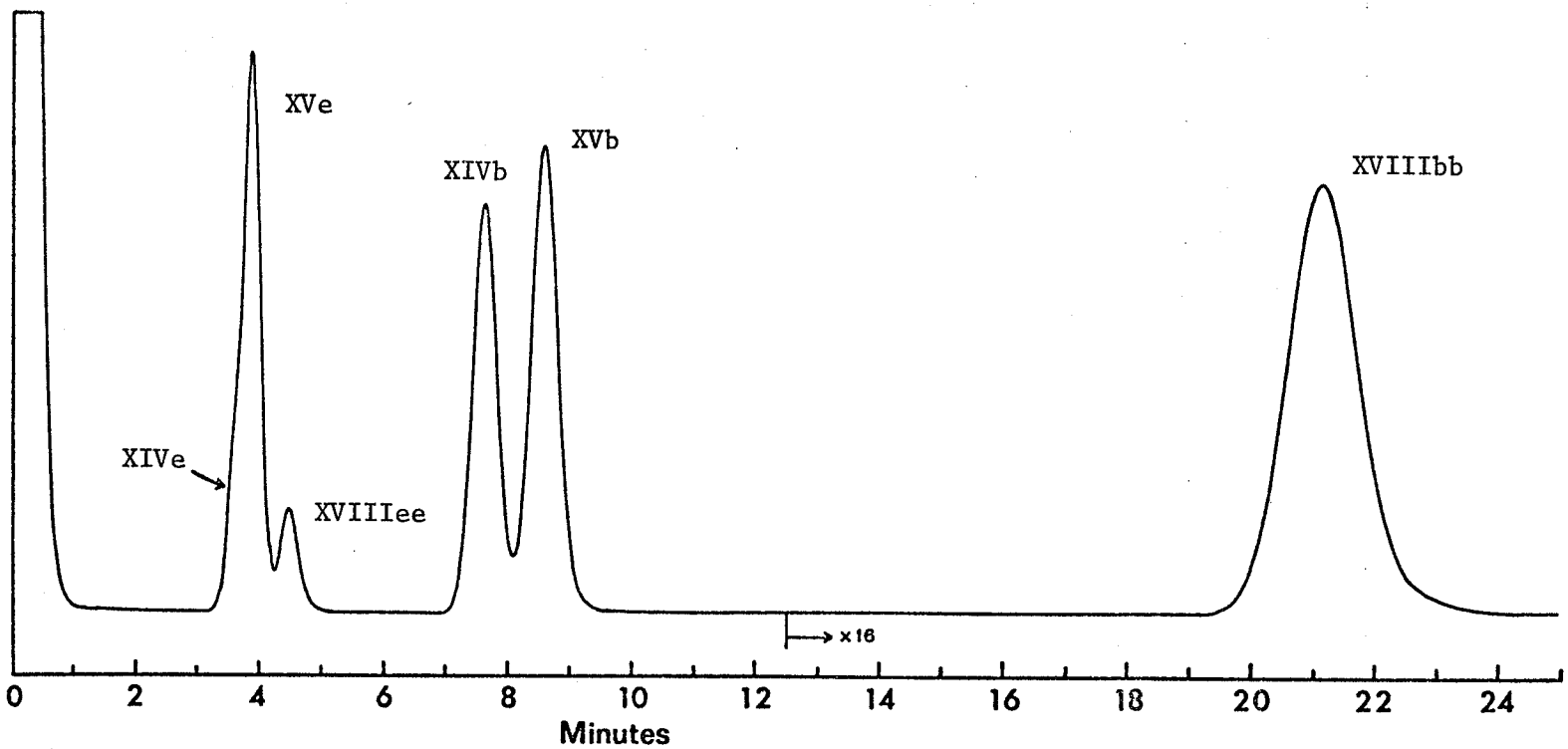


Figure 106. Gas chromatogram illustrating better separation of TBDMSi (b) versus TMSi (e) derivatives of epiandrosterone (XIV) and androstanolone (XV), and the enol silyl ether of the latter (XVIII). Conditions: column A (10% OV-1, 1m x 2mm-ID), 235°, 30 ml/min N_2 carrier.

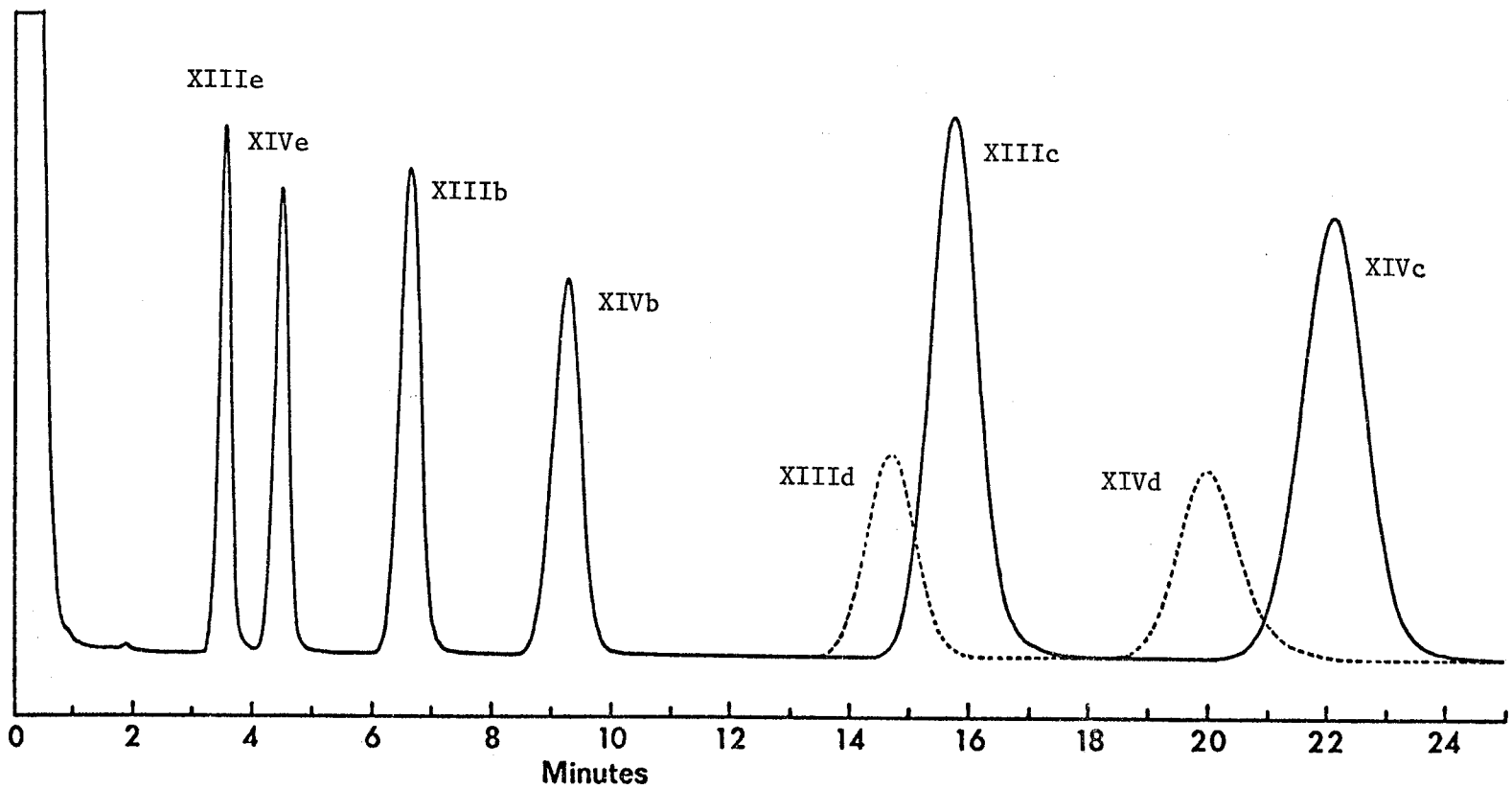


Figure 107. Gas chromatogram of a mixture of TMSi (e), TBDMSi (b), TMIPSi (d), and TMTBSi (c) derivatives of the epimeric steroids androsterone (XIII) and epiandrosterone (XIV). The peaks for the TMIPSi derivatives (dotted lines) were traced from another chromatogram. Conditions: column A (10% OV-1, 1m x 2mm-ID), 230°, 30 ml/min N_2 carrier.

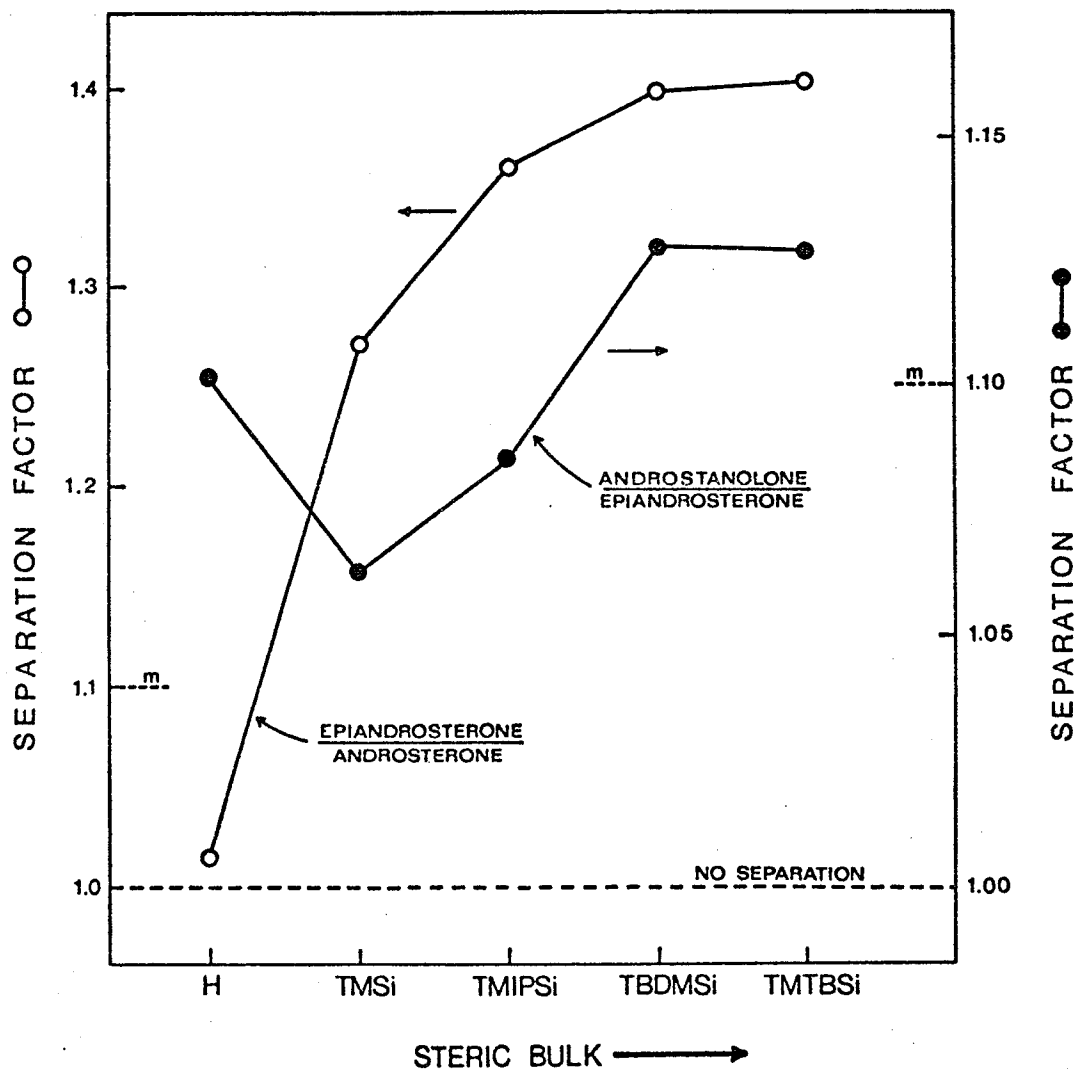


Figure 108. The effect of steric bulk of the substituents at isomeric hydroxyls upon the separation factors (ratios of retention times of isomeric pairs, as indicated). At the point marked m, isomers are 98% resolved on a packed column with 1940 plates (a typical 1m x 2mm-ID column).

sterically accessible secondary hydroxyls. The second requirement is that the derivatives have enough volatility and thermal stability to allow passage through a GC column without decomposition. Figure 109 illustrates the response curves for cholesterol and its TMSi, TBDMSi, and TMTBSi derivatives. This form of presentation emphasizes losses during chromatography, particularly for small samples. The constant relative molar responses for TMSi, TBDMSi, and TMTBSi ethers indicate good chromatographic behavior and thermal stability. With underivatized cholesterol, on the other hand, decomposition is evident with low injection quantities. In addition, the cholesterol peak tails badly at trace levels compared to the silyl derivatives as illustrated in Figure 110. The TBDMSi and TMTBSi ethers have greater FID molar response factors than do the TMSi ethers, due to a greater number of carbon and hydrogen atoms. Another advantage for SCTASi versus TMSi derivatives for quantitative work is that they are stable, easily purified, crystalline solids that can be kept as standards. This is useful in the preparation of standard solutions for FID molar response determinations.

There are some problems with compounds possessing sterically hindered hydroxyls. For example, in the silylation of XX, although only the 3,20bis-O-SCTASi ether derivatives were formed and in quantitative yield, slight decomposition during chromatography was evident. It appears that protection of all hydroxyls is necessary for quantitative work. As discussed earlier, trimethylsilylation of the 3,20bis-O-SCTASi derivatives of XX is not practical due to exchange and rearrangement reactions. Acetylation or trifluoroacetylation should be a solution, since SCTASi ethers are quite stable to acylation conditions. This has not as yet been investigated.

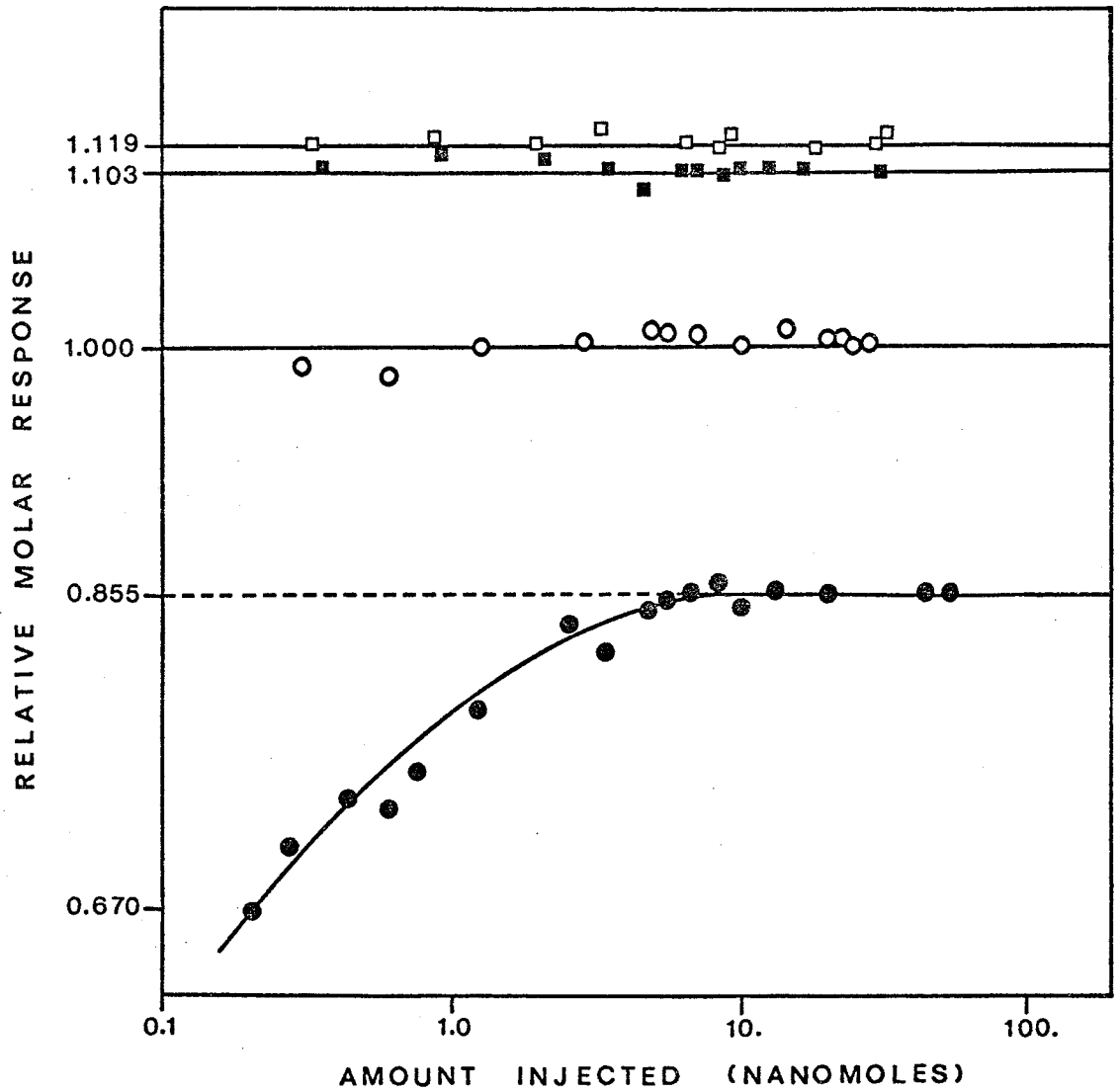


Figure 109. FID relative molar responses of cholesterol (XIa, -●-) and its TMSi (XIe, -○-), TBDMSi (XIb, -■-), and TMTBSi (XIc, -□-) derivatives plotted as a function of injection quantity (on a log scale).

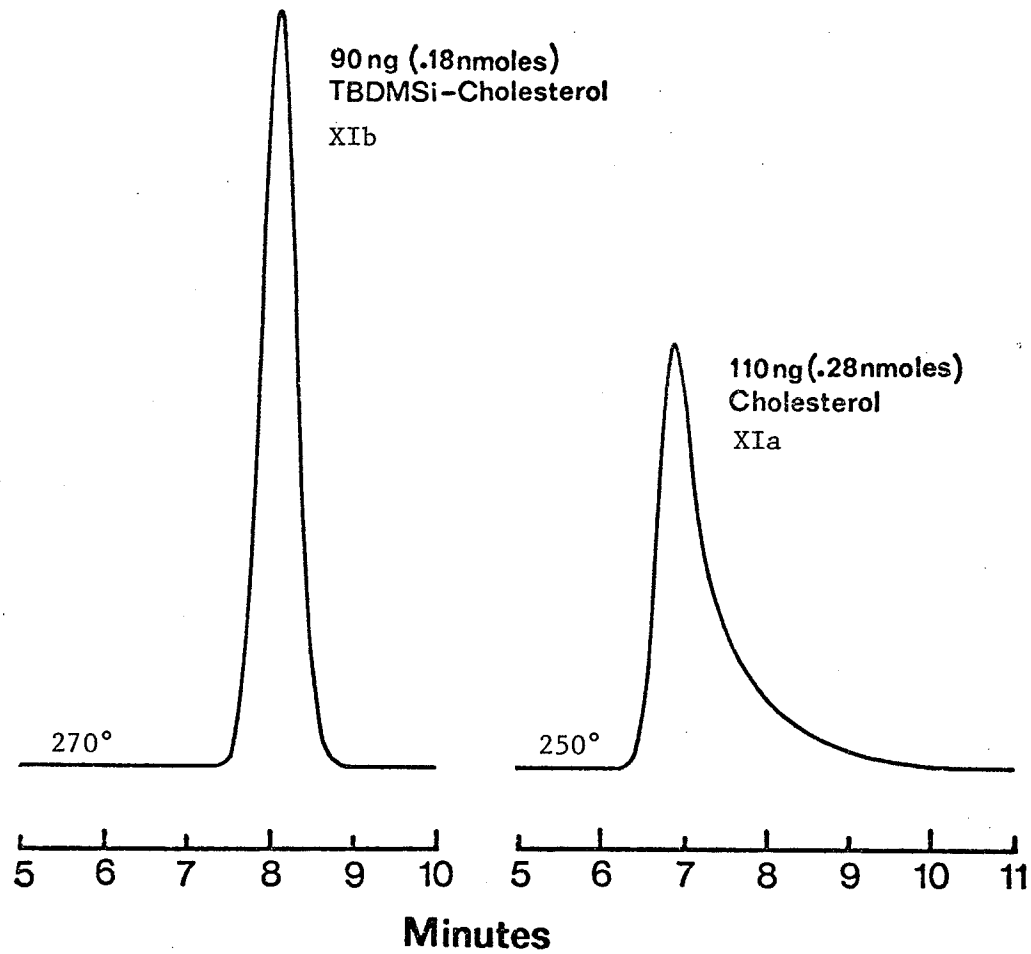


Figure 110. Partial gas chromatograms indicating severe tailing of cholesterol, XIa, at trace levels, but good peak shape and sensitivity for TBDMSi-cholesterol, XIb. Conditions: column A (10% OV-1, 1m x 2mm-ID) with 30 ml/min N_2 carrier and temperatures as indicated; electrometer sensitivity at 1 x 10^{-11}afs.

4. Mass Spectrometry

a) Nucleosides

A detailed discussion of the mass spectra of a variety of nucleoside derivatives has already been presented in Chapter IV. For the application of sterically crowded trialkylsilylation to analytical mass spectrometry, primarily the fully O- or N,O-silylated nucleosides are of interest. The mass spectra of the O-perTBDMSi derivatives of thymidine (Ibb), deoxyuridine (IIbb), deoxycytidine (IIIbb), deoxyguanosine (IVbb), deoxyadenosine (Vbba), uridine (VIbbb), 5-methyluridine (VIIbbb), and adenosine (VIIIbbba) have been given in Figures 59, 62, 64, 65, 63, 90, 93, and 94, respectively, and serve to illustrate the basic behavior of the O-perSCTASi derivatives. For cytosine, guanine, and adenine nucleosides, the N,O-perSCTASi derivatives would be the most appropriate for analytical work. In this respect, only the adenine nucleosides have as yet been investigated, but the results were quite promising (see Figures 68 and 99 for Vbbb and VIIIbbbb, respectively). For comparison, the spectra of several TMSi derivatives have been given in Figures 66 (Iee), 67 (IIee), 69 (Veee), 95 (VIeee), 96 (VIIeee), and 101 (VIIIeeee).

One of the most notable features of analytical value in the spectra of SCTASi derivatives of nucleosides is the abundant $[M-R]^+$ ion. This is useful for determining molecular weight and should be of value for selected ion recording. In comparison, both the M^+ and $[M-CH_3]^+$ ions of TMSi-nucleosides are generally of low intensity. This is well illustrated by the spectra of the uridine derivatives, VIbbb and VIeee, in Figures 90 and 95.

The spectra of the O-perSCTASi derivatives also contain a number of fragment ions that provide considerable structural information, such

as the type of base (E^+, F^+, G^+) and the positions and types of substituents in the sugar ring (A^+, D^+, K^+, $[J+H]^+$, $[J+Z]^+$, $[M-R-J]^+$, $[M-R-J-CH_2O]^+$, $RX_2SiOCH_2^+$, $HX_2SiOCH_2^+$). Deoxyribosides and ribosides are easily distinguished by the presence or absence of certain ions characteristic of the former (E^+, K^+, m/e81 (D^+)) and the latter (U^+, L^+, $[J-R_W]^+$, $[B+80]^+$, $[J+Z]^+$).

In general, SCTASi derivatives appear to be, at the very least, complementary to TMSi derivatives for the mass spectrometry of nucleosides. The two types of derivatives have quite different spectra that yield different kinds of structural information. However, as demonstrated in Chapter IV, one of the superior features of SCTASi derivatization for MS is the ease with which mixed derivatives can be prepared for detailed elucidation of fragmentation mechanisms. In addition, the SCTASi derivatives may be isolated for MS by liquid chromatographic methods (TLC, CC, HPLC) due to their excellent hydrolytic stability (even for N-SCTASi derivatives).

Finally, it should be mentioned again, that since the siliconium ion rearrangements observed in SCTASi derivatives are dependent upon the steric accessibility of silyl groups and electron dense functions, the mass spectra of isomeric substituted nucleosides are very characteristic. Thus, SCTASi derivatization has tremendous potential for structural elucidation of isomeric compounds by MS.

b) Steroids

Much has been published on the mass spectral behavior of steroids and their derivatives, not only because of their biological importance, but also from the viewpoint of understanding their fragmentation processes which are of significance to mass spectrometry as a whole. The major

fragmentation routes are now well-defined, and a number of reviews cover the numerous types of fragmentation patterns found in different steroids (10, 236, 252). However, there is still much attention being given to the formation of steroidal derivatives suitable for the location of functional goups, the determination of stereochemistry, gas chromatography, and combined GC/MS. Trimethylsilyl ethers have proven to be the most valuable and widely used derivatives of hydroxylated steroids.

This section reports the characteristic mass spectra of SCTASi derivatives of selected mono- and di-hydroxy steroids, and a tri-hydroxy steroid with a sterically hindered hydroxyl. The results demonstrate the dramatic effect of substituting a SCTASi for a TMSi group. The SCTASi group initiates some important fragmentations not, to our knowledge, previously discussed. Recently, there have been three papers which briefly outline the mass spectra of some steroid TBDMSi ethers (95-97).

i) Mono-hydroxy steroids

The mass spectral data for silyl ether derivatives of selected mono-hydroxy steroids are presented in Tables XXIV and XXV. Many ions contributing to the general background below m/e200 and not considered of structural value are not listed. The series of compounds include isomeric variations which illustrate differences between epimers, and differences when the silyloxy group is substituted onto ring A or D. Other compounds show that the spectra may be modified by the presence and position of a double bond. To allow easier comparison of the fragmentation patterns of TMSi and SCTASi derivatives, Figures 111 to 122 illustrate the mass spectra of TMSi and TBDMSi ethers of XI, XIII, XIV, XV, XVI, and XVII.

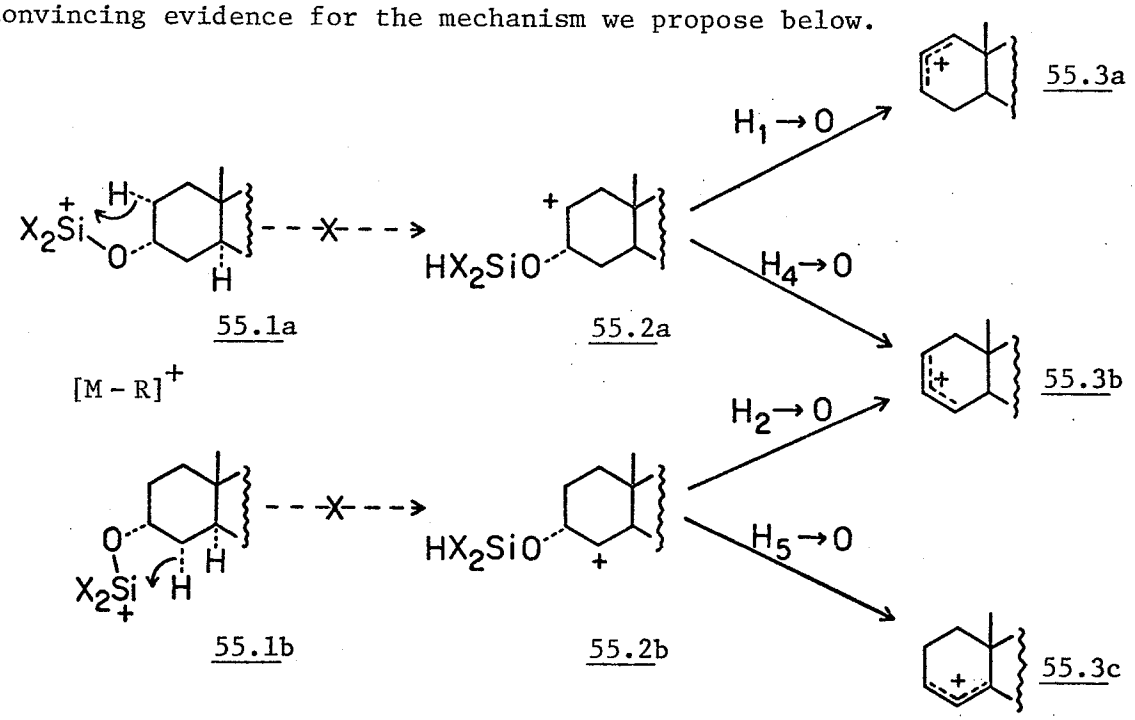
The general features of the spectra of the TMSi derivatives are in

agreement with earlier observations (12). Thus, the M^+ and $[M-CH_3]^+$ ions, while observed in each spectrum, have relative intensities which vary erratically from compound to compound. Since the expelled CH_3 group can orginate from either the TMSi group or one of the angular positions (203), both M^+ and $[M-CH_3]^+$ are potential precursors for loss of TMSiOH and, as discussed below, the stereochemistry of the molecule is an important consideration in these processes. Other characteristic fragment ions from TMSi ethers of 3β-hydroxy-Δ^5-steroids, represented as $[M-56]^+$, $[M-129]^+$, and m/e129, arising from cleavage of ring A have been well documented (203, 253-255) (also see Scheme 11).

By comparison, all of the fragmentations in the preceding paragraph are very much suppressed in the mass spectra of the SCTASi ethers. The molecular ions are very weak, but the intense ion (often the base peak) at $[M-R]^+$ serves as an indicator of the molecular weight. As discussed for the nucleoside derivatives, this ion is the ultimate precursor of most of the abundant ions. This, together with the general unavailability in steroids of electron dense centers with which the siliconium ion can interact, results in a simplification of the mass spectra since pathways from alternative precursors are suppressed. Of course, while this may be an advantage for GC/MS-SIR, it could also be a disadvantage if structurally informative fragmentations are not observed.

One of the most interesting ions arises by elimination of HX_2SiOH from $[M-R]^+$, the process confirmed in the spectra of TBDMSi ethers of cholesterol, androstanolone and epiandrosterone by the presence of a strong metastable peak (Table XXV). Although previously observed in the mass spectra of TBDMSi ether derivatives of testosterone, 17β-hydroxy-5(α and β)-androstan-3-one, 5-androsten-3β,17β-diol and 20α-hydroxy-4-pregnen-3-

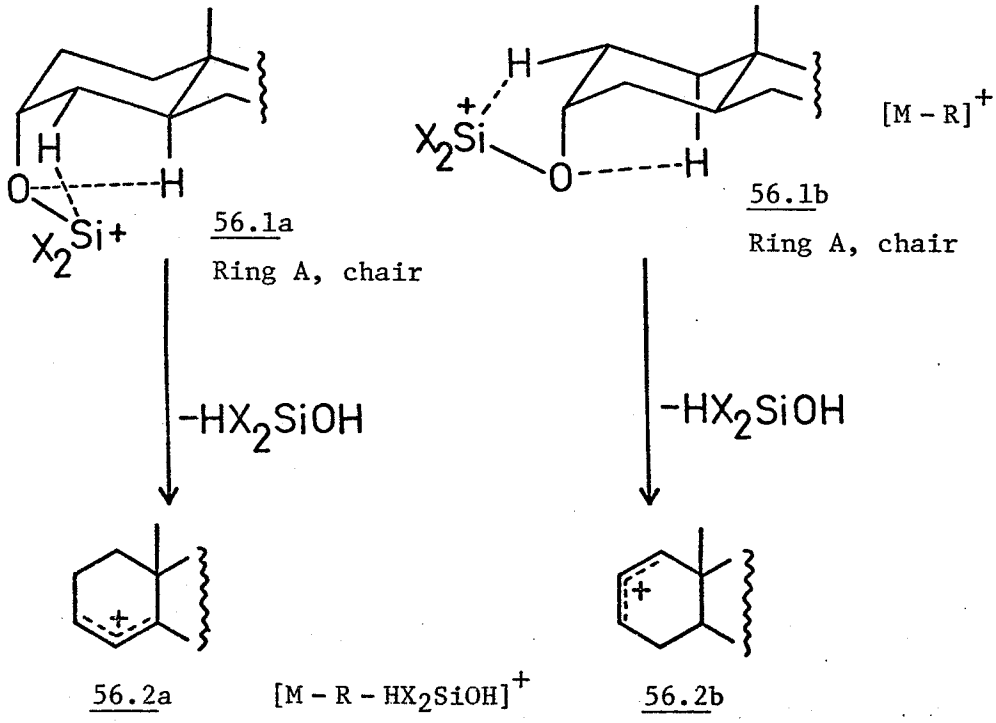
one (95), and also halomethyldimethylsilyl ethers of several steroids (43) (vide infra), its mechanistic origin was not discussed. The ion product must be formed by a double hydrogen rearrangement to the eliminated neutral species. As suggested in Scheme 55, a two step process could be a possibility. However, the sequence 55.1 → 55.2 → 55.3 should not be very favorable. Even though a stable allyl carbonium ion is produced, the step 55.1 → 55.2 would have a high activation energy, not only because Si-H bonds are weaker than C-H bonds, but mainly because the secondary carbonium ion, 55.2, would be much less stable than the initial siliconium ion, 55.1. In addition, this type of mechanism cannot explain the observed intensity variations of $[M-R-HX_2SiOH]^+$ for varying stereochemistries. This is well illustrated in Figures 114 and 116 by the dramatically different spectra of the TBDMSi ethers of the epimers XIII and XIV. The difference in the spectra is due to the preferred elimination of HX_2SiOH from $[M-R]^+$ for the former. We believe that such observations provide convincing evidence for the mechanism we propose below.



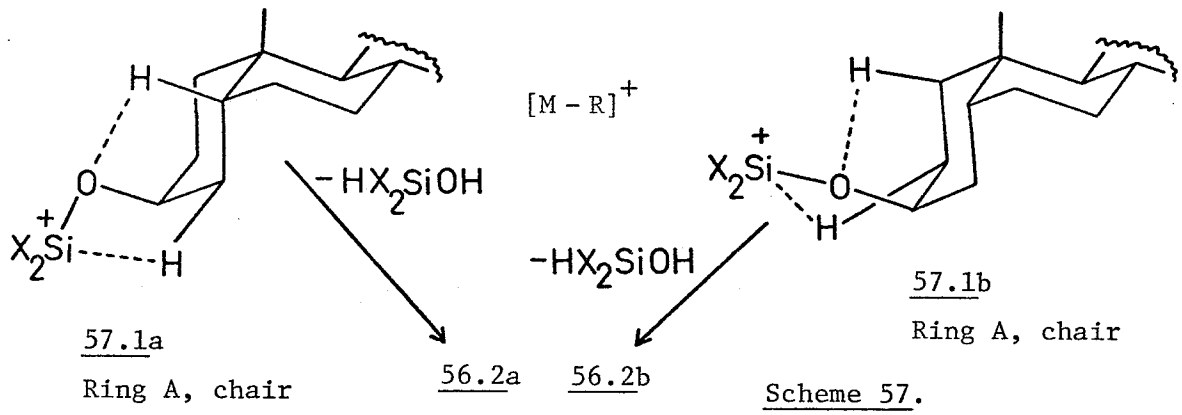
Scheme 55.

Initially, we consider the formation of $[M - R - HX_2SiOH]^+$ in the spectra of 3α-silyloxy-5α-steroids. Figure 114 and Table XXIV indicate that this ion is of very high intensity in the spectra of SCTASi ethers of androsterone (XIII). As illustrated in Scheme 56, two favorable transition states, 56.1a and 56.1b, can be proposed, based on the more probable chair conformation of ring A, from which HX_2SiOH can be eliminated via concerted bond rearrangements. Models reveal the development of little strain or steric interferences, and sufficiently close approach of axial H_1 or H_5 to O and equatorial H_2 or H_4 to Si, for transfers to occur. Consequently, the activation energy should be low compared to the alternative in Scheme 55, the driving force for the fragmentation being formation of the stable allyl carbonium ions 56.2a or 56.2b, the former being presumably more stable because of the involvement of tertiary C_5. Other

Scheme 56.

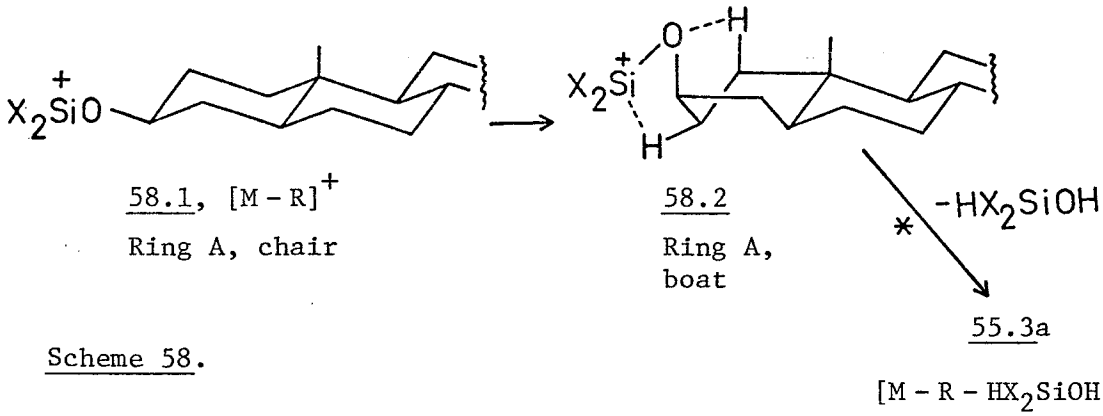


examples of the formation of $[M-R-HX_2SiOH]^+$ in the mass spectra of 3α-silyloxy-5α-steroids are found by inspection of the spectra of the halomethyldimethylsilyl ethers of androsterone and 11-ketoandrosterone (43) where intense ions of m/e 271 and 285 respectively, assignable to 56.2(a or b) are observed. In a similar way $[M-R-HX_2SiOH]^+$ is the base peak in the mass spectrum of the chloromethyldimethylsilyl ether derivative of the 3β-hydroxy-5β-steroid epietiocholanone (43) for which the favorable multicenter transition states 57.1a and 57.1b can be proposed, in which an axial hydrogen is transferred to oxygen and an equatorial hydrogen to silicon, in the preferred chair conformation of ring A.



Scheme 57.

Next we consider the formation of $[M-R-HX_2SiOH]^+$ in the mass spectra of 3β-silyloxy-5α-steroids. For epiandrosterone (XIV), this ion, though still important, has a much lower intensity in the spectra of the SCTASi ethers (Table XXIV and Figure 116) and halomethyldimethylsilyl ethers (43) than in the spectra of the isomers discussed above. In these compounds the silyloxy group occupies an equatorial position in the chair conformation of ring A so that conversion to the boat form is required before concerted bond rearrangements leading to elimination of HX_2SiOH can occur. Once the chair → boat transformation, 58.1 → 58.2, has occurred models confirm the favorable nature of the transition state, 58.2, but

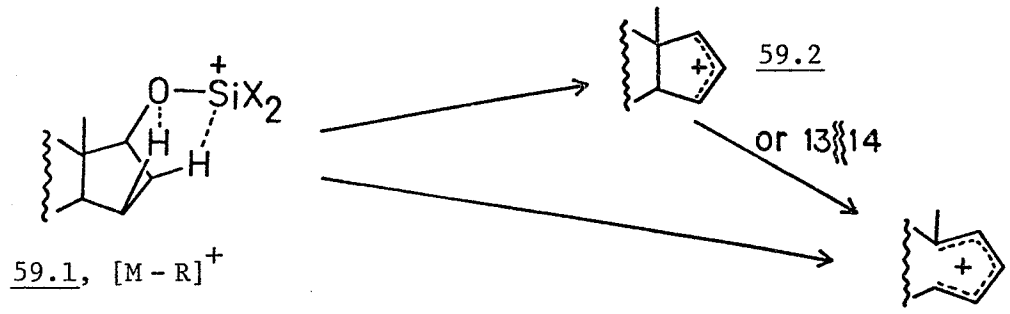


Scheme 58.

because the boat conformation is less probable the intensity of $[M-R-HX_2SiOH]^+$ is reduced. Additional support for this proposal is obtained from the spectra of the halomethyldimethylsilyl derivatives of the 3α-hydroxy-5β-steroids etiocholanone and 11-ketoetiocholanone (43), where the silyloxy group occupies an equatorial position in the chair form, and where the abundance of $[M-R-HX_2SiOH]^+$ is quite low.

The presence of a Δ^5 double bond has a significant effect on the foregoing fragmentations. The mass spectra of the silyl ethers of cholesterol, XI, and epicholesterol, XII, (Table XXIV) are very similar, the relative intensities of the $[M-R-HX_2SiOH]^+$ ions, in both cases, being reduced to levels observed in the spectra of the epiandrosterone derivatives. This may mean that the participation of C_5 in allyl carbonium ion formation (which is not available to cholesterol, epicholesterol and epiandrosterone) is important for androsterone derivatives, or that the tendency of C_5 to adopt trigonal planar hybridization modifies the conformational preferences of ring A.

$[M-R-HX_2SiOH]^+$ is of high abundance in the spectra of the SCTASi ethers of androstanolone, XV (Table XXIV and Figure 118), and an analogous mechanism in Scheme 59 is proposed for a 17β-silyloxy substituent.



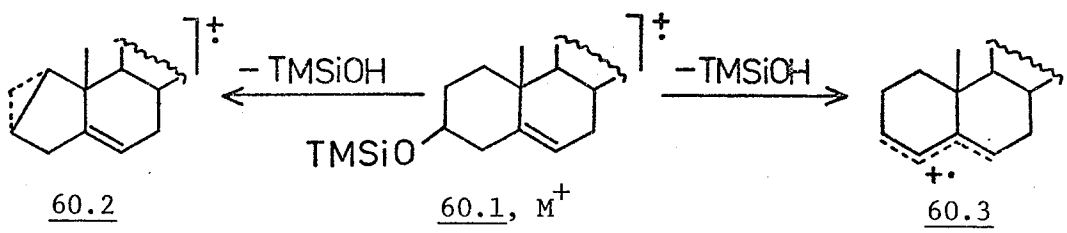
Scheme 59.

Because ring D is nearly planar this sequence should occur with about equal facility for a 17α-silyloxy substituent. This expectation is substantiated by the almost identical intensities for the analog of this species in the mass spectra of steroids epimeric at C_{17} reported in Section B5 of this chapter. To rationalize the high abundance of this ion, and also a subsequent decomposition (vide infra), structure 59.3 is a likely alternative to structure 59.2.

The foregoing observations suggest that favorable conditions for HX_2SiOH elimination from a given ring occur when the silyloxy group is axially located in a chair conformation and a stable allyl carbonium ion can be formed. More work is required to further define the conditions for this elimination but these results hold promise that HX_2SiOH loss, because of the multi-center nature of the transition state in the elimination reaction, may be the most powerful diagnostic rearrangement thus far reported for stereochemical elucidation, particularly for distinguishing between epimers.

The foregoing proposals are based on consideration of earlier discussions of the stereochemically influenced loss of H_2O or TMSiOH in the mass spectra of many sterols and their TMSi ethers. For the former

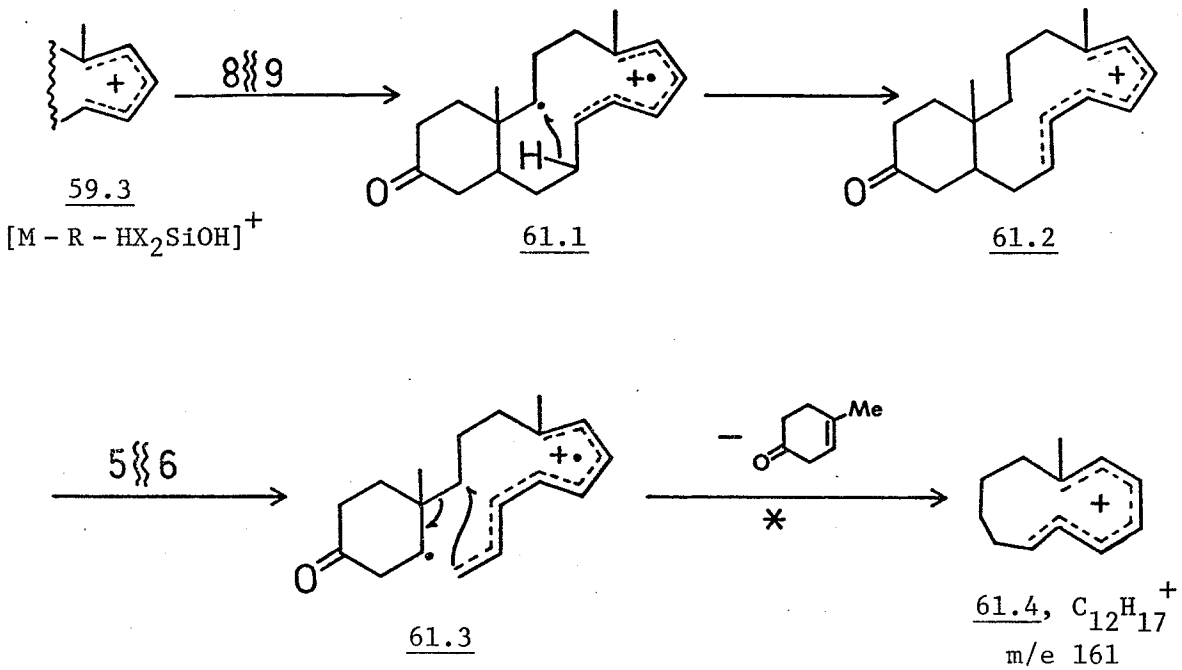
elimination, deuterium labeling studies revealed that H_2O loss occurred preferably via a 1,3 mechanism and was influenced by the approach of the oxygen atom to hydrogens in axial positions and by the probability of the required boat or chair conformation of the steroid ring (256). Deuterium labeling studies of TMSiOH elimination are sparse but available evidence (203) indicates that from the TMSi ether of cholesterol both a 1,3 elimination (involving transfer of H_1 to oxygen, 60.1 → 60.2) and a 1,2 elimination (involving abstraction of H_4, 60.1 → 60.3) occur. Presumably, the driving force for the unusual latter fragmentation is delocalization of the ion radical center over the Δ^5 double bond, C_3 and C_4 atoms, effectively lowering the activation energy component of the reaction. Studies of ^{18}O-labeled TMSi ether derivatives of several steroids (257) also support the preceeding arguments. Elimination of TMSiOH occurs much less readily from the 3β-silyloxy region than from the 3α-silyloxy or 17β-silyloxy regions of skeletally saturated 5α-steroids, i.e. sufficiently close approach of axial silyloxy group and hydrogen is preferred.



Scheme 60.

A prominent ion at m/e 161 in the mass spectra of the SCTASi ethers of XV (see Figure 118) parallels the high abundance of the $[M-R-HX_2SiOH]^+$ ion (which is confirmed to be its precursor by the observation of a strong metastable peak). We propose that this characteristic ion is formed by fission of ring B, initiated by structure 59.3 via the mechanism given

Scheme 61.

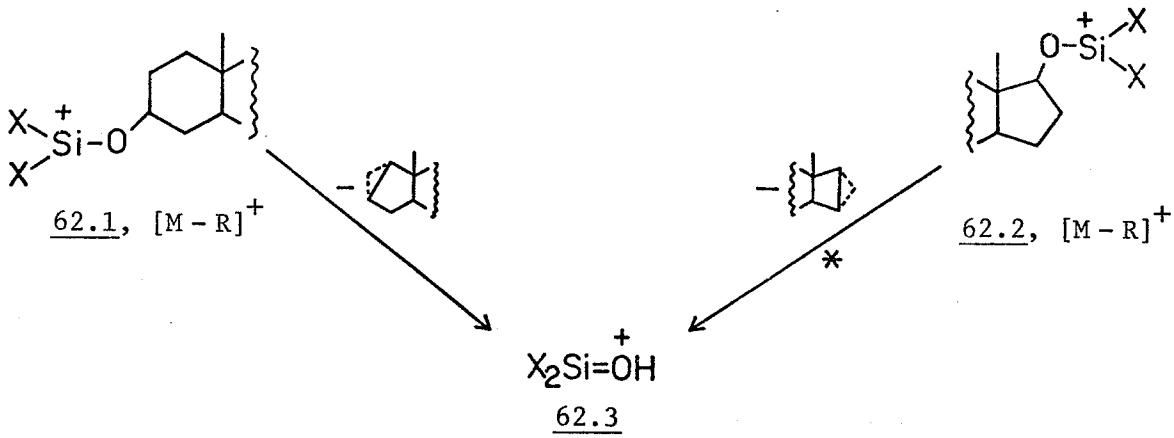


in Scheme 61. Each step in this sequence should be of low activation energy. Thus, the fission of the 8-9 bond extends the length of the conjugated system as does the transfer of H_7 to C_9 via a 6-membered cyclic transition state (61.1 → 61.2). 61.2 → 61.3 extends the conjugated system, while for 61.3 → 61.4 a stable neutral is eliminated. This mechanism would also account for the low abundance of its analog in the mass spectra of the testosterone derivatives, in accord with unfavorable vinylic 5-6 bond cleavage suppressing the sequence 61.2 → 61.4. To our knowledge this type of fragmentation of the steroid skeleton has not been previously reported for even-electron ions.

Ions of low abundance corresponding to loss of H_2O from various precursors are observed in these spectra. Extensive rearrangement is presumably required for their appearance. Apparently the presence of a suitably located double bond, or a keto function enhances their formation.

Perhaps, in the latter case, enolization is a necessary step in the reaction.

Other ion types of high intensity listed in Table XXIV not accounted for by the previously described processes appear to be mostly residues of the steroid nucleus or the silyl group. The X_2SiOH^+ ion is of particularly high abundance and is probably formed from $[M-R]^+$ by processes such as those in Scheme 62 (supported by a metastable peak for XVb).



Scheme 62.

TABLE XXIV. Partial mass spectra of trialkylsilyl derivatives of mono-hydroxy steroids.[a]

Steroid (Derivative)	Ion type: M^+	$(M-CH_3)^+$	$(M-56)^+$	$(M-R)^+$	$(M-R-H_2O)^+$
XI. Cholesterol					
e. TMSi	458/30	443/8.6	402/-	b	425/-
b. TBDMSi	500/0.6	485/2.3	444/37[i]	443/100	425/2.6
c. TMTBSi	526/0.3	511/0.7	470/40[i]	469/100	451/3.5
d. TMIPSi	512/1.2	497/-	456/-	469/100	451/1.5
XII. Epicholesterol					
e. TMSi	458/28	443/7.8	402/-	b	425/-
b. TBDMSi	500/0.30	485/2.0	444/37[i]	443/100	425/1.3
XIII. Androsterone					
e. TMSi	362/25	347/32	306/4.2	b	329/-
b. TBDMSi	404/2.4	389/1.3	348/17.6[i]	347/56	329/0.24
c. TMTBSi	430/2.3	415/0.71	374/22[i]	373/70	355/-
d. TMIPSi	416/3.9	401/-	360/-	373/66	355/0.44
XIV. Epiandrosterone					
e. TMSi	362/45	347/100	306/4.8	b	329/-
b. TBDMSi	404/2.2	389/1.7	348/28[i]	347/100	329/0.2
c. TMTBSi	430/6.7	415/0.98	374/31[i]	373/100	355/0.45
d. TMIPSi	416/5.9	401/-	360/-	373/100	355/0.44
XV. Androstanolone					
e. TMSi	362/10.3	347/16.4	306/4.8	b	329/-
b. TBDMSi	404/0.70	389/2.0	348/18.7[i]	347/59	329/0.71
c. TMTBSi	430/2.4	415/1.6	374/25[i]	373/75	355/1.7
d. TMIPSi	416/4.5	401/1.3	360/1.2	373/75	355/1.0
XVI. Dehydroepiandrosterone					
e. TMSi	360/9.0	345/2.4	304/8.8	b	327/-
b. TBDMSi	402/0.39	387/4.1	346/29[i]	345/100	327/3.6
XVII. Testosterone					
e. TMSi	360/37	345/12.5	304/7.5	b	327/-
b. TBDMSi	402/1.3	387/1.9	346/28[i]	345/100	327/0.18

[a] Finnigan model 1015 quadrupole mass spectrometer, corrected for mass discrimination.

[b] Same as $(M-CH_3)^+$

[c] Same as RX_2Si^+

[i] Uncorrected intensity. Contains significant isotopic contribution from ion type of lower mass.

continued . . .

TABLE XXIV. (continued)

		$(M-RX_2SiOH)^+$	$(M-R-HX_2SiOH)^+$	$(M-CH_3-RX_2SiOH)^+$	$(M-R-HX_2SiOH-CH_4)^+$	$(M-R-HX_2SiOH-H_2O)^+$	$(M-RX_2SiOC_3H_4)^+$	$RX_2SiOC_3H_4^+$	X_2SiOH^+
XI	e.	368/41	367/1.8	353/18.2	351/-	349/-	329/44	129/100	75/27
	b.	368/5.5[i]	367/10.5	353/1.7	351/-	349/-	329/0.6	171/5.7	75/71
	c.	368/11.7	367/8.8	353/4.0	351/0.3	349/-	329/0.7	197/3.4	101/62
	d.	368/7.7	367/9.7	353/2.6	351/-	349/-	329/1.9	183/11.0	101/51
XII	e.	368/38	367/2.2	353/15.8	351/-	349/-	329/42	129/100	75/41
	b.	368/3.5[i]	367/11.3	353/1.0	351/-	349/-	329/0.59	171/5.4	75/94
XIII	e.	272/79	271/50	257/22	255/6.2	253/6.7	233/12.4	129/37	75/100
	b.	272/21[i]	271/100	257/2.0	255/4.9	253/8.2	233/-	171/2.5	75/69
	c.	272/20[i]	271/100	257/1.8	255/6.9	253/8.6	233/-	197/-	101/47
	d.	272/24[i]	271/100	257/2.4	255/6.4	253/7.9	233/0.36	183/1.1	101/43
XIV	e.	272/31	271/16.9	257/12.0	255/7.2	253/3.5	233/9.1	129/31	75/93
	b.	272/1.6[i]	271/7.5	257/1.5	255/5.3	253/3.3	233/-	171/3.5	75/97
	c.	272/1.0[i]	271/5.4	257/0.94	255/6.5	253/2.6	233/-	197/0.90	101/42
	d.	272/1.8[i]	271/6.1	257/2.2	255/8.2	253/3.1	233/-	183/0.82	101/58
XV	e.	272/25	271/4.4	257/19.7	255/1.6	253/0.60	233/0.46	129/100	75/22
	b.	272/14.6[i]	271/66	257/1.1	255/7.3	253/5.6	233/-	171/8.1	75/100
	c.	272/22[i]	271/95	257/1.9	255/9.4	253/6.0	233/-	197/5.2	101/100
	d.	272/24[i]	271/84	257/6.3	255/10.1	253/6.1	233/-	183/17.8	101/100
XVI	e.	270/7.5	269/0.5	255/5.6	253/0.77	251/0.14	231/11.5	129/100	75/11.8
	b.	270/2.5[i]	269/9.7	255/0.9	253/18.9	251/3.4	231/0.6	171/16.1	75/87
XVII	e.	270/29	269/1.7	255/7.4	253/3.3	251/1.1	231/1.7	129/100	75/25
	b.	270/3.7[i]	269/13.6	255/0.37	253/2.3	251/2.7	231/-	171/19	75/61

TABLE XXIV. (continued)

		RX_2Si^+	MeX_2Si^+	$C_{12}H_{17}^+$, 61.4	Other ions			Base peak as $\%\Sigma_{50}$
XI	e.	73/40	c		159/21	147/19	145/30	6.61
	b.	115/3.8	73/14.6		159/14.7	147/10.8	145/12.1	12.62
	c.	141/3.6	99/12		159/21	147/21	145/22	9.54
	d.	127/6.1	99/17.5		159/18.6	147/13.0	145/15.8	10.43
XII	e.	73/51	c		159/25	147/17.4	145/29	6.28
	b.	115/5.8	73/20		159/21	147/8.7	145/15.9	11.22
XIII	e.	73/69	c		215/25	155/35		5.60
	b.	115/2.3	73/12.5		161/8.8			15.73
	c.	141/1.4	99/13.8					13.10
	d.	127/2.8	99/13.9					13.42
XIV	e.	73/51	c		215/16.7	155/28		7.35
	b.	115/2.7	73/15.0		161/15.2			15.86
	c.	141/0.90	99/9.8					20.25
	d.	127/2.5	99/15.4					16.50
XV	e.	73/43	c	161/12.7	149/23			12.00
	b.	115/8.9	73/35	161/76	147/18.3	145/18.7		10.51
	c.	141/7.1	99/26	161/94				7.99
	d.	127/19.2	99/40	161/84				7.35
XVI	e.	73/26	c					20.15
	b.	115/7.2	73/27					11.09
XVII	e.	73/74	c		226/26	147/38		7.59
	b.	115/6.5	73/30		145/16.5			13.85

TABLE XXV. Metastable transitions in the mass spectra of TBDMSi ethers of mono-hydroxy steroids.[a]

Transition	Cholesterol (XIb)	Epiandrosterone (XIVb)	Androstanolone (XVb)
$M^+ \rightarrow (M-R)^+$		404→347,298.0,≃298 m	404→347,298.0,298.3 s
$(M-CH_3)^+ \rightarrow (M-CH_3-42)^+$		389→347,309.5,≃310 vw	
$(M-CH_3)^+ \rightarrow (M-CH_3-HXRSiOH)^+$			389→271,188.8,≃188 m
$(M-R)^+ \rightarrow (M-R-H_2O)^+$	443→425,407.7,≃408 m		347→329,311.9,≃312 m
$(M-R)^+ \rightarrow (M-R-44)^+$			347→303,264.6,≃265 m
$(M-R)^+ \rightarrow (M-R-HX_2SiOH)^+$	443→367,304.0,≃304 s	347→271,211.7,≃212 s	347→271,211.7,≃212 vs
$(M-R-HX_2SiOH)^+ \rightarrow (M-R-HX_2SiOH-H_2O)^+$		271→253,236.2,236 m	271→253,236.2,≃236 s
$(M-R-HX_2SiOH)^+ \rightarrow 161$			271→161,95.65,≃96 s
$(M-R)^+ \rightarrow X_2Si{=}OH^+$			347→75,16.21,16.3 w

[a] Data given are: $m_p \rightarrow m_d$, m* (calc), m* (obs)

vs = very strong, s = strong, m = moderate, w = weak, vw = very weak

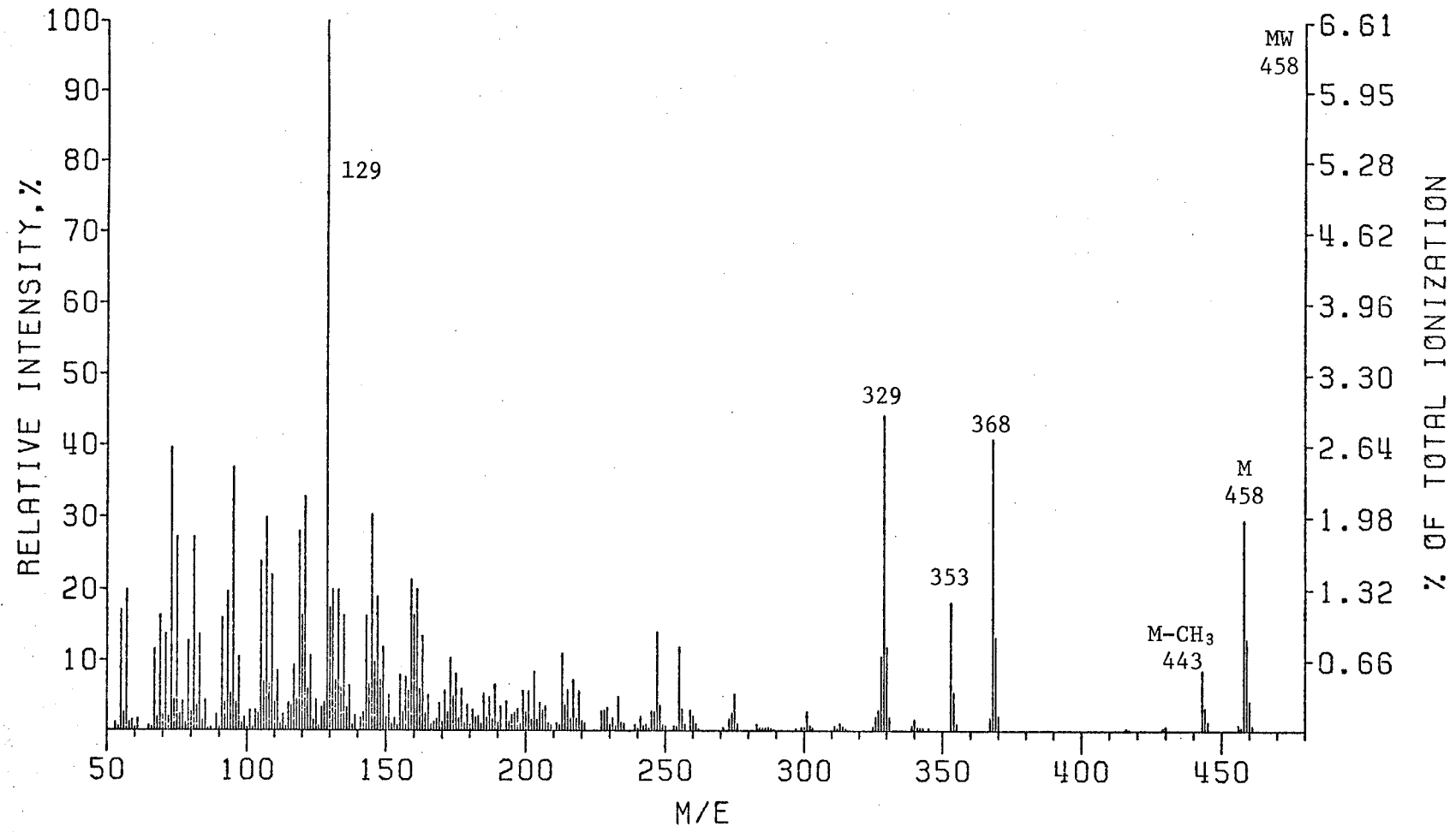


Figure 111. Mass spectrum of TMSi-cholesterol (XIe) recorded on the Finnigan MS at 70 eV.

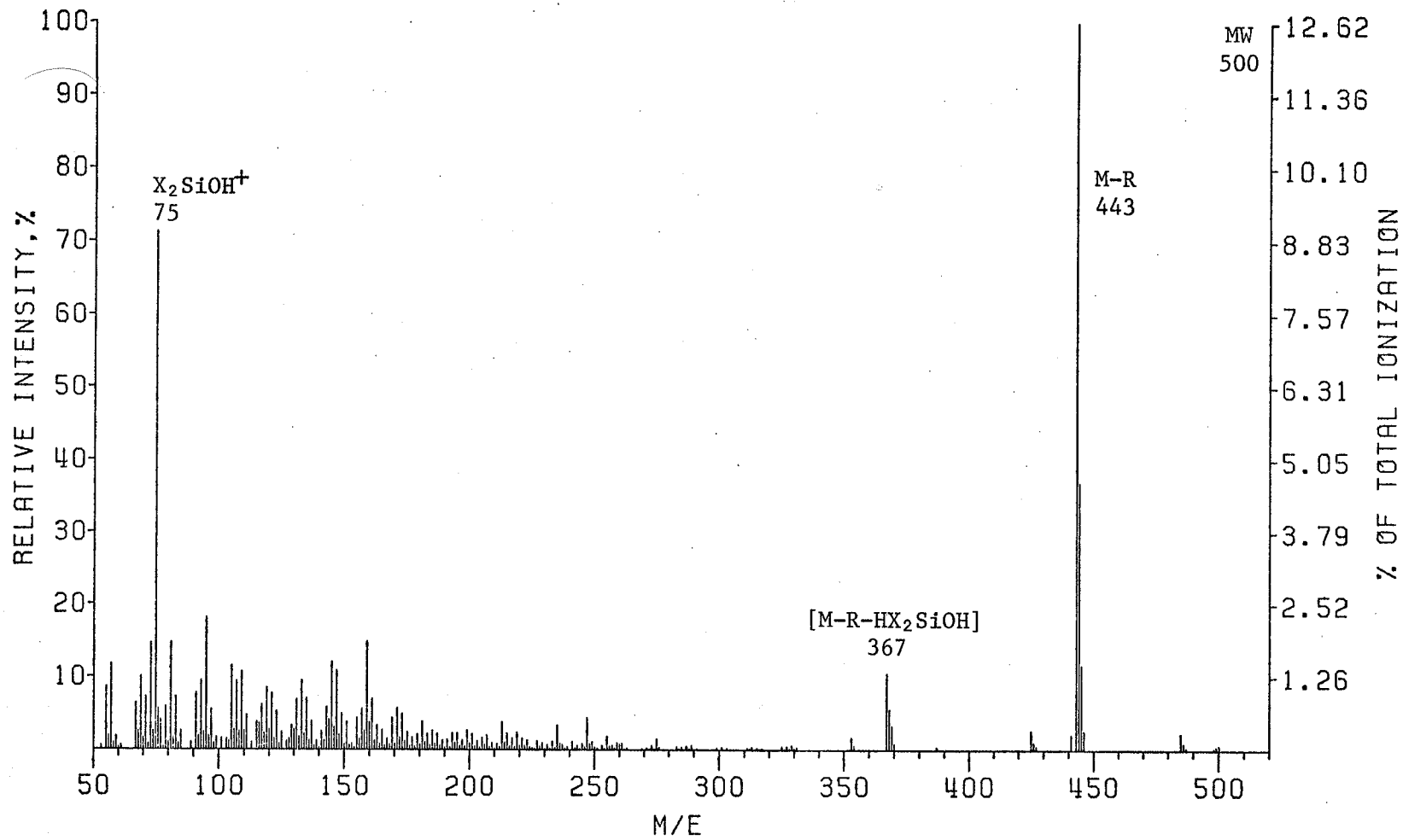


Figure 112. Mass spectrum of TBDMSi-cholesterol (XIb) recorded on the Finnigan MS at 70 eV.

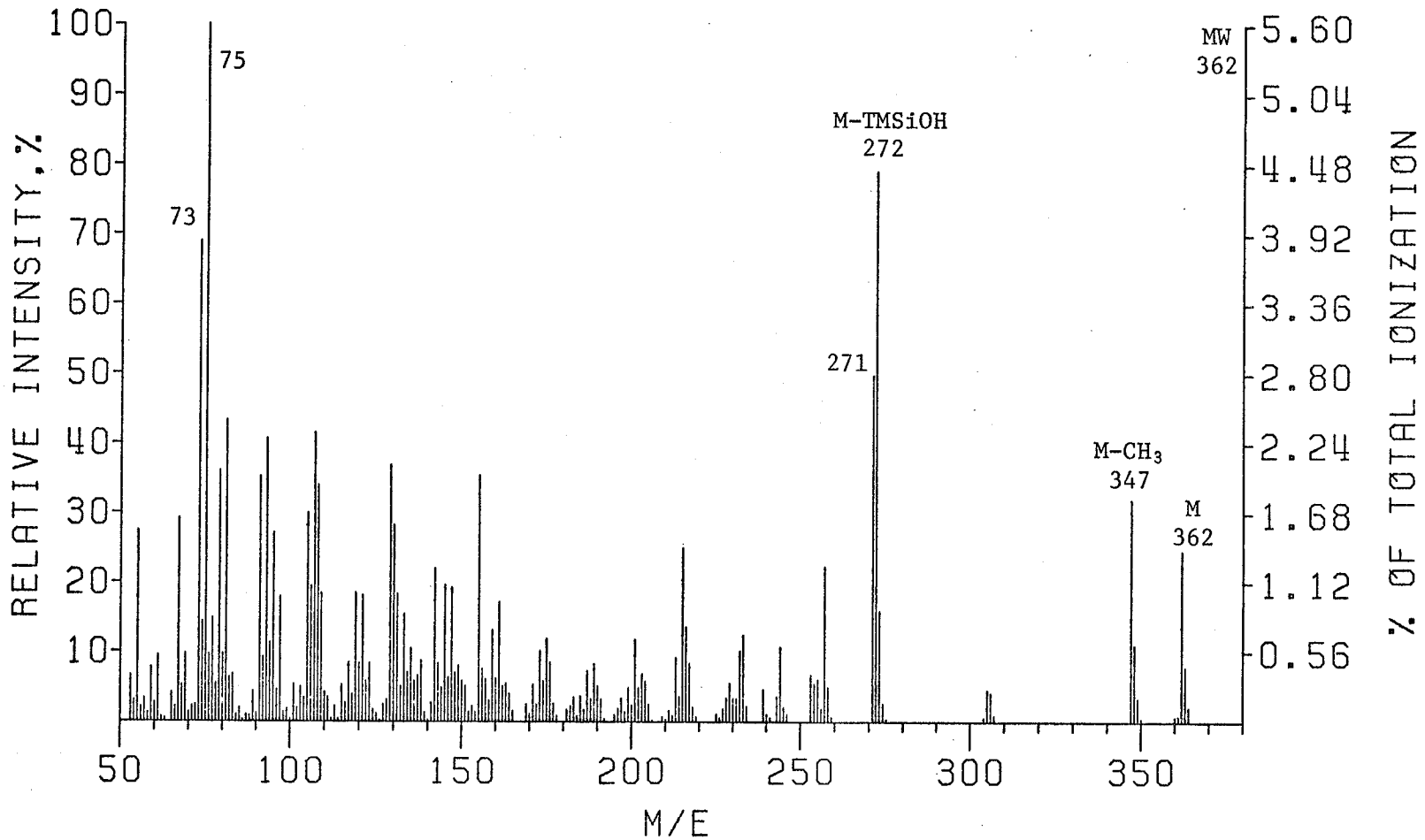


Figure 113. Mass spectrum of TMSi-androsterone (XIIIe) recorded on the Finnigan MS at 70 eV.

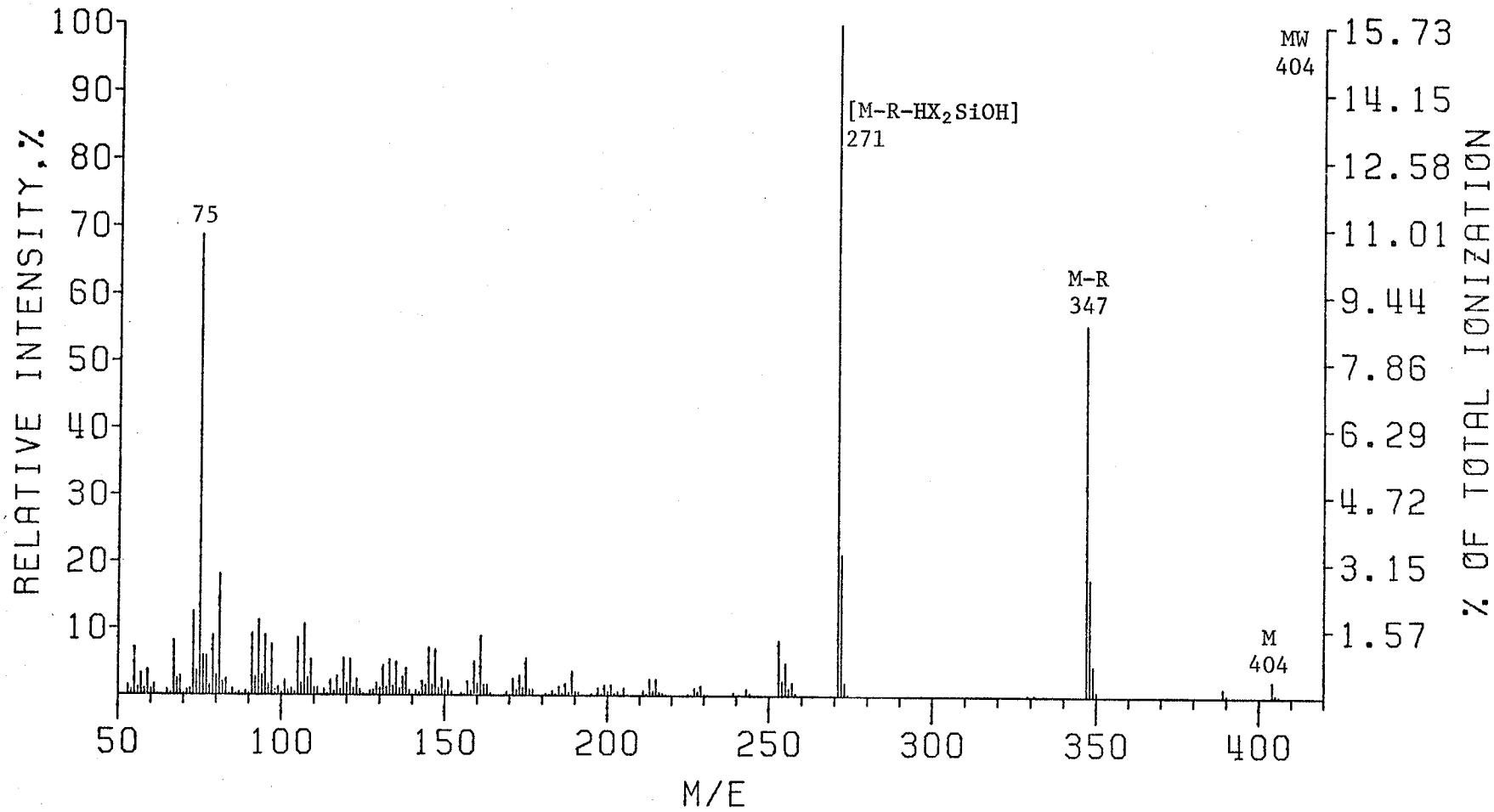


Figure 114. Mass spectrum of TBDMSi-androsterone (XIIIb) recorded on the Finnigan MS at 70 eV.

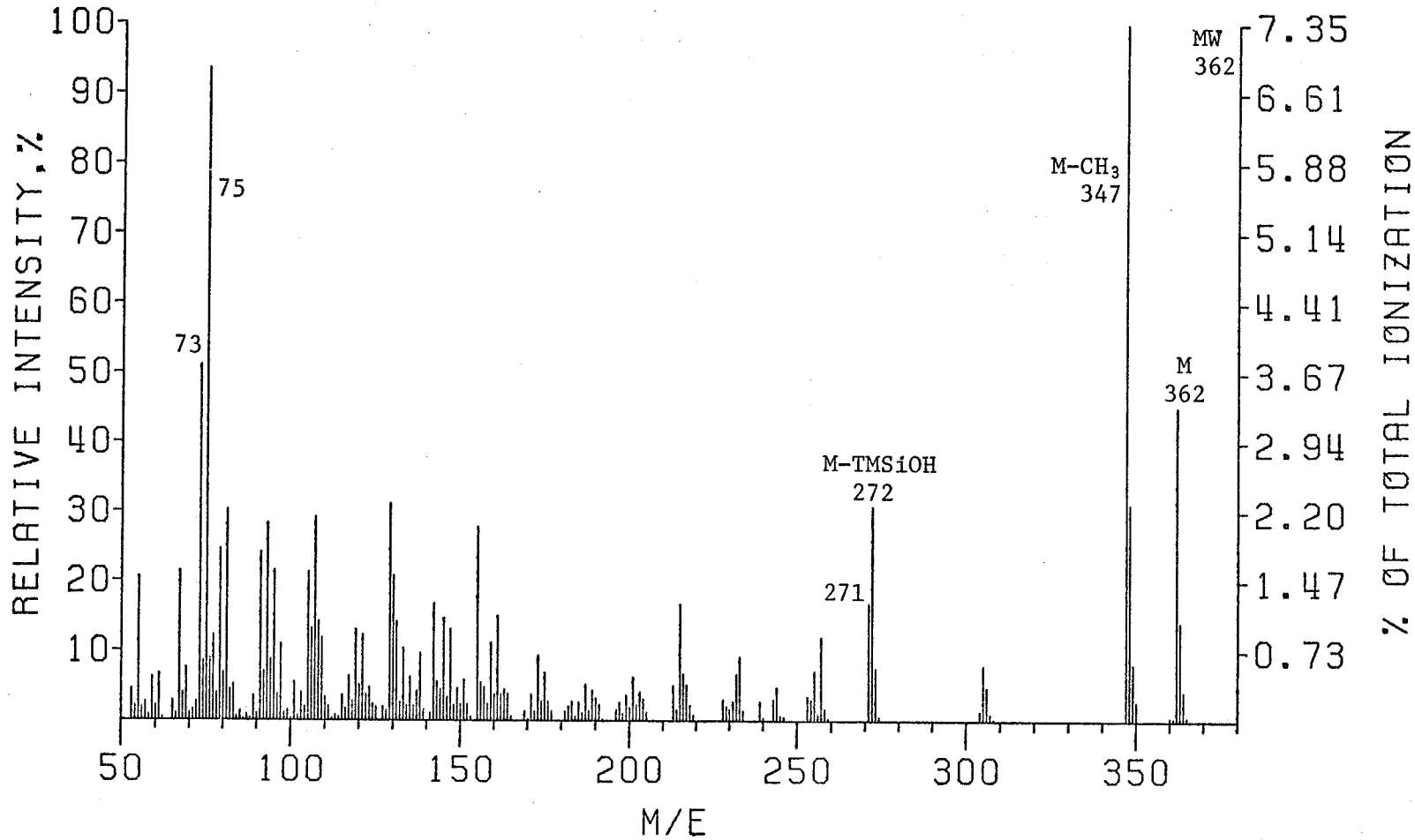


Figure 115. Mass spectrum of TMSi-epiandrosterone (XIVe) recorded on the Finnigan MS at 70 eV.

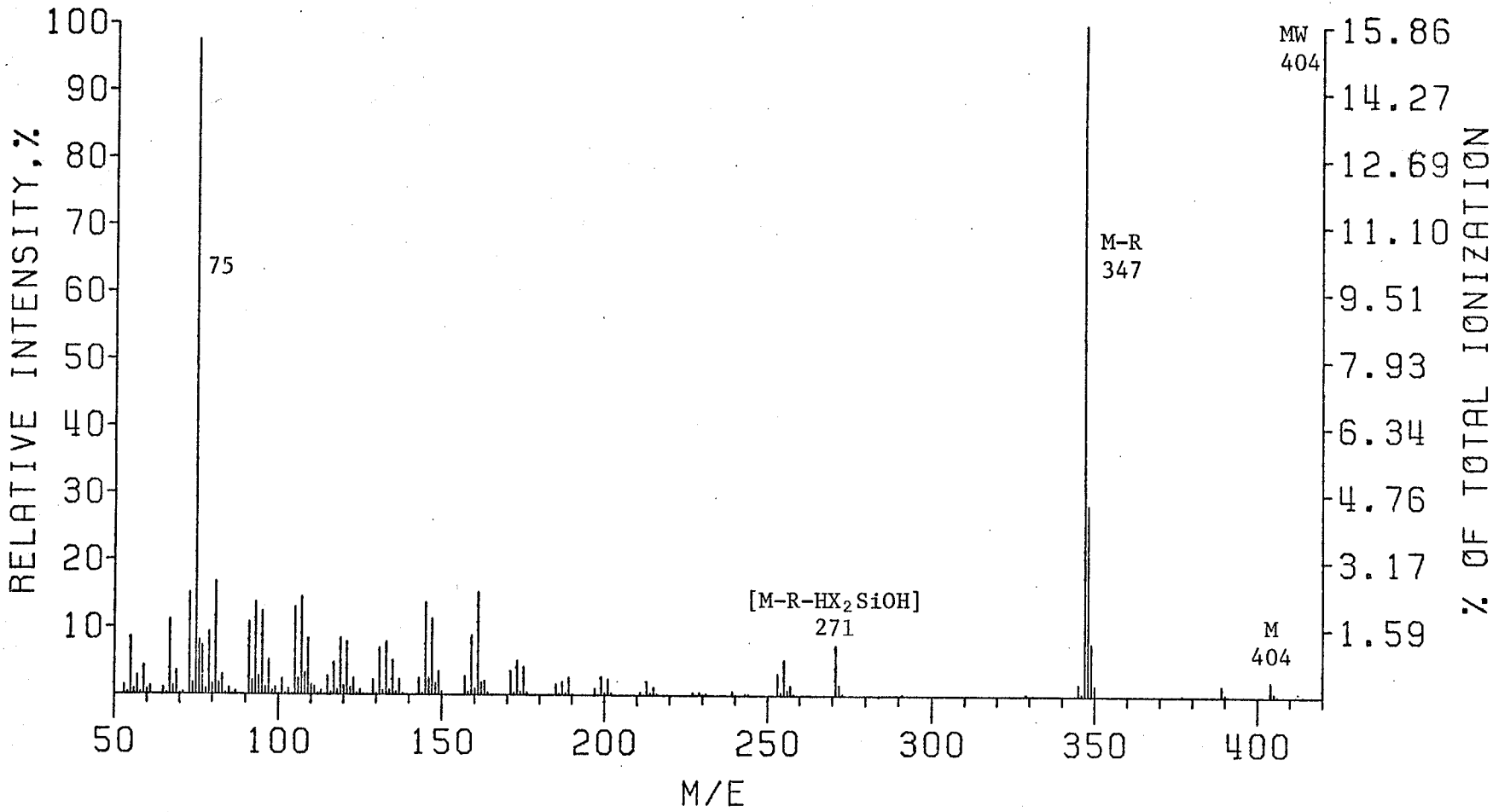


Figure 116. Mass spectrum of TBDMSi-epiandrosterone (XIVb) recorded on the Finnigan MS at 70 eV.

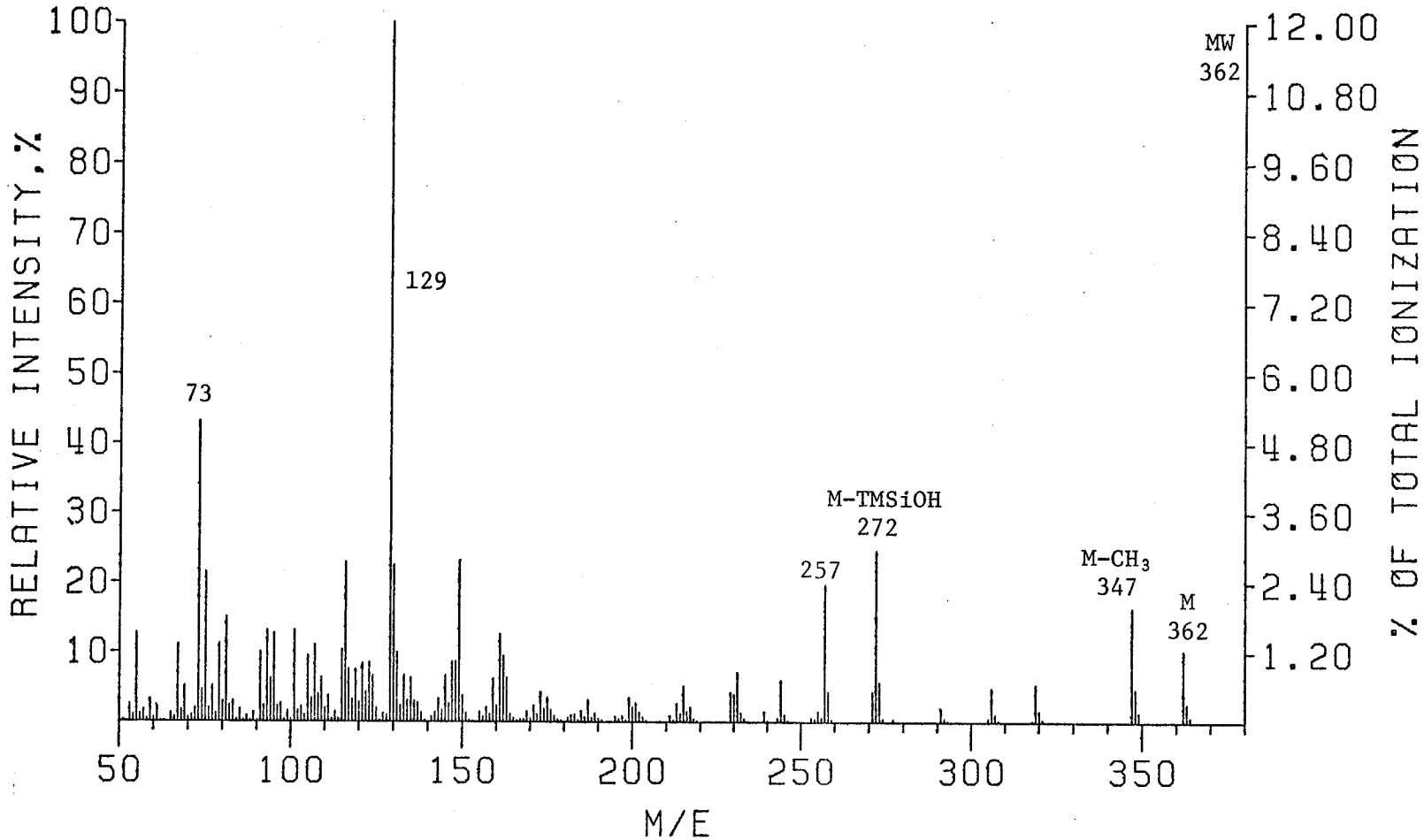


Figure 117. Mass spectrum of TMSi-androstanolone (XVe) recorded on the Finnigan MS at 70 eV.

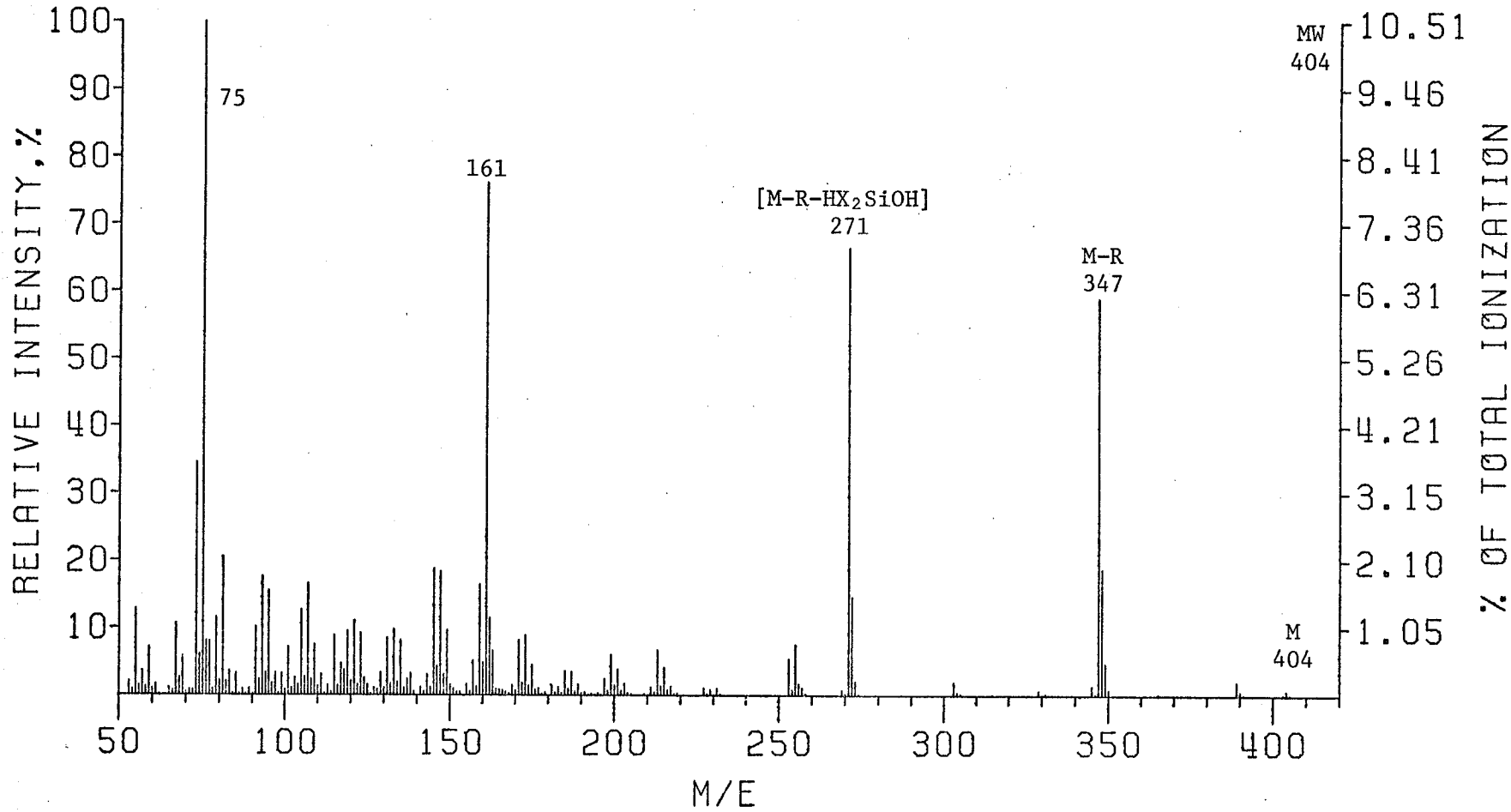


Figure 118. Mass spectrum of TBDMSi-androstanolone (XVb) recorded on the Finnigan MS at 70 eV.

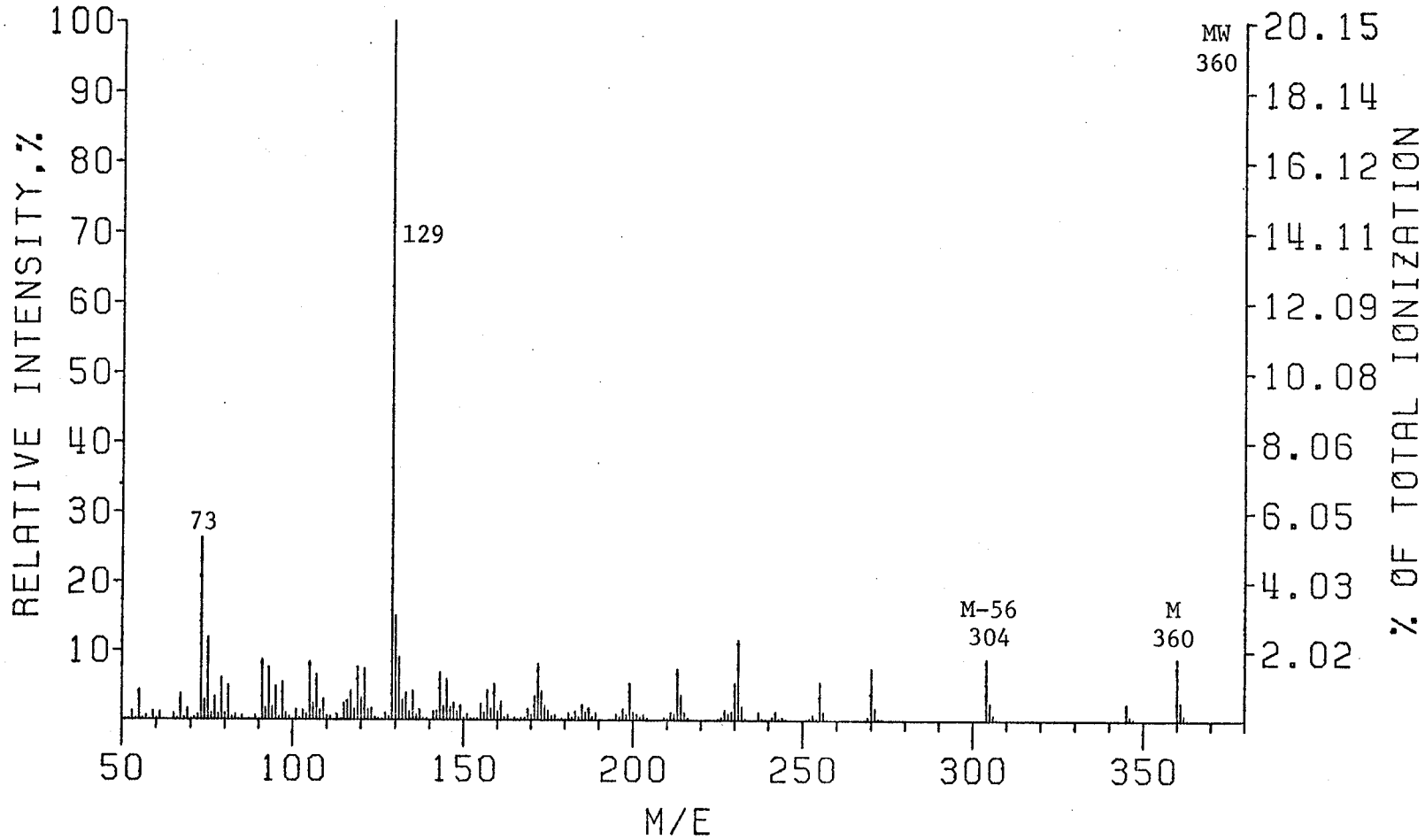


Figure 119. Mass spectrum of TMSi-dehydroepiandrosterone (XVIe) recorded on Finnigan MS at 70 eV.

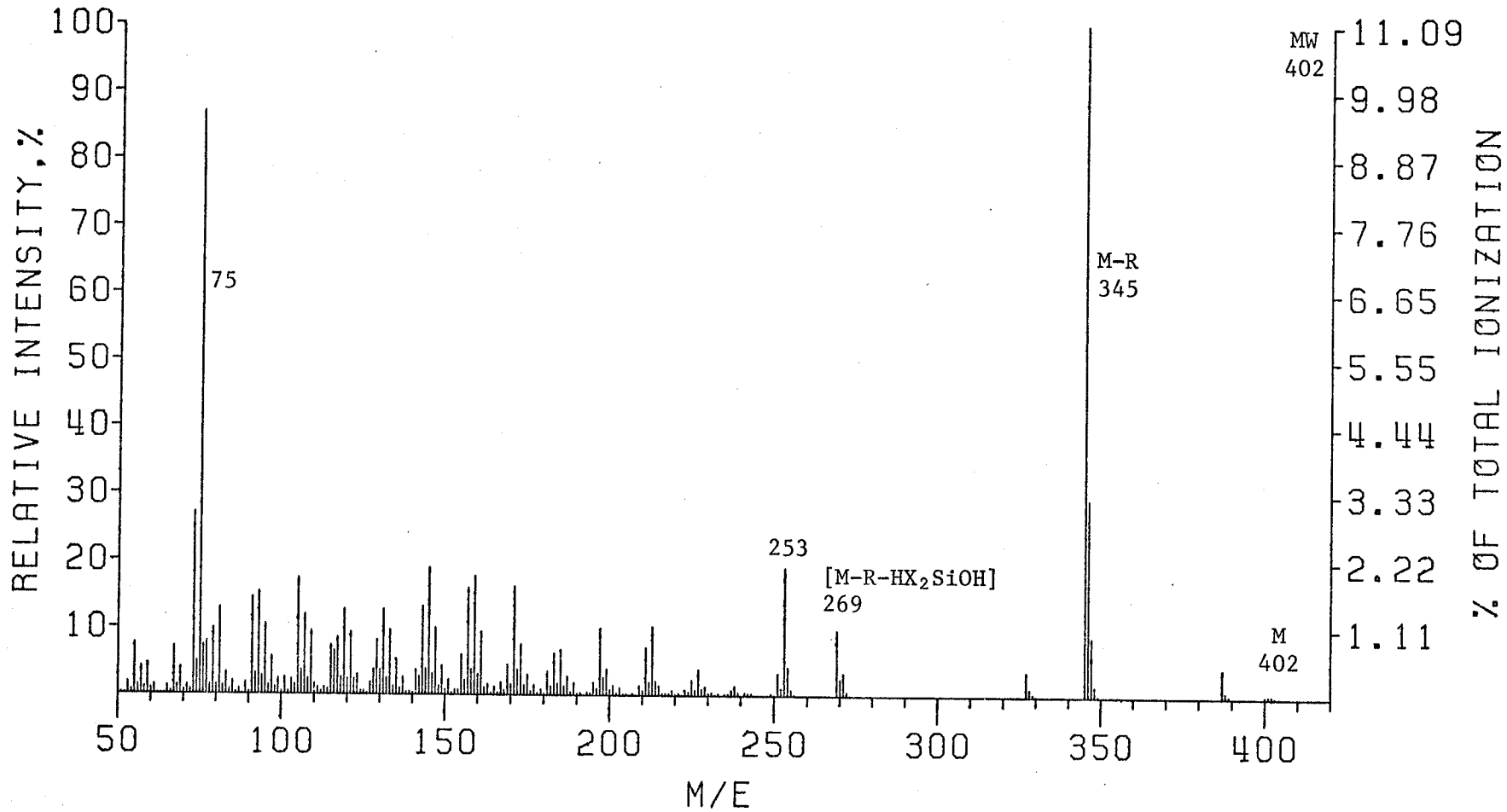


Figure 120. Mass spectrum of TBDMSi-dehydroepiandrosterone (XVIb) recorded on Finnigan MS at 70 eV.

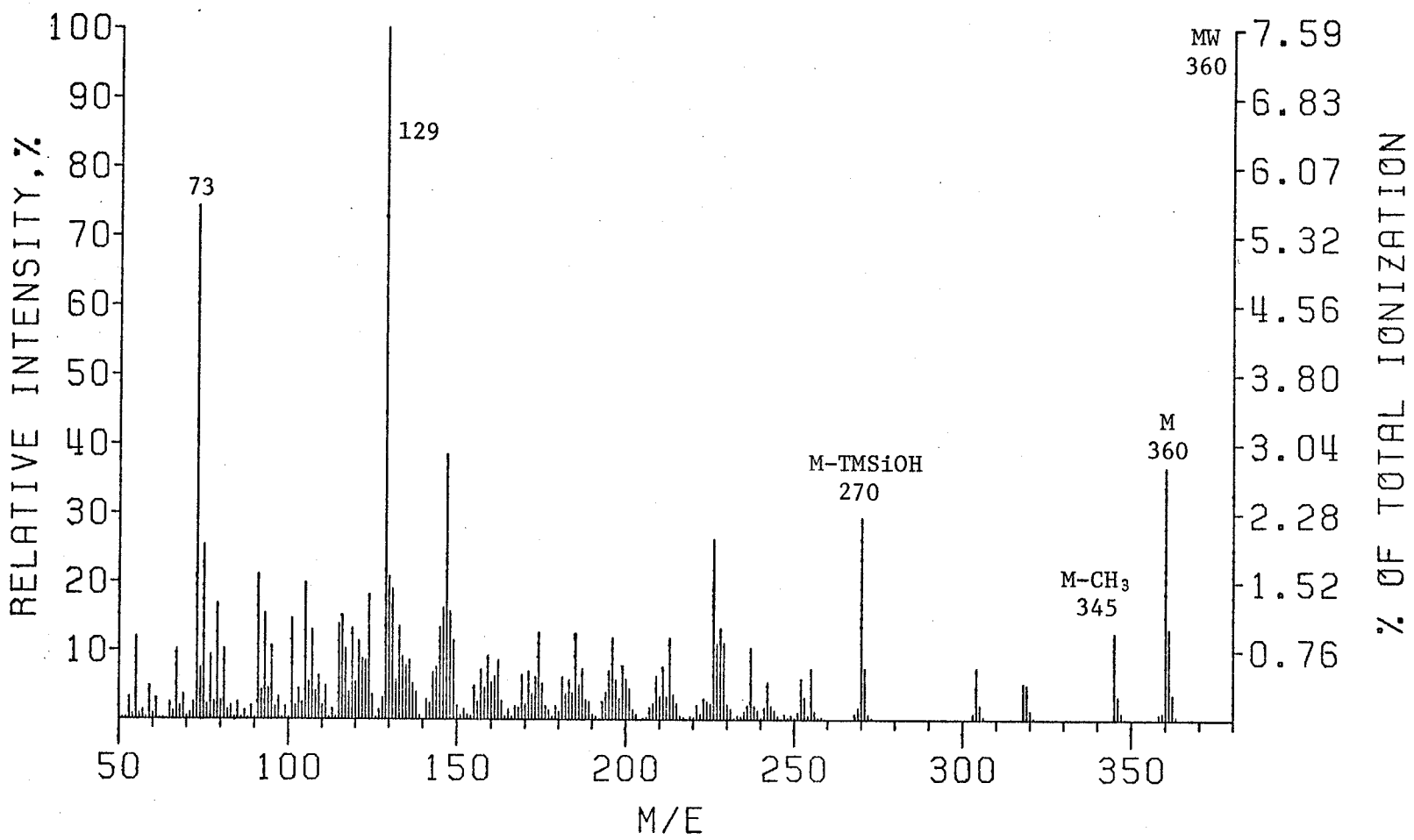


Figure 121. Mass spectrum of TMSi-testosterone (XVIIe) recorded on the Finnigan MS at 70 eV.

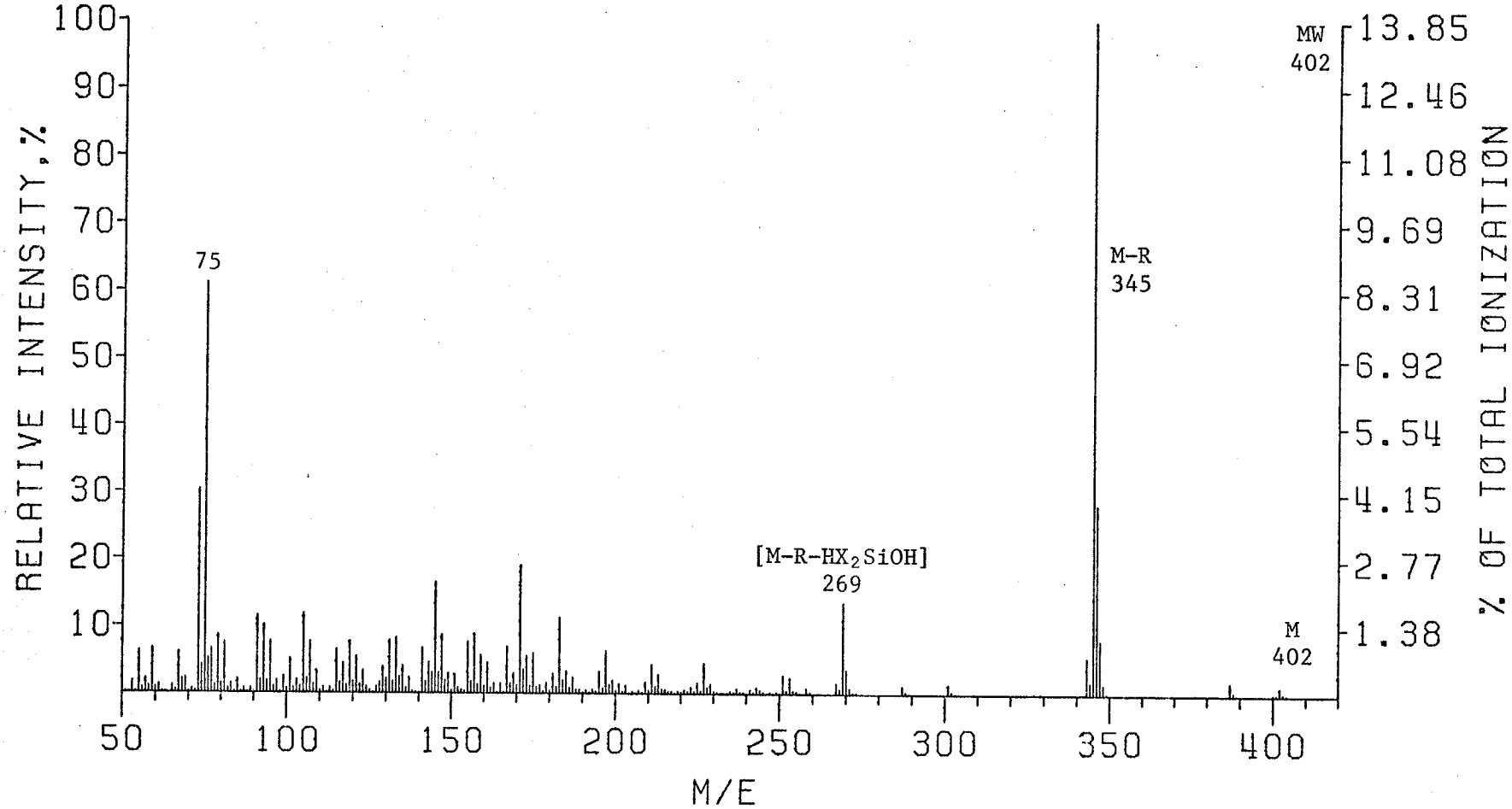
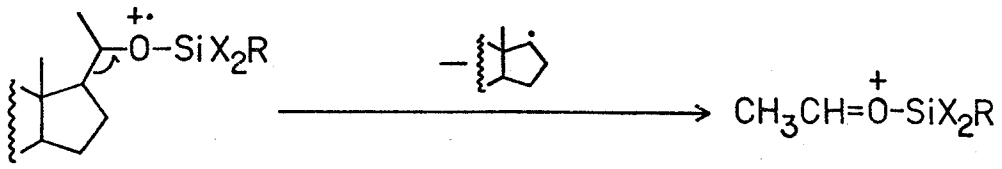


Figure 122. Mass spectrum of TBDMSi-testosterone (XVIIb) recorded on Finnigan MS at 70 eV.

ii) Di-hydroxy steroids

The mass spectral data for the silyl ether derivatives of 5β-pregnane-3α,20α-diol, XIX, are presented in Tables XXVI to XXVIII. Figures 123 to 126 illustrate the striking difference between the spectra of the TMSi and SCTASi ether derivatives and emphasize the potential of the latter for analytical work. The spectra also illustrate the mass shifts that occur upon change of R and X_2 substituents on silicon. The high mass region of the spectrum of the TMSi ether contains ions only of very low abundance. The ion of m/e 117 (Scheme 63) characterizes the side chain attached to C_{17} but other abundant ions of diagnostic value are lacking. In contrast, the spectra of the SCTASi ethers have an abundant ion at $[M-R]^+$, which serves to indicate the molecular weight. In the high mass region the only other really prominent ion corresponds to $[M-R-RX_2SiOH-HX_2SiOH]^+$. Several routes to this species are possible as shown by Scheme 64. There is evidence that both proposed structures 64.4 and 64.8 occur. A metastable peak indicates sequential loss, first of RX_2SiOH and then of HX_2SiOH from $[M-R]^+$. Furthermore, mass spectra of the partially silylated steroid, and mixed silyl derivatives (Table XXVIII), show that in addition to the previously discussed eliminations of RX_2SiOH or HX_2SiOH from ring A of a 3α-silyloxy-5β-steroid, elimination of either of these species from the region of ring D also occurs. An analog of 64.7 has also been observed in the mass spectrum of the TBDMSi

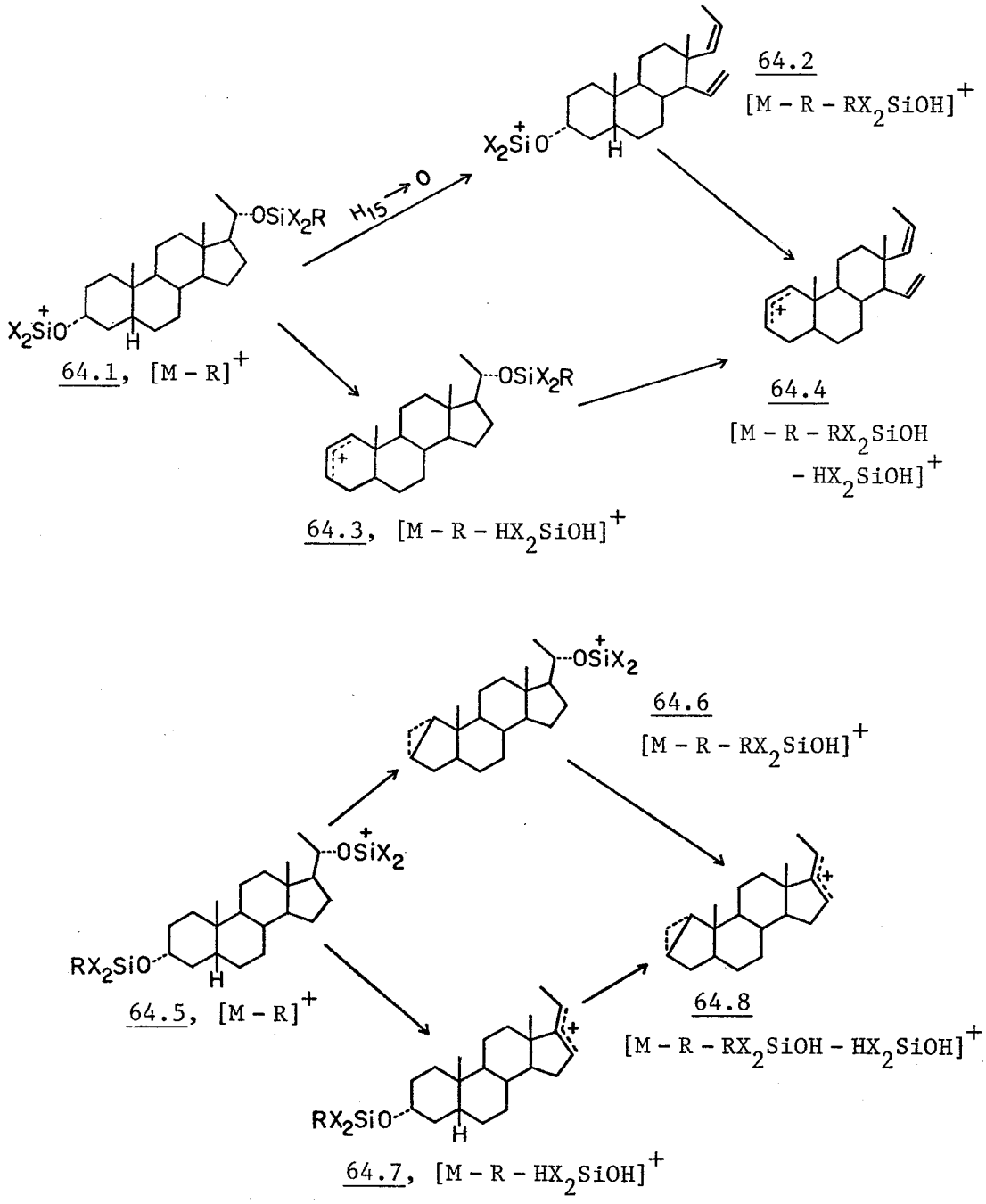


63.1, M^+

63.2, m/e 117 - TMSi
m/e 159 - TBDMSi
m/e 185 - TMTBSi

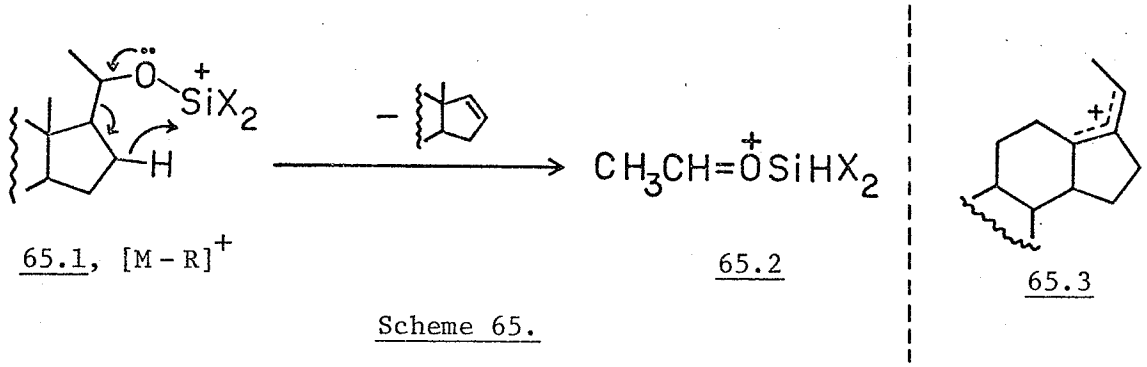
Scheme 63.

Scheme 64.


64.2
[M – R – RX2SiOH]+
X2SiO
H
OSiX2R
H15 → O
X2SiO
H
64.1, [M – R]+
OSiX2R
64.4
[M – R – RX2SiOH
– HX2SiOH]+
64.3, [M – R – HX2SiOH]+
OSiX2
64.6
[M – R – RX2SiOH]+
OSiX2
RX2SiO
H
64.5, [M – R]+
64.8
[M – R – RX2SiOH – HX2SiOH]+
RX2SiO
H
64.7, [M – R – HX2SiOH]+

ether of 20α-hydroxy-4-pregnene-3-one (95).

The fragmentation in Scheme 63, which characterizes the side chain, also occurs readily in the mass spectra of the SCTASi derivatives. There is also another abundant ion, 65.2, indicative of the C_{17} side chain and we propose the mechanism in Scheme 65 as its mode of formation.

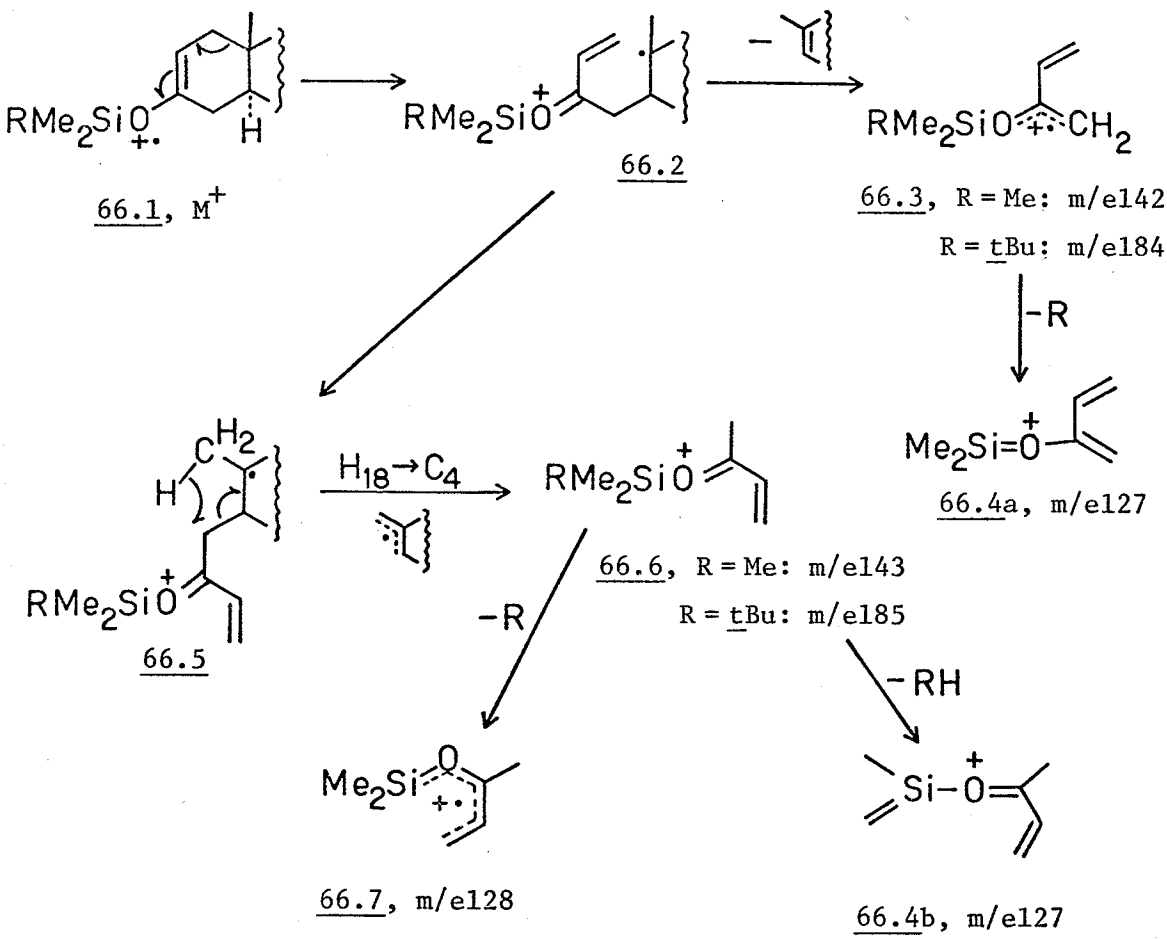


Scheme 65.

Most of the other ions in the spectra arise by now familiar pathways, and many involve losses of CH_3, R, H_2O or RX_2SiOH. There are two other fragmentations for which tentative proposals are made. Table XXVII lists metastable peaks for the elimination of 90 amu and 42 amu from $[M-R]^+$. The former may be an analog of the HX_2SiOH elimination in which the C_{18} methyl group is lost, rather than H from C_{16}, to give ion 65.3. This ion has the same mass as $[M-CH_3-RX_2SiOH]^+$ in the spectra of the TBDMSi ether derivatives, but analogs were of negligible intensity in the case of the other derivatives. The loss of 42 amu from $[M-R]^+$ may involve elimination of C_3H_6 from the intact TBDMSi group. We have observed a metastable peak for a similar transition in the spectra of TBDMSi ether derivatives of simple molecules where its origin is not ambiguous, e.g. for the transition 189 → 147 in the mass spectrum of $(\underline{t}BuMe_2Si)_2O$.

The silyl ether derivatives of compound XVIII (Scheme 53 and Table XXVI) were obtained as secondary products in the silylation of androstanolone (XV) via enolization of the ketone. Two possible enols were

considered, having either a Δ^2 or Δ^3 double bond. The enol was assigned as 5α-androst-2-en-3,17β-diol from the mass spectra of the silyl ethers, XVIIIee and XVIIIbb, on the basis of the fragmentations in Scheme 66. Ions at m/e 143, 142, and 127, corresponding to species 66.6, 66.3, and 66.4(a or b) respectively, are very abundant in the mass spectrum of the TMSi ether. The presence of a Δ^2 double bond initiates fission of the 1-10 bond, 66.1 → 66.2. 66.2 may then decompose either by fission of the 4-5 bond to give the stable radical ion, 66.3, and a stable neutral species, or by hydrogen transfer, possibly via the 5-membered cyclic transition state, 66.5, to give an allyl radical and the stable ion 66.6.



Scheme 66.

As shown, 66.3 and 66.6 are the likely precursors of m/e 127, either form (66.4a or b) of which should be very stable, while the radical ion, 66.7, of m/e 128 is only prominent when it is formed by elimination of the bulky t-butyl radical from 66.6. As anticipated, 66.3, 66.6 and 66.4 are more abundant for TMSi ethers than TBDMSi ethers. Our assignment of a Δ^2 rather than a Δ^3 double bond is in accord with acetylation of 3-keto-steroids where the yield of the Δ^2 form of the enol acetate approached 100% (258). The Δ^2 enol was about 2.1 kcal/mole more stable than the Δ^3 enol.

Another noteworthy feature of the spectrum of the TBDMSi ether of XVIII, which is useful in recognizing that an enol ether system is present, is the unusually high abundance of the molecular ion. Presumably, this is caused by the stabilizing influence of the Δ^2 double bond on a positive charge on the silyloxy group by charge delocalization. Other ions in the high mass region can be explained by mechanisms already given, the high abundance of $[M-R-HX_2SiOH]^+$ being consistent with the presence of the 17β-silyloxy substituent.

An interesting feature of the spectra of bis-(SCTASi)-ethers is the relatively high abundance of the double siliconium ions $[M-2R]^{2+}$, reflecting the considerable stability of siliconium ions, especially when remote from other functional groups, and from each other. They were recognized by isotope peaks at half-integral m/e values and their disappearance when the electron energy was reduced to 20 eV.

TABLE XXVI. Partial mass spectra of bis-O-trialkylsilyl derivatives of di-hydroxy steroids.[a]

Steroid:	5β-Pregnane-3α,20α-diol				5α-Androst-2-en-3,17β-diol	
Derivative:	TMSi	TBDMSi	TMTBSi	TMIPSi	TMSi	TBDMSi
Ion type # :	XIXee	XIXbb	XIXcc	XIXdd	XVIIIee	XVIIIbb
M^+	464/0.12	548/-	600/-	572/-	434/39	518/43
$(M-CH_3)^+$	449/1.8	533/2.5	585/1.5	557/-	419/6.9	503/5.7
$(M-29)^+$	435/-	519/-	571/-	543/-	405/10.3	489/1.2
$(M-R)^+$	b	491/89	543/95	529/100	b	461/61
$(M-R-42)^+$	407/-	449/3.7	501/-	487/-	377/-	419/-
$(M-RX_2SiOH)^+$	374/0.88	416/-	442/1.0[i]	428/0.59[i]	344/1.5	386/22[i]
$(M-R-HX_2SiOH)^+$	373/-	415/1.0	441/2.0	427/2.2	343/1.7	385/62
$(M-CH_3-RX_2SiOH)^+$	359/0.51	401/2.1[c]	427/-	413/-	329/0.82	371/1.8
$(M-R-RX_2SiOH)^+$	d	359/7.5	385/13.3	385/14.9	d	329/5.8
$(M-RX_2SiOC_2H_4)^+$	347/3.0	389/-	415/-	401/-	NA	NA
$(M-RX_2SiOC_2H_5)^+$	346/3.3	388/-	414/-	400/-	NA	NA
$(M-R-RX_2SiOH-H_2O)^+$	341/0.05	341/1.3	367/2.9	367/2.5	311/-	353/-
$(M-2RX_2SiOH)^+$	284/4.2	284/6.8[i]	284/9.2[i]	284/8.8[i]	254/1.3	254/2.6[i]
$(M-R-RX_2SiOH-HX_2SiOH)^+$	283/1.2	283/29	283/35	283/31	253/0.5	253/12.1
$(M-CH_3-2RX_2SiOH)^+$	269/3.5	269/2.5	269/2.5	269/3.0	239/2.0	239/1.1
$(M-R-RX_2SiOC_4H_5)^+$	NA	NA	NA	NA	277/0.28	277/2.2
$RX_2SiOC_4H_6^+$, 66.6	NA	NA	NA	NA	143/100	185/16.4
$RX_2SiOC_4H_5^+$, 66.3	NA	NA	NA	NA	142/74	184/1.3

continued . . .

TABLE XXVI. (continued)

Ion type	XIXee	XIXbb	XIXcc	XIXdd	XVIIIee	XVIIIbb
$X_2SiOC_4H_6^+$, 66.7	NA	NA	NA	NA	128/6.4[i]	128/23
$X_2SiOC_4H_5^+$, 66.4	NA	NA	NA	NA	127/37	127/16.9
$RX_2SiOC_3H_4^+$	129/1.3	171/2.4	197/1.1	183/1.0	129/29	171/8.2
$RX_2SiOC_2H_4^+$, 63.2	117/100	159/46	185/70	171/71	NA	NA
$HX_2SiO{=}CHCH_3^+$, 65.2	103/1.6	103/28	129/46	129/45	NA	NA
$X_2Si{=}OH^+$	75/11.0	75/100	101/100	101/79	75/32	75/100
RX_2Si^+	73/15.4	115/8.9	141/5.8	127/17	73/60	115/10.2
MeX_2Si^+	e	73/26	99/21	99/27	e	73/82
$(M-2R)^{2+}$	217/0.91	217/11.5	243/12.9	243/12.3	202/19.7	202/26
Other peaks		145/26	285/10.2	285/9.8		417/2.3
		117/38				201/25
Base peak as $\%\Sigma_{50}$	38.08	12.03	9.45	9.94	10.10	8.16

[a] Quadrupole mass spectrometer, corrected for mass discrimination

[b] Same as $(M-CH_3)^+$

[c] At least partly assigned to structure 65.3

[d] Same as $(M-CH_3-RX_2SiOH)^+$

[e] Same as RX_2Si^+

[i] Uncorrected intensity. Contains significant isotopic contribution from ion type of lower mass.

NA = not applicable

TABLE XXVII. Metastable transitions in the mass spectra of bis-TBDMSi ethers of di-hydroxy steroids.[a]

Transition	XIXbb	XVIIIbb
$M^+ \rightarrow (M-R)^+$		518→461, 410.3, 410 vs
$M^+ \rightarrow (M-R-HX_2SiOH)^+$		518→385, 286.1, 286 m
$(M-R)^+ \rightarrow (M-R-42)^+$	491→449, 410.6, 411 s	
$(M-R)^+ \rightarrow (M-R-44)^+$		461→417, 377.2 ≃ 378 w
$(M-R)^+ \rightarrow (M-R-HX_2SiOH)^+$		461→385, 321.5, ≃ 322 vs
$(M-R)^+ \rightarrow (M-R-90)^+$	491→401, 327.5, ≃ 328 m	
$(M-R)^+ \rightarrow (M-R-RX_2SiOH-HX_2SiOH)^+$	491→283, 163.1, ≃ 164 m	
$(M-R-HX_2SiOH)^+ \rightarrow (M-R-HX_2SiOH-RX_2SiOH)^+$		385→253, 166.3, 167 vw
$(M-R-RX_2SiOH)^+ \rightarrow (M-R-RX_2SiOH-HX_2SiOH)^+$	359→283, 223.1, 223 m	

[a]Data given are: $m_p \rightarrow m_d$, m*(calc), m*(obs)

vs = very strong, s = strong, m = moderate, w = weak, vw = very weak

TABLE XXVIII.

Partial mass spectra of partial and mixed silyl derivatives of 5β-pregnane-3α,20α-diol.[a]

3α-substituent:		TBDMSiO	HO		TBDMSiO	TMSiO
20α-substituent:		HO	TBDMSiO		TMSiO	TBDMSiO
Ion type	m/e	XIXba	XIXab	m/e	XIXbe	XIXeb
M^+	434	0.97	0.17	506	0.87	0.38
$(M-CH_3)^+$	419	1.7	0.97	491	3.1	4.2
$(M-R)^+$	377	83	44	449	52	89
$(M-R-H_2O)^+$	359	2.8	2.9	NA	-	-
$(M-R-TMSiOH)^+$	NA	-	-	359	4.0	10.4
$(M-R-HX_2SiOH)^+$	301	8.7	4.1	373	0.53	1.3
$C_{21}H_{31}^+$, 64.4 or 64.8	283	29	8.2	283	14.1	26
$Me_3SiO{=}CHCH_3^+$	NA	-	-	117	100	31
$X_2Si{=}OH^+$	75	100	100	75	71	100
$(M-R-CH_3)^{2+}$	181	0.8	-	217	2.5	13.0
Other ions	285	-	9.1			
	257	8.6	13.9			
Base peak as $\%\Sigma_{50}$		10.88	8.05		16.15	10.90

[a] $RX_2Si \equiv \underline{t}BuMe_2Si$ NA = not applicable

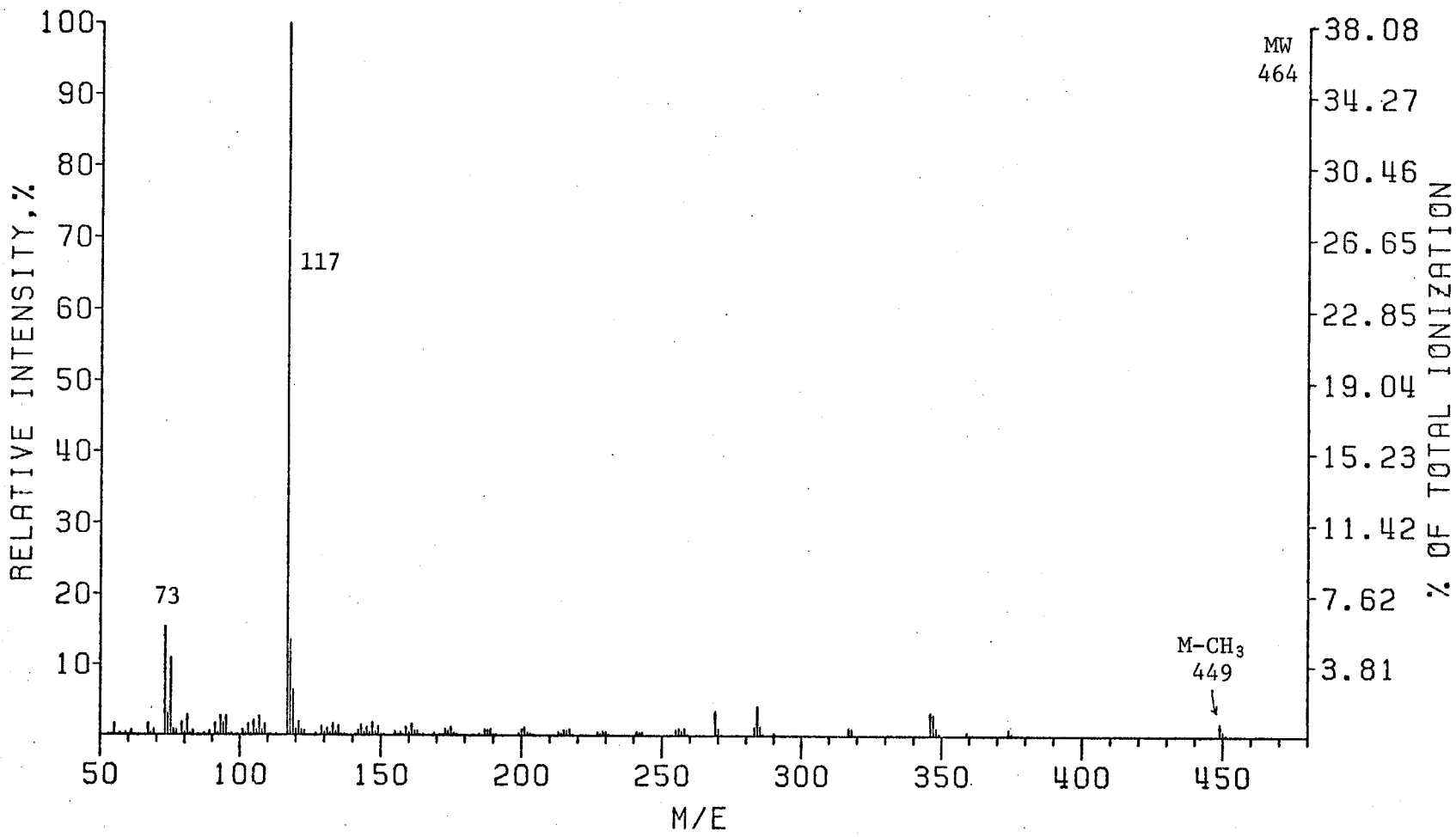


Figure 123. Mass spectrum of bis-TMSi-pregnanediol (XIXee) recorded on the Finnigan MS at 70 eV.

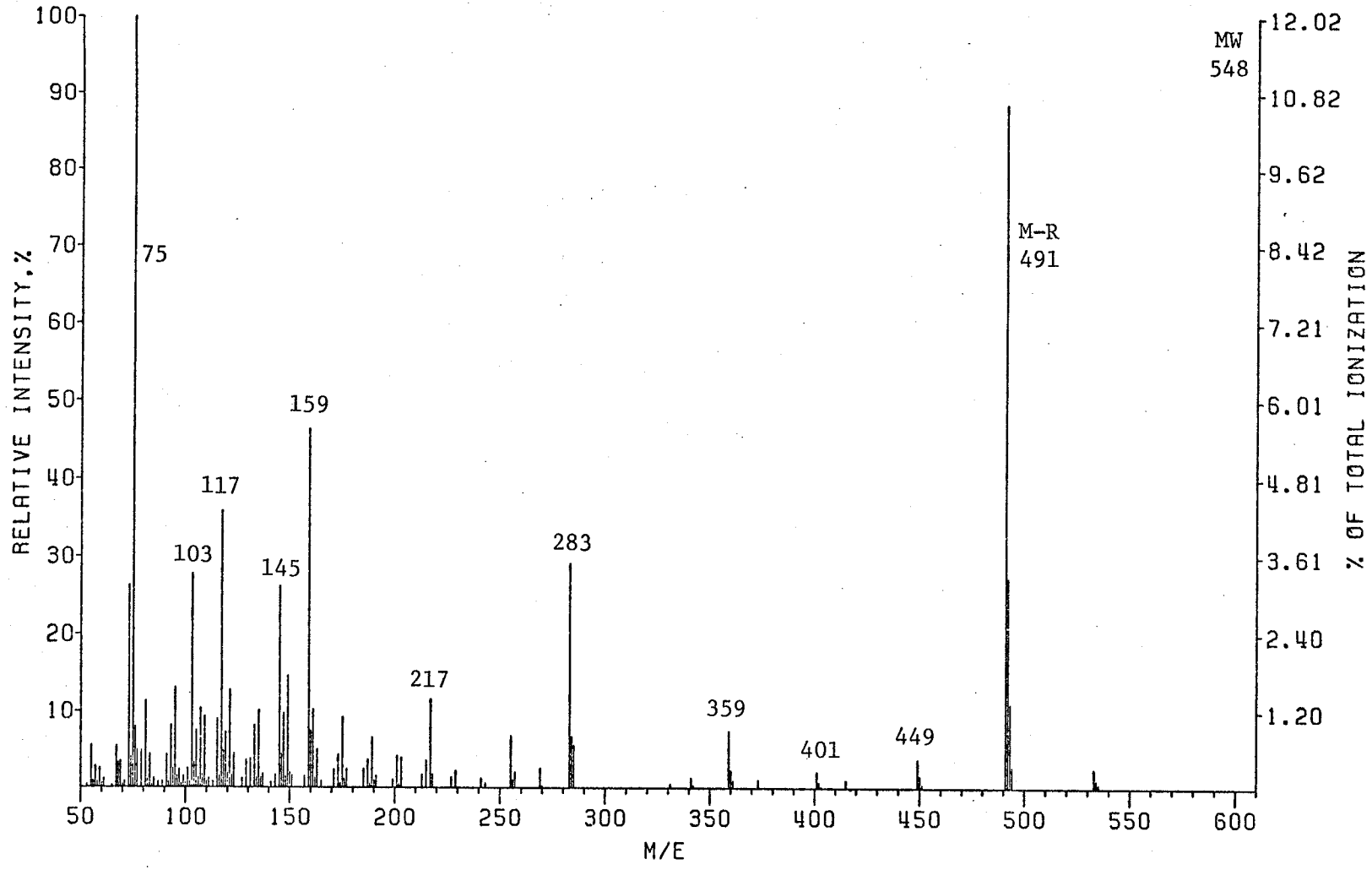


Figure 124. Mass spectrum of bis-TBDMSi-pregnanediol (XIXbb) recorded on Finnigan MS at 70 eV.

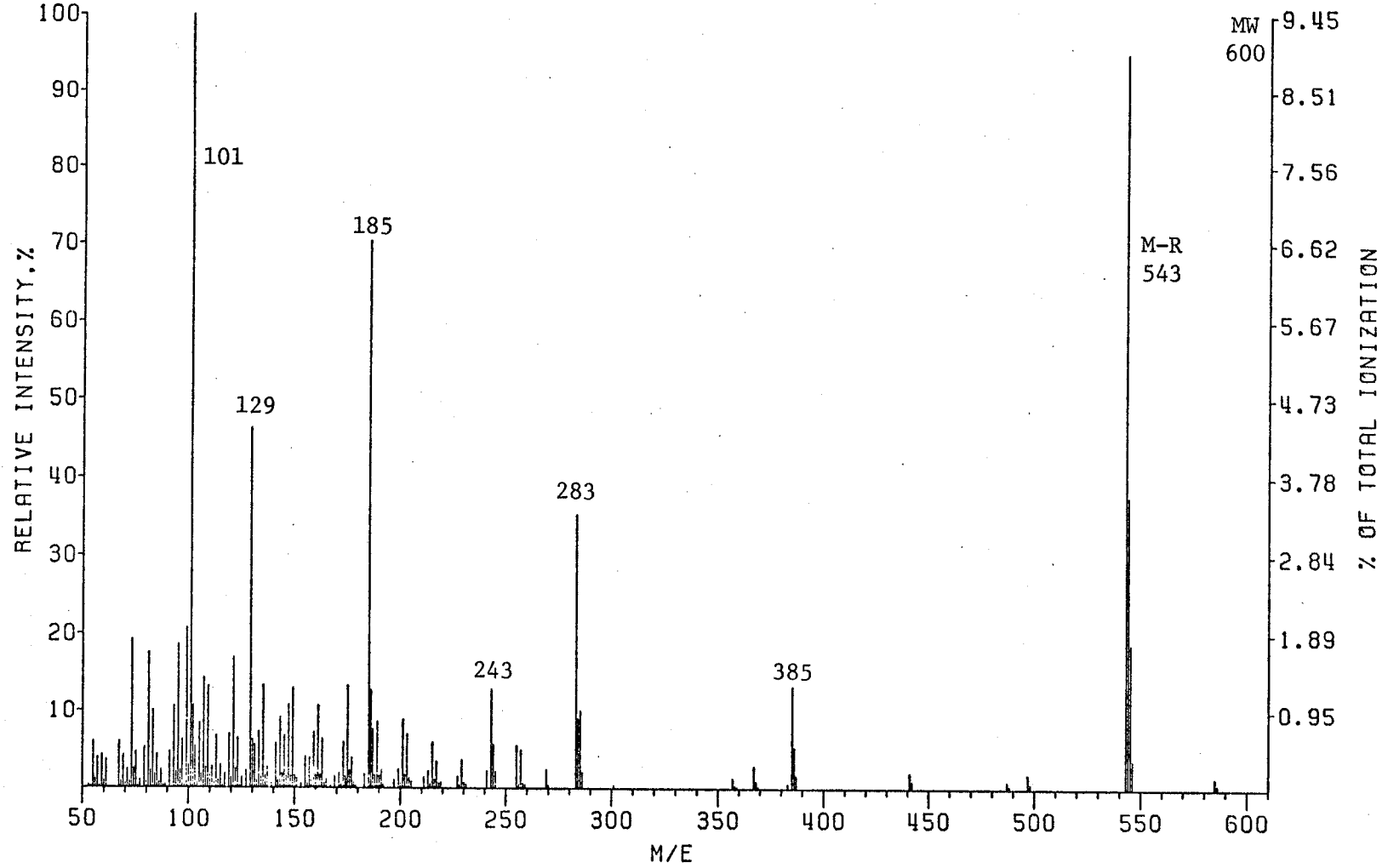


Figure 125. Mass spectrum of bis-TMTBSi-pregnanediol (XIXcc) recorded on Finnigan MS at 70 eV.

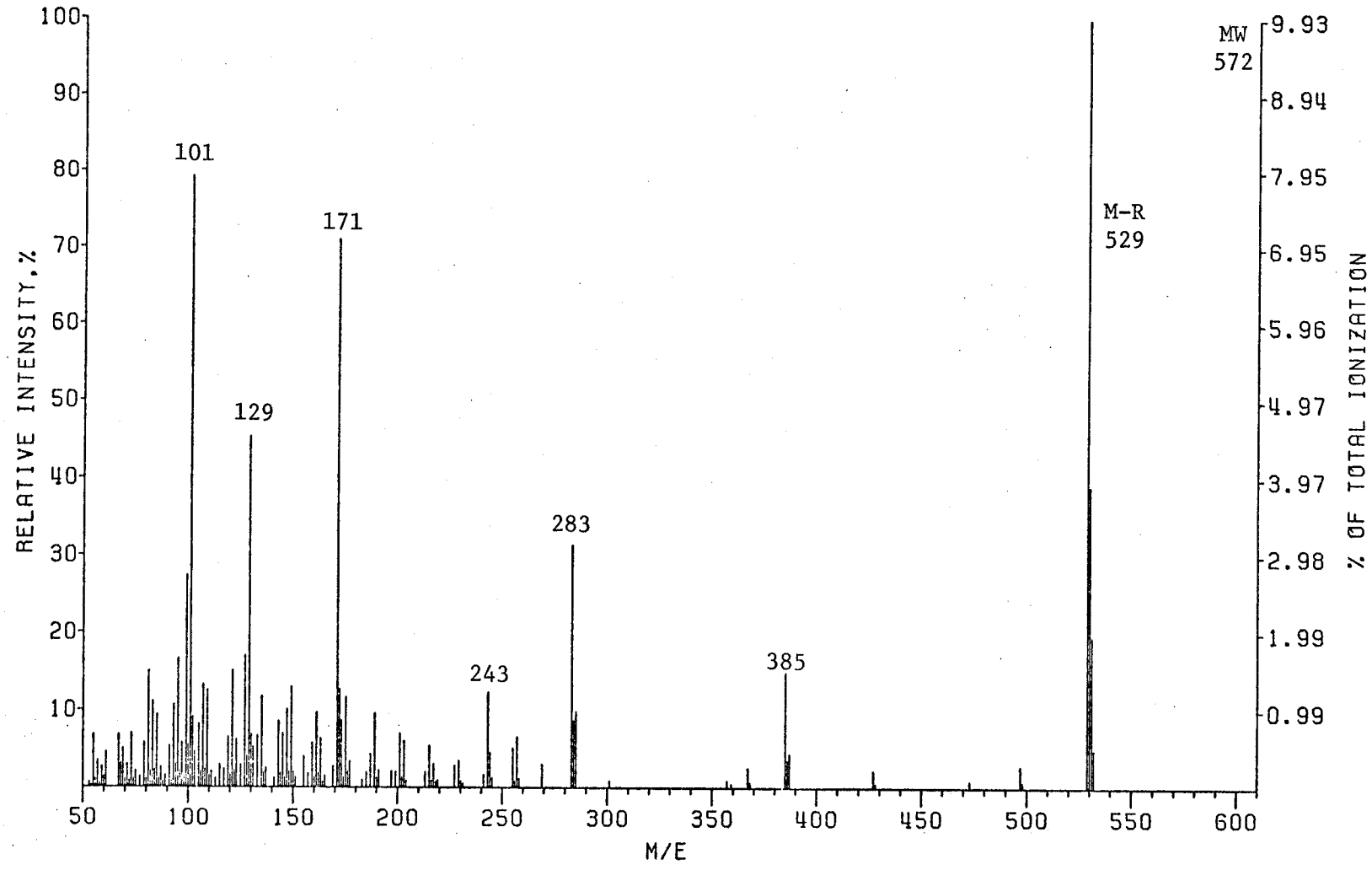


Figure 126. Mass spectrum of bis-TMIPSi-pregnanediol (XIXdd) recorded on Finnigan MS at 70 eV.

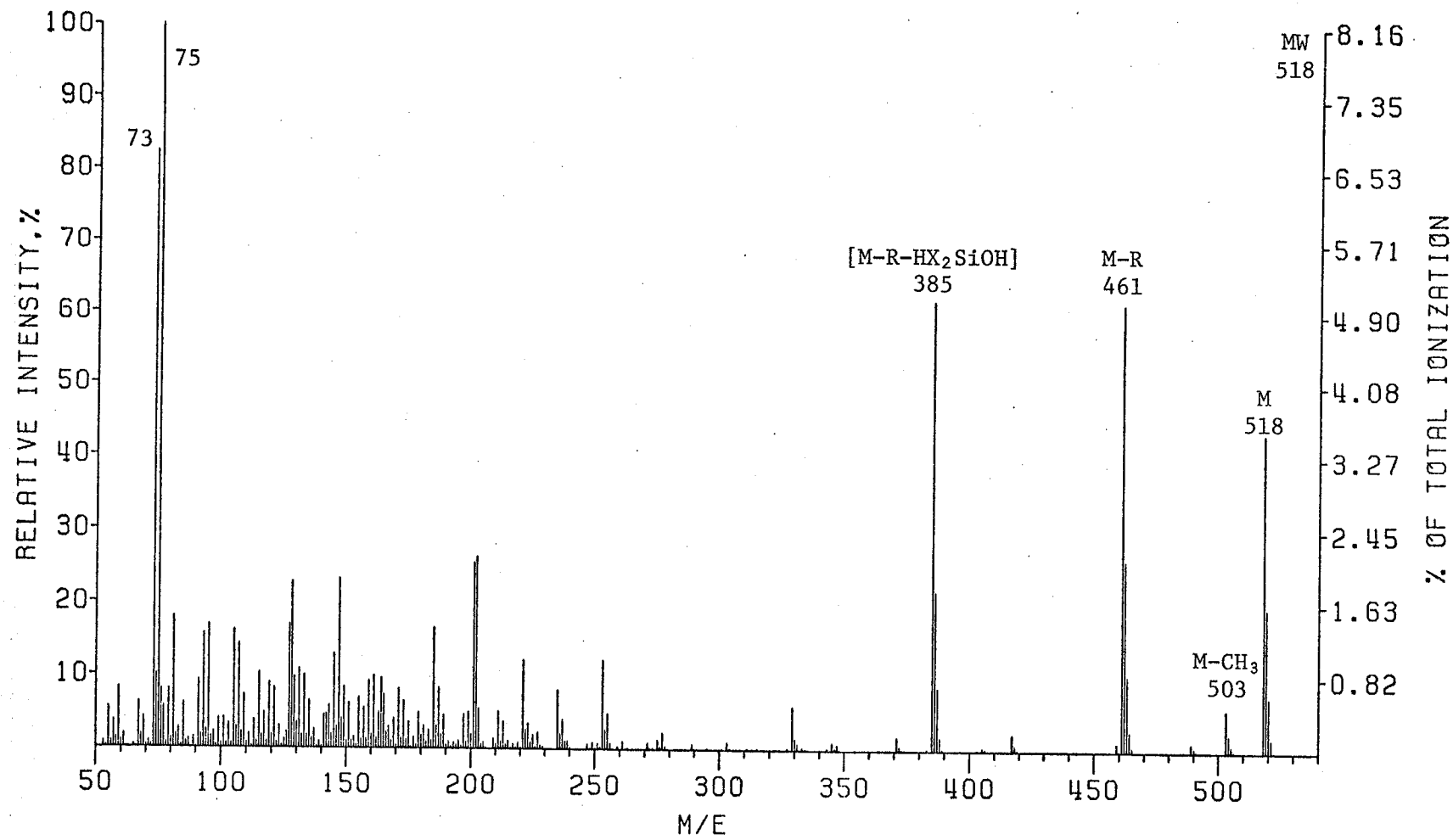


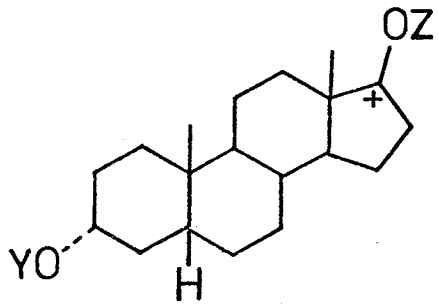
Figure 127. Mass spectrum of TBDMSi enol ether of XVb (XVIIIbb) recorded on Finnigan MS at 70 eV.

iii) 5β-Pregnane-3α,17α,20α-triol (XX)

This steroid was studied because of the analytical problems associated with the difficulty of silylation of highly hindered hydroxyl functions (176, 246, 247), and the interesting observations on the silylation and mass spectral behavior of 17α,20(α,β)-dihydoxy steroids (248). As discussed earlier, attempts to convert the 17α-hydroxyl of XX to its SCTASi ether were unsuccessful and only the 3α- and 20α-hydroxyls were silylated. Trimethylsilylation of these partial silyl derivatives gave the 17α-TMSi-3α,20α-bis-SCTASi ether derivatives (but in non-quantitative yield due to side reactions).

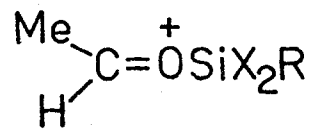
The spectra of the bis- and tris-silyl ether derivatives were useful in clarifying some of the observed fragmentations. Data are given in Table XXIX, and spectra of the derivatives XXeae, XXeee, XXbab, and XXbeb are illustrated in Figures 128 to 131.

In the spectra of all derivatives of XX, the ions in the high mass region are of low abundance, even for the SCTASi derivatives. Many of the ions correspond to losses of TMSiOH or RX_2SiOH from M^+ or $[M-R]^+$. A major fragmentation, which establishes the identity of the 20α-oxy function, involves cleavage of the 17-20 bond with abundant ions resulting from retention of the charge on either fragment, 67.1 or 67.2, as has been observed in related steroids (248, 259, 260). Ion 67.1, abbreviated to Q^+, is the ultimate precursor of many prominent ions formed, where applicable, by sequential elimination of H, H_2O, TMSiOH, RX_2SiOH or $Me_2Si{=}CH_2$. A metastable peak was earlier reported (248) for elimination of H from Q^+ of TMSi derivatives. Two major ion types which arise in this are observed at m/e 273 and 255. For the latter, which is the base peak in several of the spectra, a 1,3 axial elimination of ZOH with



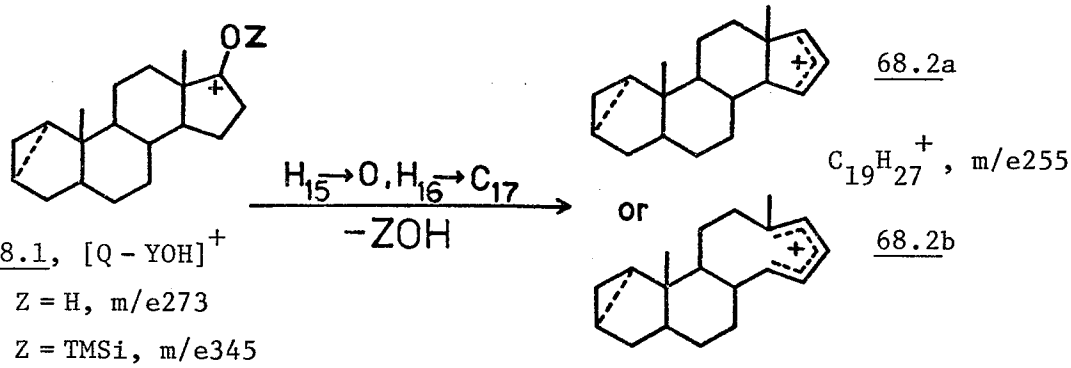
67.1, Q^+ Z = H, TMSi

Y = TMSi, TBDMSi, TMTBSi



67.2

Scheme 67.



68.1, $[Q - YOH]^+$

Z = H, m/e273

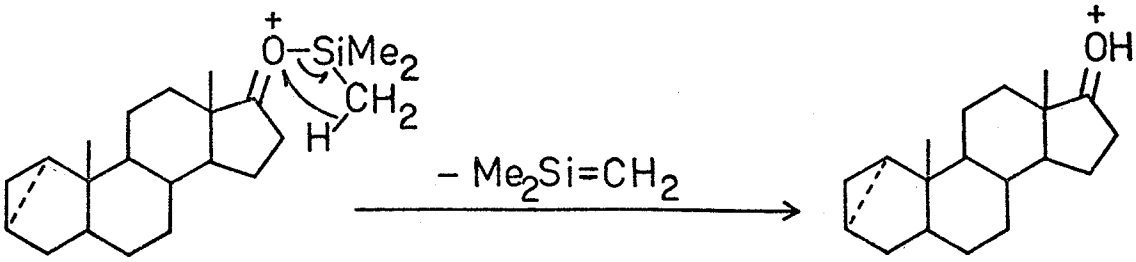
Z = TMSi, m/e345

68.2a

$C_{19}H_{27}^+$, m/e255

68.2b

Scheme 68.



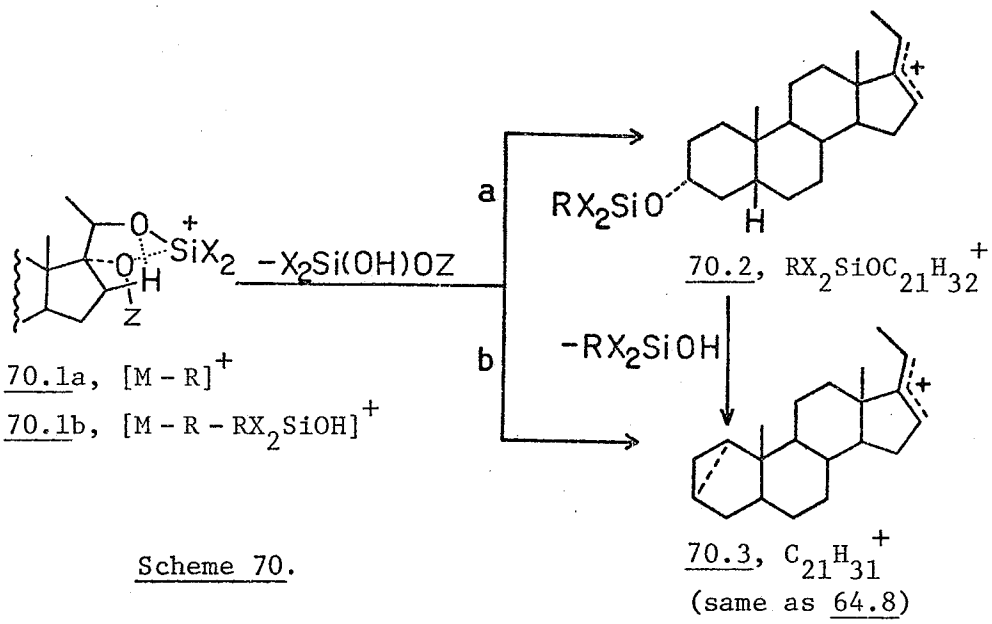
69.1, $[Q - YOH]^+$

69.2, $C_{19}H_{28}OH^+$

Scheme 69.

a concomitant 1,2 H shift is proposed, 68.1 → 68.2(a or b), to give a stable carbonium ion. For the formation of m/e 273 from a 17 silyl ether derivative, elimination of $Me_2Si{=}CH_2$ is proposed (Scheme 69).

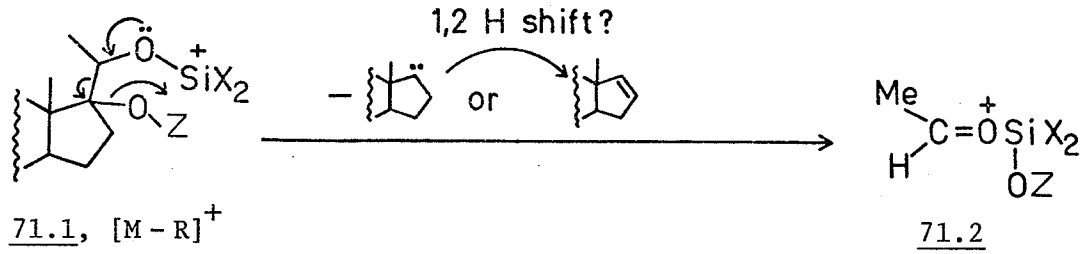
The 17α and 20α silyloxy functions are close enough to interact. A siliconium ion, once formed by loss of R, has a great tendency to interact with accessible electron-dense sites, such as the lone pair of an oxygen atom. A number of important ions arise in this way. Thus, stable allyl carbonium ions 70.2 and 70.3 could be formed via a muti-centered



Scheme 70.

transition state 70.1 (a or b) in which the 17α-oxy function is transferred to Si and H_{16} is transferred to O_{20} with elimination of $X_2Si(OH)OZ$. A second ion type owes its genesis to 71.1 → 71.2, in which the 17α-oxy function migrates to silicon. Other high intensity ions are $RX_2SiO{=}SiMe_2^+$ and $Me_3SiO{=}SiX_2^+$, which are typical of many compounds containing silyloxy groups close enough to interact (see Chapter IV). The former ion is of much lower abundance than the latter because the tBu radical is lost more readily than $CH_3\cdot$ Furthermore, we have observed that $tBuMe_2SiO{=}SiMe_2^+$

decomposes by elimination of C_3H_6 to give $Me_3SiO{=}SiMe_2^+$.



71.1, $[M-R]^+$ 71.2

Scheme 71.

TABLE XXIX. Mass spectral data for partial and mixed silyl derivatives of 5β-pregnane-3α,17α,20α-triol.

3α,20α-substituents:	TMSiO	TBDMSiO	TMTBSiO	TMSiO	TBDMSiO	TMTBSiO
17α-substituent:	HO	HO	HO	TMSiO	TMSiO	TMSiO
Ion type[a] # :	XXeae	XXbab	XXcac	XXeee	XXbeb	XXcec
M^+	480/1.8	564/0.2	616/0.60	552/3.2	636/1.8	688/0.28
$(M-CH_3)^+$	465/0.5	549/-	601/0.15	537/1.6	621/0.70	673/0.28
$(M-R)^+$	NA	507/2.1	559/5.3	NA	579/4.9	631/2.3
$RX_2SiOC_{21}H_{32}^+$, 70.2	NA	415/1.6	441/2.9	NA	415/-	441/-
$(M-R-RX_2SiOH)^+$	NA	375/2.1	401/1.0	NA	447/-	473/0.19
Q^+, 67.1	363/10.5	405/0.96	431/0.31	435/62	477/64	503/100
$(Q-H)^+$	362/14.2	404/1.1	430/0.47	434/-	476/-	502/-
$C_{21}H_{31}^+$, 70.3	283/4.1	283/37	283/22	283/4.5	283/19.3	283/21
$(Q-H_2O)^+$	345/3.5	387/2.5	413/1.7	NA	NA	NA
$(Q-H-R)^+$	NA	347/4.7	373/0.86	NA	419/-	445/-
$(Q-TMSiOH)^+$	b	NA	NA	345/12.6	387/3.5	413/3.5
$(Q-RX_2SiOH)^+$	NA	b	b	NA	345/5.4	345/5.6
$C_{19}H_{28}OH^+$, 68.1 or 69.2	273/35	273/11.4	273/2.5	273/-	273/4.6	273/-
$(Q-H-TMSiOH)^+$	272/19.0	NA	NA	344/-	NA	NA
$C_{19}H_{27}^+$, 68.2	255/100	255/61	255/19.1	255/100	255/100	255/95
$RX_2SiOSiMe_2^+$	NA	NA	NA	NA	189/11.2	215/17.0
$Me_3SiOSiX_2^+$	NA	NA	NA	147/33	147/82	173/51

continued . . .

TABLE XXIX. (continued)

Ion type[a]	XXeae	XXbab	XXcac	XXeee	XXbeb	XXcec
$C_2H_4OSiX_2OZ^+$, 71.2	119/29	119/100	145/100	191/6.0	191/30	217/19.4
$RX_2SiOC_2H_4^+$, 67.2	NA	159/81	185/31	NA	159/25	185/15.0
$TMSiOC_2H_4^+$, 67.2	117/61	NA	NA	117/18.9	(117/6.5)[c]	NA
RX_2Si^+	NA	115/17.8	141/6.2	NA	115/10.9	141/7.4
X_2SiOH^+	NA	75/82	101/45	NA	75/39	101/40
Me_2SiOH^+	75/42	d	NA	75/17.9	d	75/11.6
Me_3Si^+	73/64	73/58	NA	73/51	73/65	73/58
Other ions	215/26	215/11.1		215/15.8	215/19.3	
Base peak as $\%\Sigma_{50}$	8.67	8.47	13.00	11.82	7.71	7.37

[a] $RX_2Si \equiv$ TBDMSi or TMTBSi

[b] Same as $C_{19}H_{28}OH^+$

[c] Possibly formed by rearrangement of 17α and 20α silyloxy groups (248).

[d] Same as X_2SiOH^+

NA = not applicable

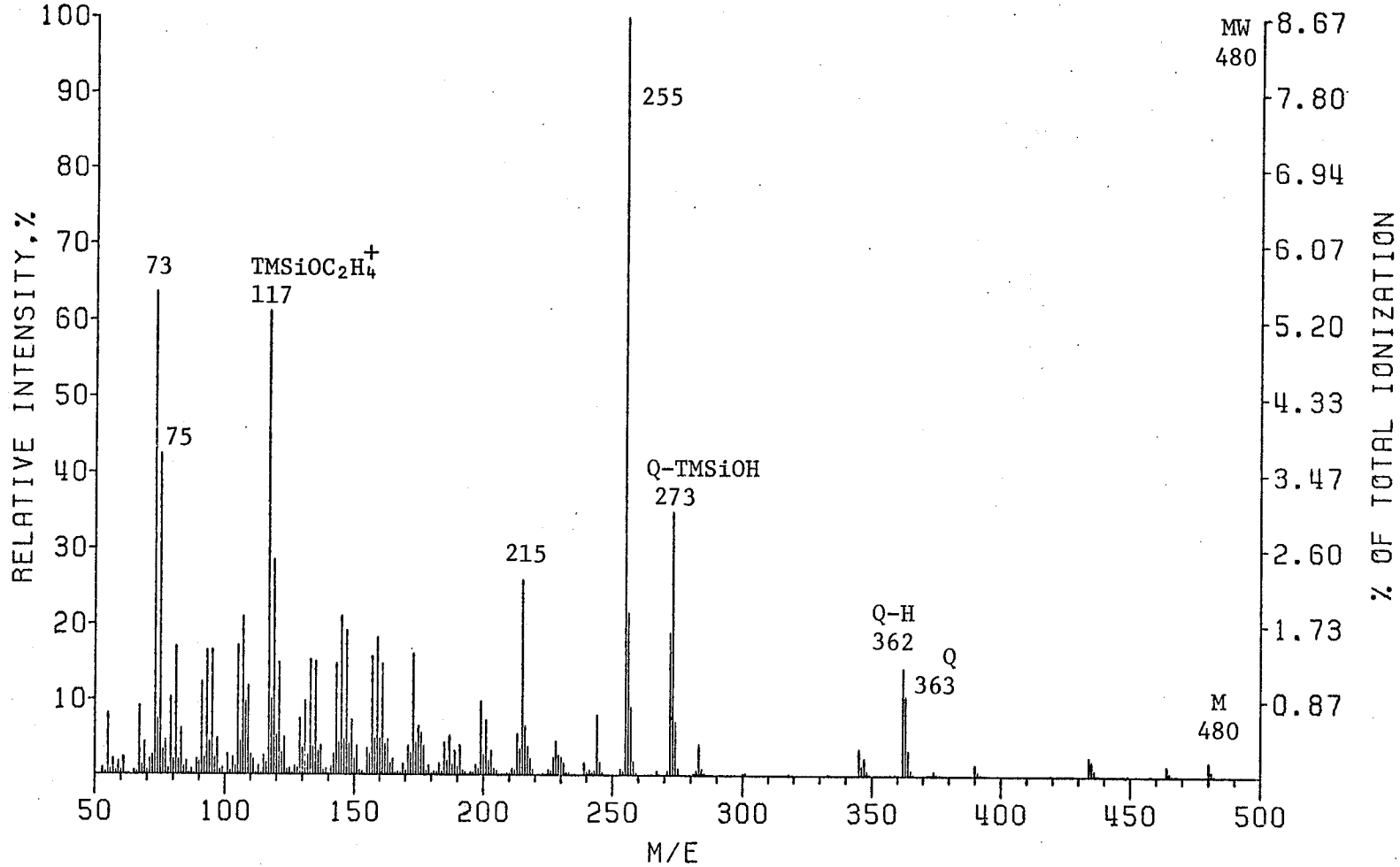


Figure 128. Mass spectrum of 3,20-bis-TMSi-pregnanetriol (XXeae) recorded on Finnigan MS at 70 eV.

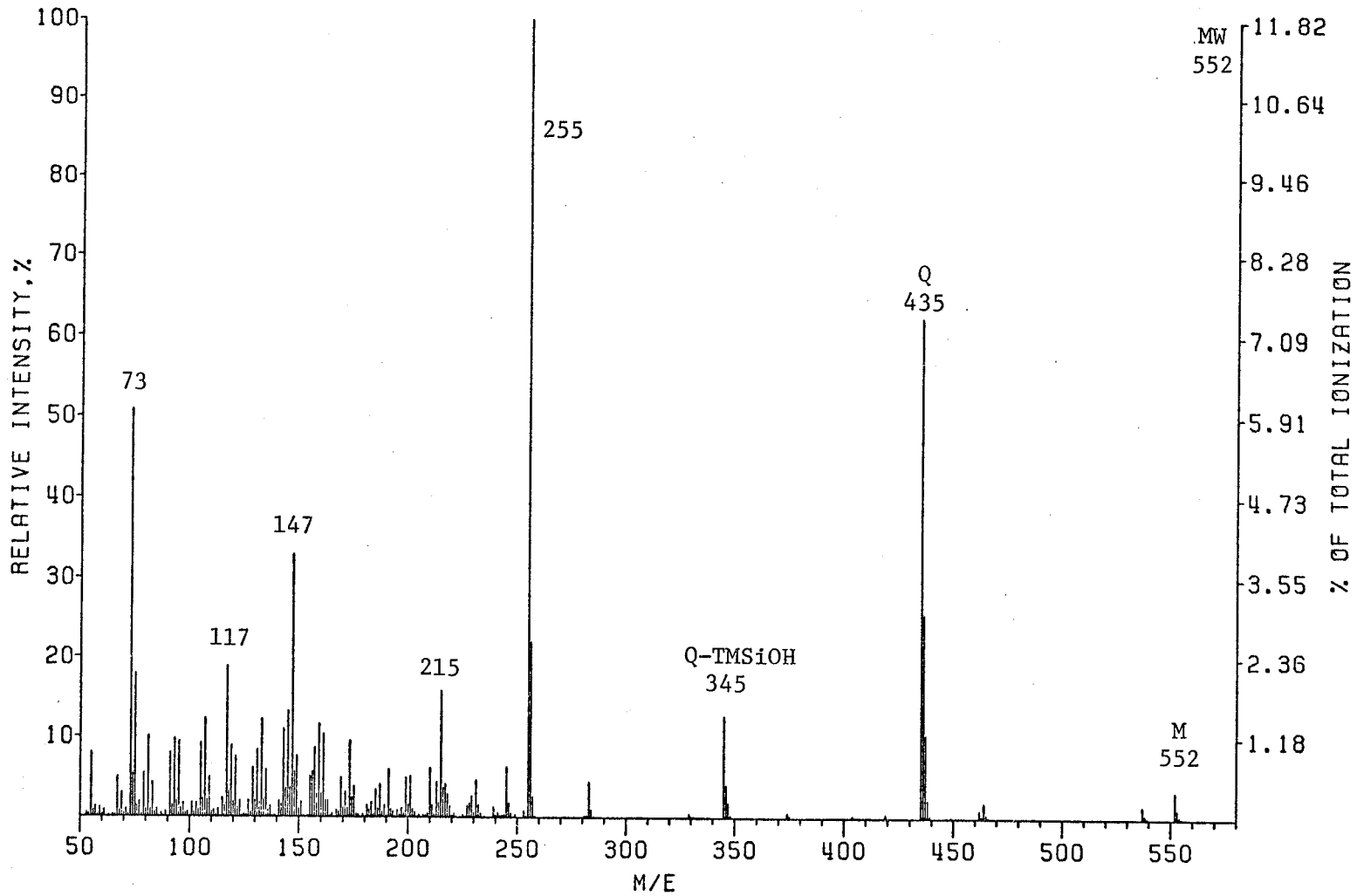


Figure 129. Mass spectrum of 3,17,20-tris-TMSi-pregnanetriol (XXeee) recorded on Finnigan MS at 70 eV.

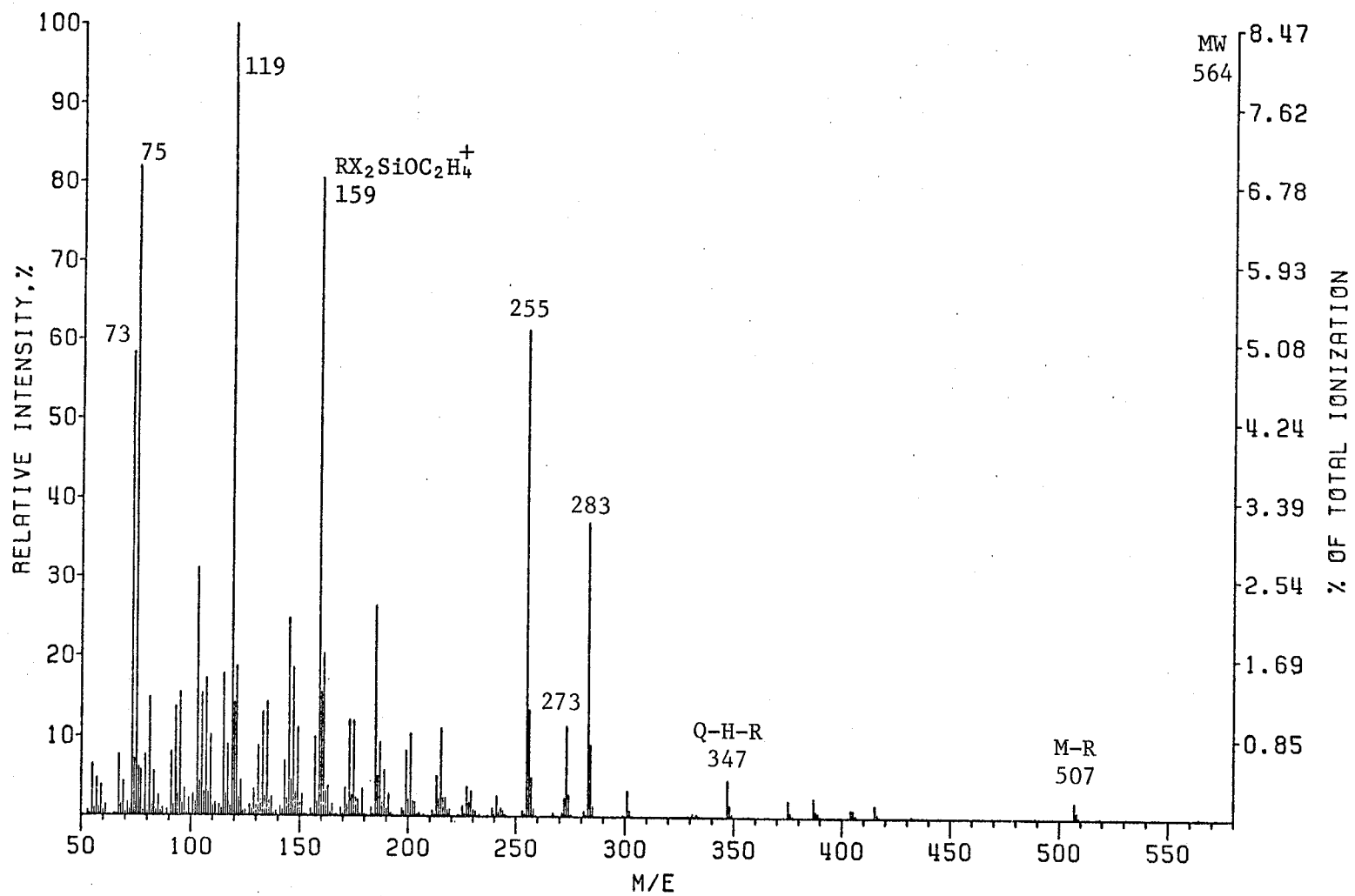


Figure 130. Mass spectrum of 3,20-bis-TBDMSi-pregnanetriol (XXbab) recorded on Finnigan MS at 70 eV.

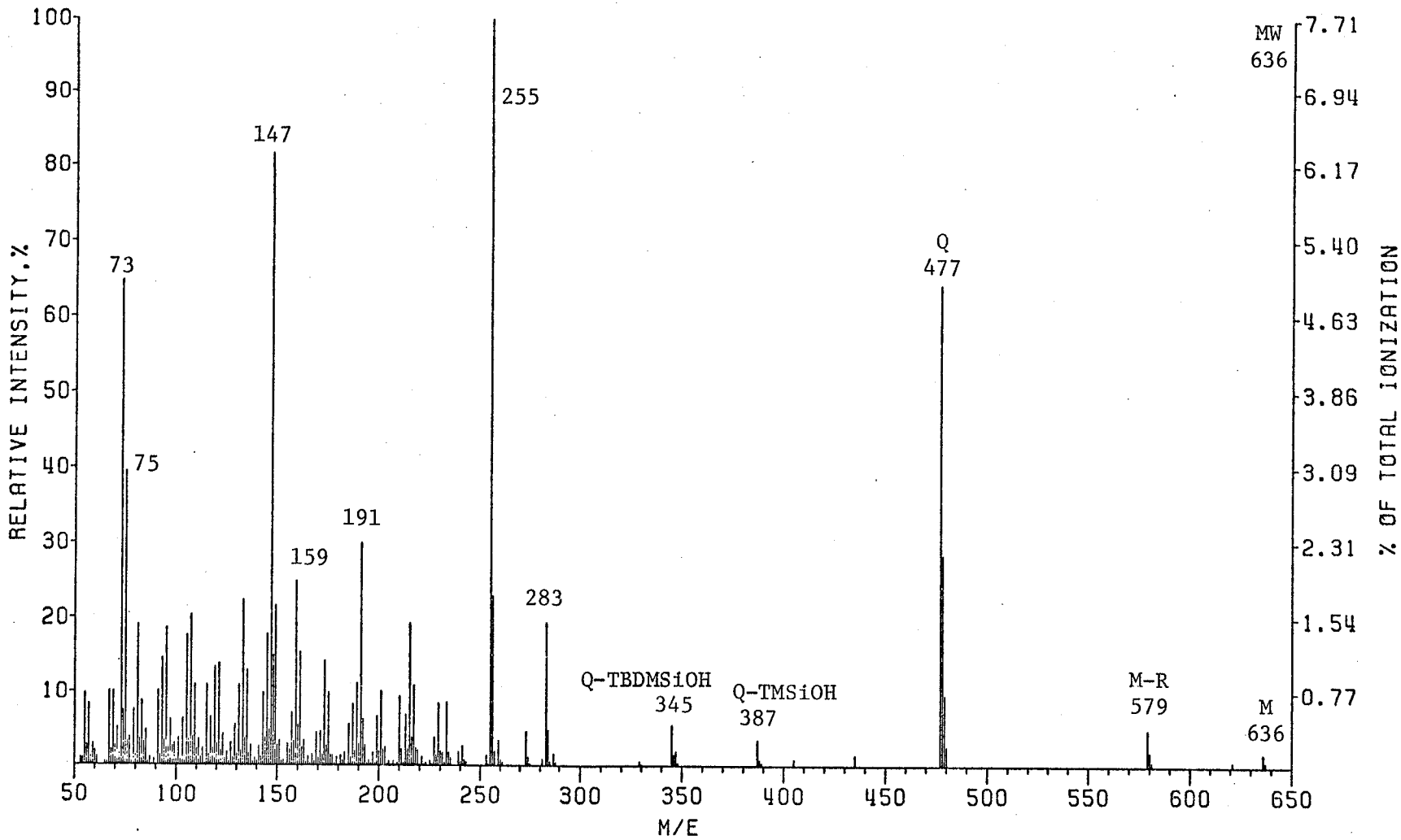


Figure 131. Mass spectrum of 17-TMSi-3,20-bis-TBDMSi-pregnanetriol (XXbeb) recorded on Finnigan MS at 70 eV.

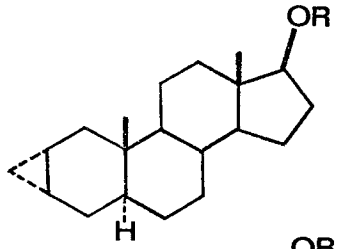
5. Application of SCTASi Derivatives to Steroid Metabolites

A number of metabolites of 2α,3α-cyclopropano-5α-androstan-17β-ol (XXIII) were earlier identified in the crude neutral steroid fraction from β-glucuronidase hydrolysis of the urine from orally dosed rabbits (261, 262). The steroidal materials were extracted into ether, purified by column chromatography and crystallization, and identified by elemental analysis, IR, NMR and MS. Of the various products, XXIV-XXVIII (see Scheme 72) were the major components, as estimated by GC. A simplification of the analysis was achieved by eliminating epimeric hydroxyl isomers via Jones oxidation, and subsequent GC determination of the resulting ketones. A simpler and more accurate method of quantitative analysis would normally be by GC of TMSi derivatives. Trimethylsilylation of the crude extract of the rabbit urine and analysis by GC on OV-1 and OV-17 columns gave the chromatograms shown in Figures 132a and 132b, respectively. The OV-17 column gave the better separation because the more polar ketosilyloxysteroids are retarded with respect to silyloxysteroids. However, two positionally isomeric diols, XXIV and XXV, were inseparable as TMSi ethers on either column, although both do separate from XXVI, the epimer of XXIV. Higher resolution columns did not yield separation of XXIV and XXV but did help in other parts of the chromatogram.

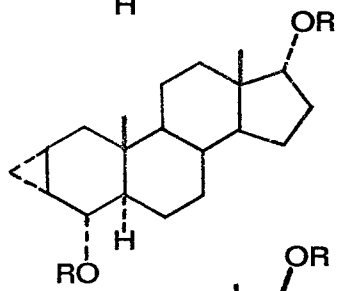
Preparation of the TBDMSi derivatives of the steroid metabolites in the crude extract resulted in the chromatograms in Figures 133b and 134, with peak identities assigned by comparison with derivatives of isolated compounds and checked by MS. Almost complete separations of XXIV and XXV were achieved on both of the short columns used. The OV-1 column (Figures 133b and 134a) gave the greater number of resolved peaks and a better overall separation than that of the TMSi derivatives on

Scheme 72

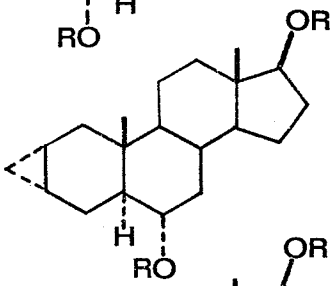
Substituents: (R) a = H ; b = TBDMSi ; c = TMTBSi ;
d = TMIPSi ; e = TMSi



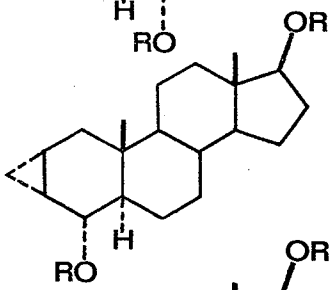
#[a]	Parent Compound
XXIII_r	2α,3α-cyclopropano-5α-androstan-17β-ol



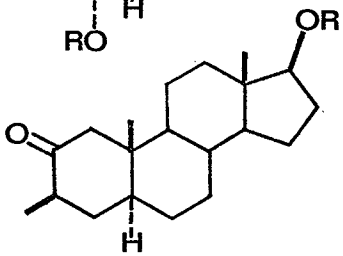
XXIV_r	2α,3α-cyclopropano-5α-androstan-4α,17α-diol



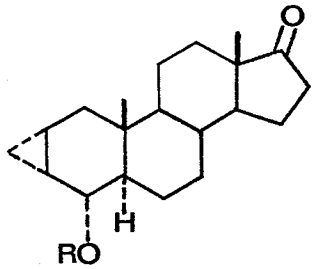
XXV_r	2α,3α-cyclopropano-5α-androstan-6α,17β-diol



XXVI_r	2α,3α-cyclopropano-5α-androstan-4α,17β-diol



XXVII_r	17β-hydroxy-3β-methyl-5α-androstan-2-one



XXVIII_r	4α-hydroxy-2α,3α-cyclopropano-5α-androstan-17-one

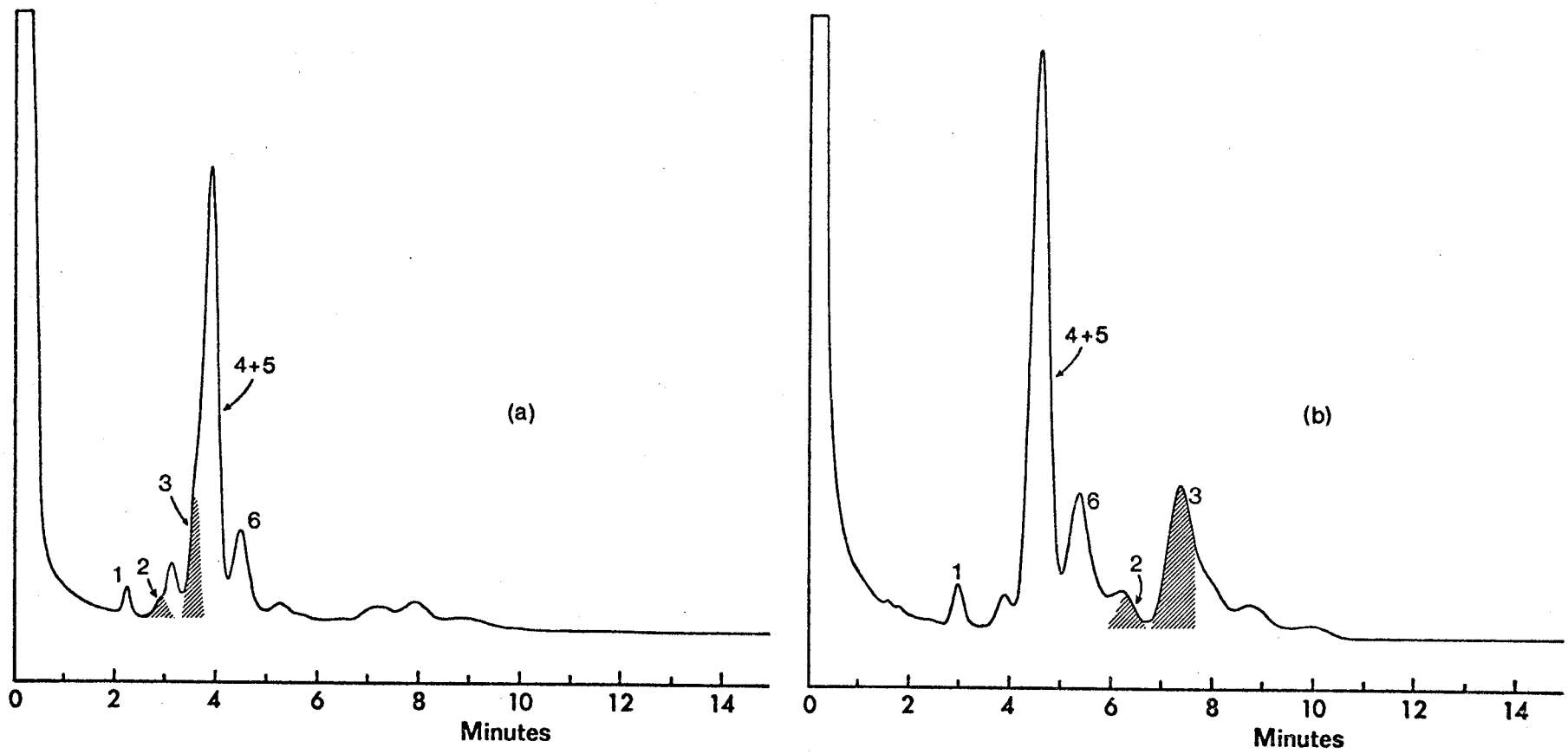


Figure 132. Gas chromatograms of trimethylsilylated crude extract of steroid metabolites: (a) column A (10% OV-1, 1m x 2mm-ID), 230°; (b) column B (10% OV-17, 1m x 2mm-ID), 230°. Ketonic compounds are denoted by cross-hatching. Peak identity (underivatized steroid):

1 = XXIII	4 = XXIV (4α,17α-diol)
2 = XXVII	5 = XXV (6α,17β-diol)
3 = XXVIII	6 = XXVI (4α,17β-diol)

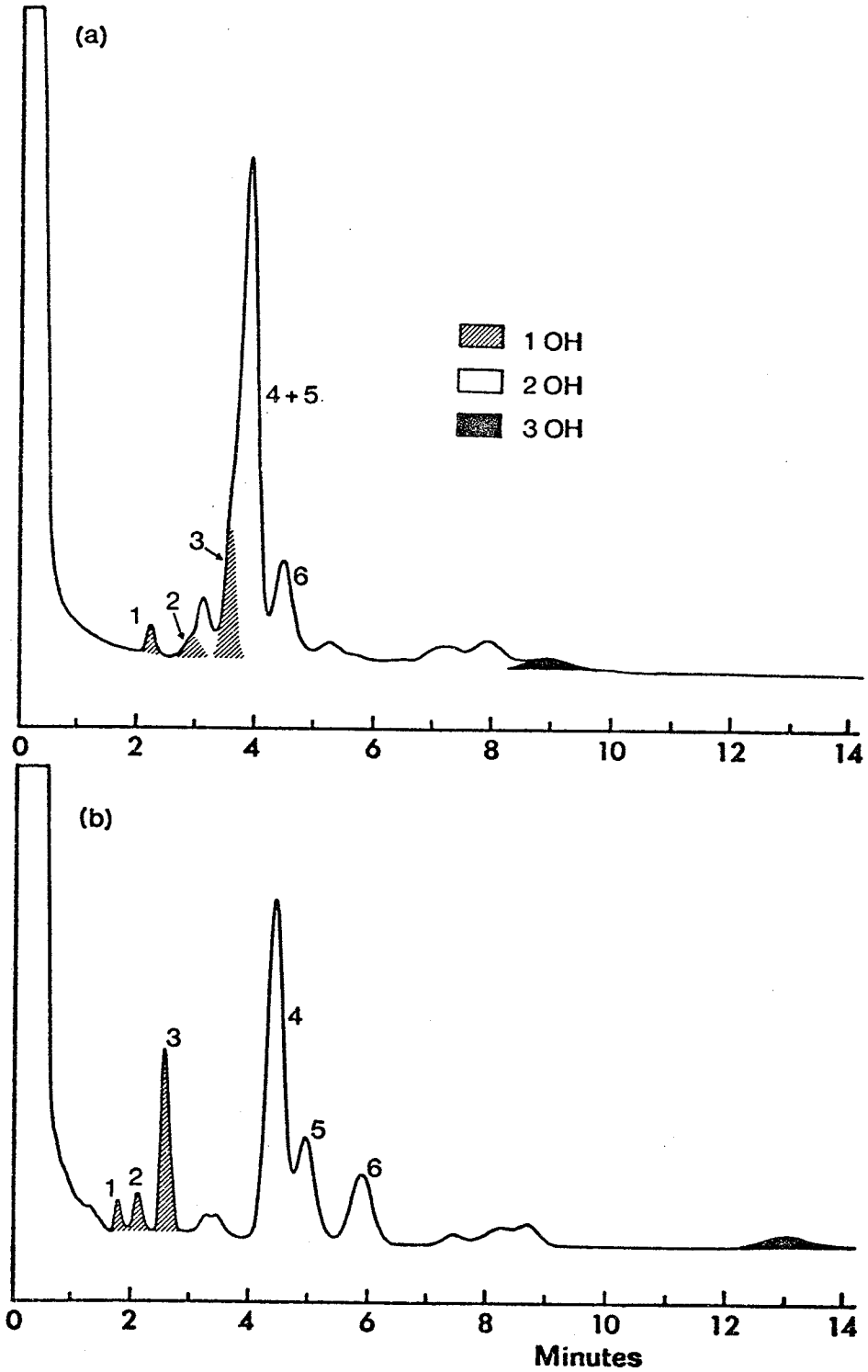


Figure 133. The influence of the type of silyl group upon the GC of crude extract of steroid metabolites: (a) TMSi derivatives on column A (10% OV-1) at 230°; (b) TBDMSi derivatives on same column at 260°. Shading indicates the number of hydroxyls present in each compound (determined by δI values, MS, and structural elucidation by other techniques). Peak identities the same as in Figure 132.

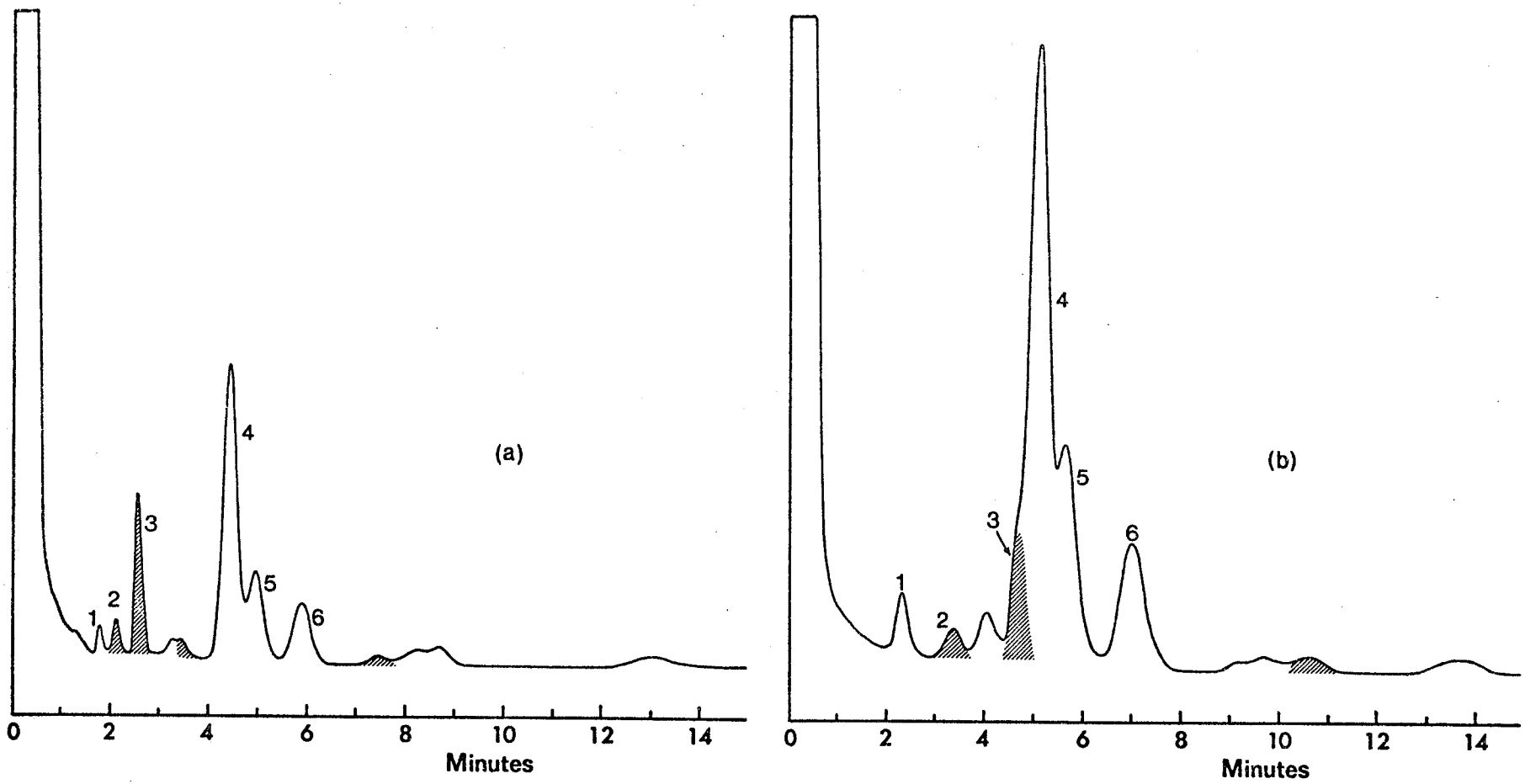


Figure 134. Gas chromatograms of TBDMSi derivatized crude extract of steroid metabolites: (a) column A (10% OV-1, 1m x 2mm-ID), 260°; (b) column B (10% OV-17, 1m x 2mm-ID), 260°. Ketonic compounds are denoted by cross-hatching. Peak identities are the same as in Figure 132.

either column. Retention indices were, of course, increased relative to TMSi ethers but the greater retention increments for alcohol to TBDMSi ether conversion allowed better separation of mono-, di- and tri-silyloxy-steroids (compare Figures 133a and 133b). On the OV-1 phase the three diols were shifted to a region of the chromatogram apparently free from other substances. However, on the OV-17 phase (Figure 134b) the more polar ketosilyloxysteroid, XXVIII, was sufficiently retarded that it interfered with the derivative of XXIV. Retention indices, reported for all derivatives in Table XXX, are very useful in supporting the assigned structures. Retention increments in changing either the column ($\Delta I_{OV17-OV1}$) or the derivative ($\delta I_{TBDMSi-TMSi}$) indicate the presence or absence of ketone functions and the number of silyl groups, respectively. ($\Delta I = 30-150$ for silyloxysteroids, $\Delta I = 200-300$ for ketosilyloxysteroids; $\delta I = 300$, 550 and 700 for one, two and three silyl groups respectively.)

In this study it was also possible to demonstrate the usefulness of SCTASi derivatives for TLC-GC separation schemes. Using cyclohexane/ethyl acetate (9:1) as a developing solvent, the TBDMSi derivatized extract was subjected to TLC. Figure 135b shows the GC analysis on OV-1 of the non-polar fraction isolated by elution of a band at $R_f = 0.55$ to 0.65 clearly showing the absence of the more polar ketosteroids, which were contained in a TLC band at $R_f = 0.40$ to 0.50. This "group separation" is also possible with TMSi derivatives, but due to the susceptibility of these derivatives to hydrolysis on handling it is usually necessary to resilylate the eluted material prior to further analysis by GC (25). The TBDMSi derivatives, on the other hand, are recovered without any decomposition, allowing direct analysis of the eluate from the silica gel.

To further investigate isomer separations achieved by the change

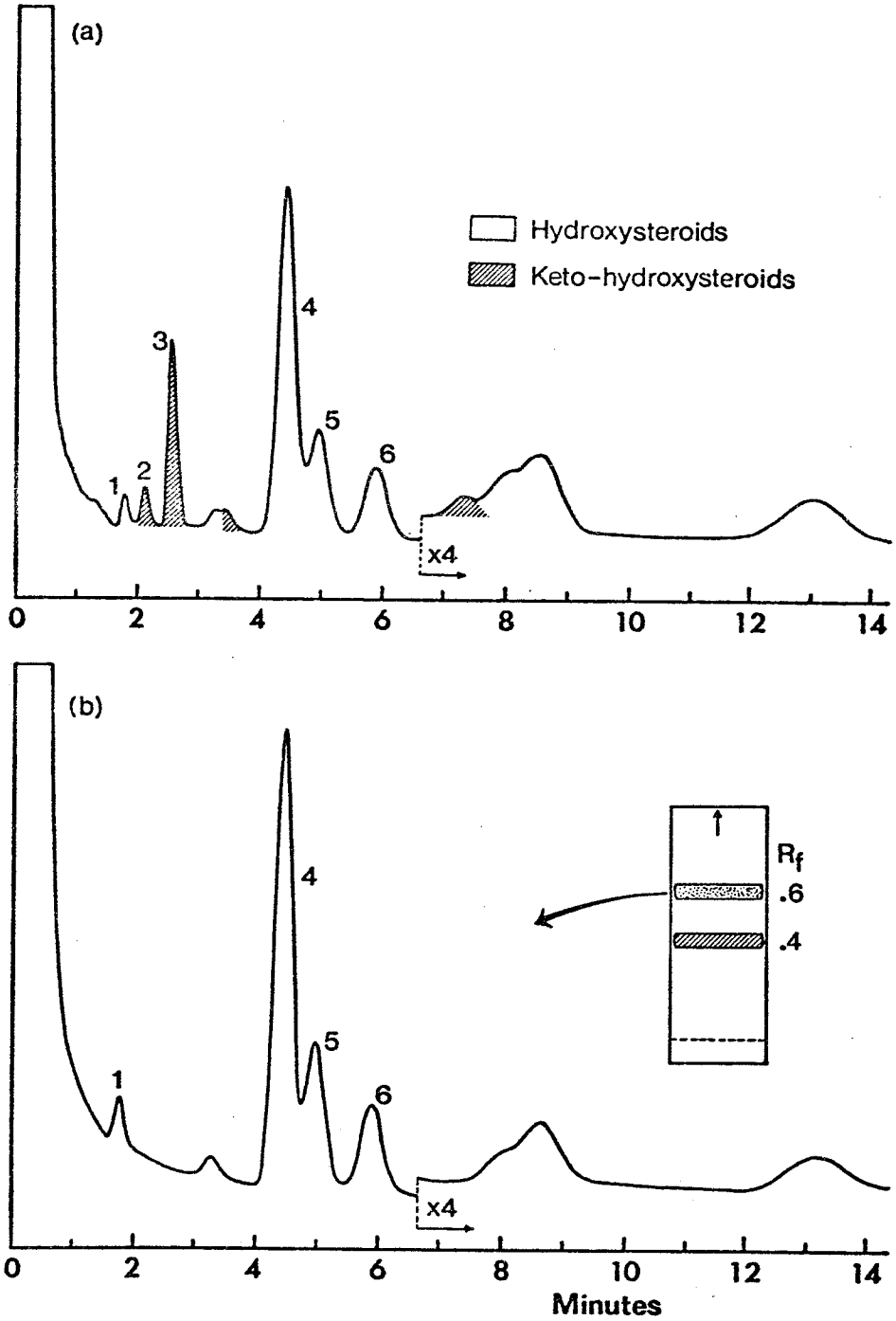


Figure 135. Gas chromatograms of (a) TBDMSi derivatized crude extract of steroid metabolites on column A (10% OV-1, 1m x 2mm-ID) at 260°; (b) non-ketonic fraction of TBDMSi derivatives of metabolites isolated by TLC (R_f = 0.55 to 0.65), with same GC conditions. Peak identities the same as in Figure 132.

TABLE XXX. Chromatographic data for steroid metabolite derivatives.

Parent Compound[a] Name	O-Silyl[b] Derivative	Comp'd[a] No.	R_f TLC[c]	GC DATA[d] I_{OV-1} 230°	I_{OV-1} 260°	I_{OV-1} 280°	I_{OV-17} 230°	I_{OV-17} 260°	I_{OV-17} 280°	$\Delta I_{OV17-OV1}$[e]
2α,3α-cyclopropano-5α-androstan-17β-ol										
	TMSi	XXIIIe	0.62	2464			2598			134 (230°)
	TBDMSi	XXIIIb	0.62		2780			2919		139 (260°)
2α,3α-cyclopropano-5α-androstan-4α,17α-diol										
	TMSi	XXIVe	0.59	2651			2731			80 (230°)
	TBDMSi	XXIVb	0.59		3154	3179		3218		64 (260°)
	TMIPSi	XXIVd	0.59			3735			3928	193 (280°)
	TMTBSi	XXIVc	0.59			3790			3952	162 (280°)
2α,3α-cyclopropano-5α-androstan-6α,17β-diol										
	TMSi	XXVe	0.61	2654			2732			78 (230°)
	TBDMSi	XXVb	0.61		3201	3229		3260		59 (260°)
	TMIPSi	XXVd	0.61			3761			3944	183 (280°)
	TMTBSi	XXVc	0.61			3838			3986	148 (280°)
2α,3α-cyclopropano-5α-androstan-4α,17β-diol										
	TMSi	XXVIe	0.58	2699			2787			88 (230°)
	TBDMSi	XXVIb	0.58		3269	3303		3337		68 (260°)
	TMIPSi	XXVIb	0.58			3846			4044	198 (280°)
	TMTBSi	XXVIc	0.58			3930			4095	165 (280°)

continued...

TABLE XXX (continued)

Derivative	#	R_f TLC	I_{OV-1} 230°	I_{OV-1} 260°	I_{OV-1} 280°	I_{OV-17} 230°	I_{OV-17} 260°	I_{OV-17} 280°	$\Delta I_{OV17-OV1}$ [e]
17β-hydroxy-3β-methyl-5α-androstan-2-one									
TMSi	XXVIIe	0.42	2550			2835			285 (230°)
TBDMSi	XXVIIb	0.42		2849			3049		200 (260°)
4α-hydroxy-2α,3α-cyclopropano-5α-androstan-17-one									
TMSi	XXVIIIe	0.43	2630			2888			258 (230°)
TBDMSi	XXVIIIb	0.43		2930			3190		260 (260°)

[a] See Scheme 72 for structures and numbering system.

[b] No mixed derivatives used; refer to compound number to determine the number of silyl groups.

[c] TLC development solvent: cyclohexane/ethyl acetate (9:1,v/v).

[d] GC data expressed as Kovats' retention indices.

[e] Temperature given in parentheses.

TABLE XXXI. Separation factors (α) for isomer pairs.

Derivative[a]	Col.Temp.	OV-1		OV-17	
		XXV/XXIV	XXVI/XXIV	XXV/XXIV	XXVI/XXIV
TMSi	230°	1.008	1.152	1.002	1.188
TMIPSi	280°	1.059	1.280	1.039	1.320
TBDMSi	260°	1.124	1.335	1.119	1.373
TMTBSi	280°	1.112	1.367	1.083	1.406

[a] Arranged in order of increasing steric crowding around silicon.

of silyl groups, the separation factors for the derivatives of three isomeric diols, XXIV-XXVI, were measured for each of the four silyl groups: TMSi, TMIPSi, TBDMSi and TMTBSi. The results in Table XXXI, expressed as separation factors for isomer pairs, imply that, up to a point, separation of isomers increases with increasing steric crowding around silicon. Possibly, as proposed earlier for other steroid isomers, the bulkier (sterically) that the silyl group is, the more the steroids are forced into conformations which accentuate the differences between isomers. In the case of the epimers, XXIV and XXVI, the best separation was achieved with the most sterically crowded TMTBSi derivatives. For the more difficult separation between XXIV and XXV, TBDMSi derivatives gave the best separation.

The mass spectra of the silyl derivatives of the diol metabolites XXIV-XXVI were studied in order to assess their effectiveness in distinguishing between positional isomers and between epimers. The mass spectra of the TMSi and TBDMSi ethers are shown in Figures 136-141, with a summary of the more important ions for all derivatives presented in Table XXXII. The epimers XXIV and XXVI are virtually indistinguishable by the mass spectra of their TMSi ethers, while differences in the spectra of their SCTASi ethers are observed. These are mainly variations in the intensities of certain ion types. The isomer XXV has mass spectra readily distinguishable from those of XXIV or XXVI as either the TMSi or SCTASi ether derivatives.

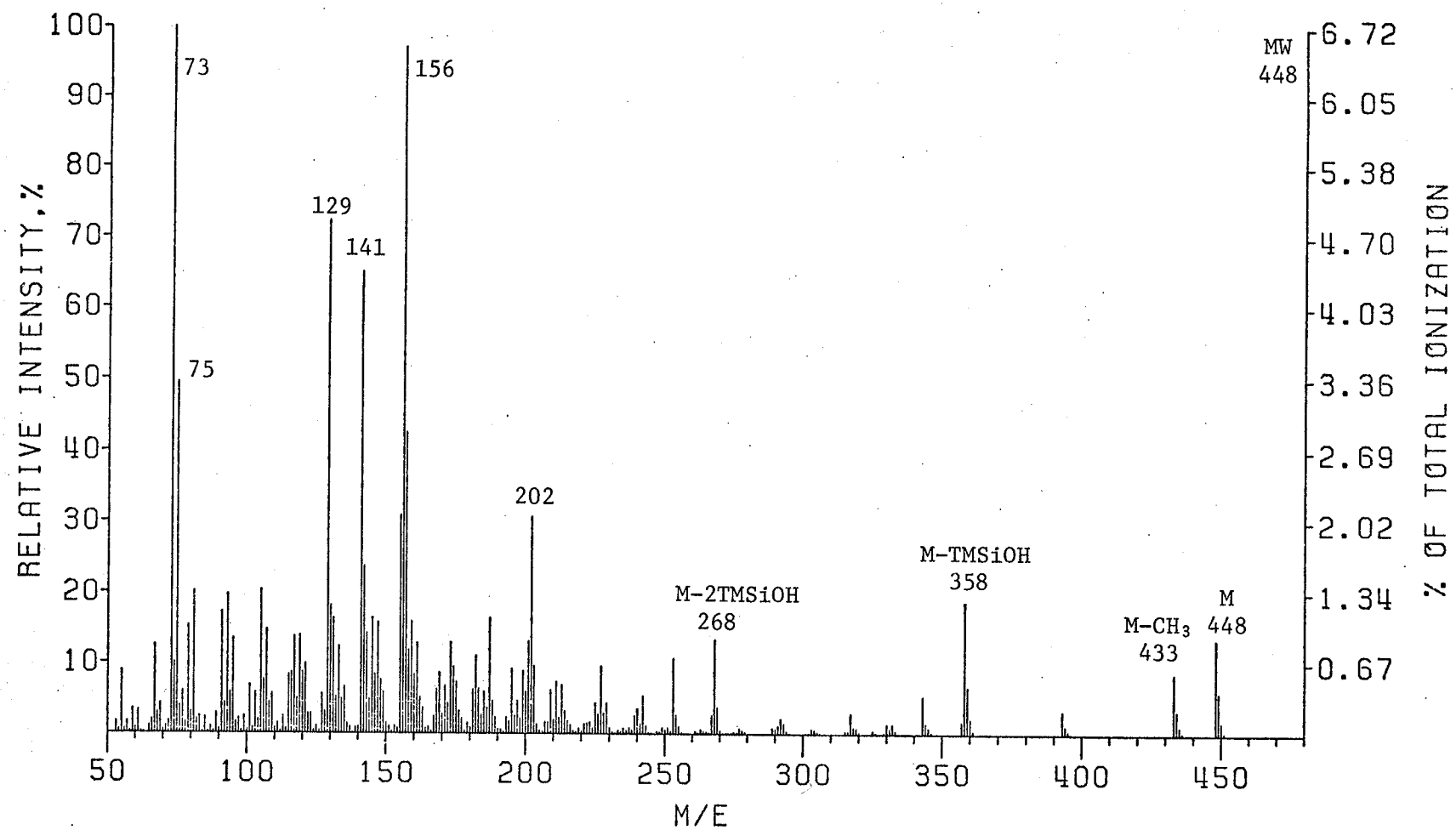


Figure 136. Mass spectrum of TMSi derivative of the 4α,17α-diol metabolite (XXIVe) recorded on Finnigan MS at 70 eV.

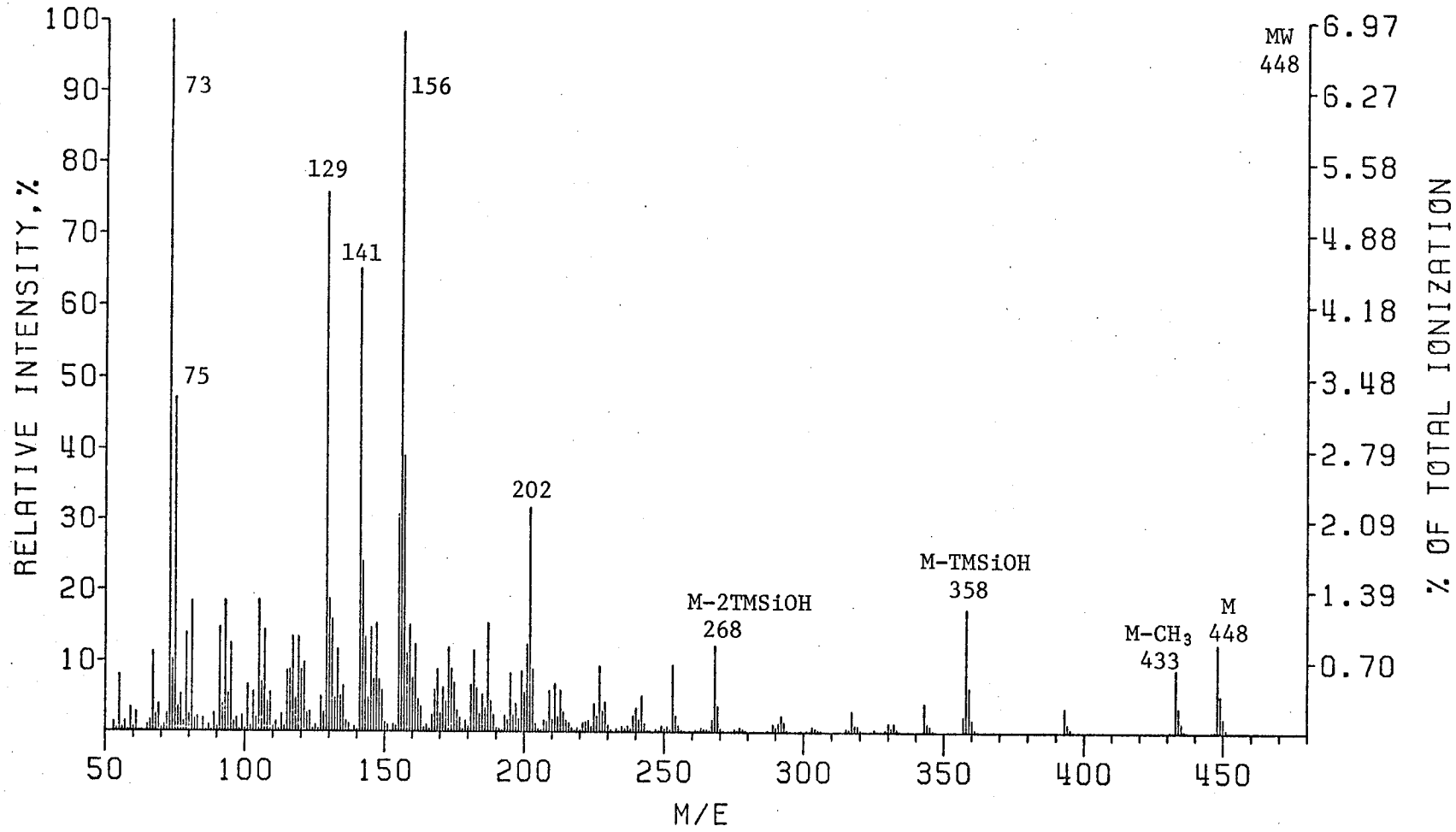


Figure 137. Mass spectrum of TMSi derivative of the 4α,17β-diol metabolite (XXVIe) recorded on Finnigan MS at 70 eV.

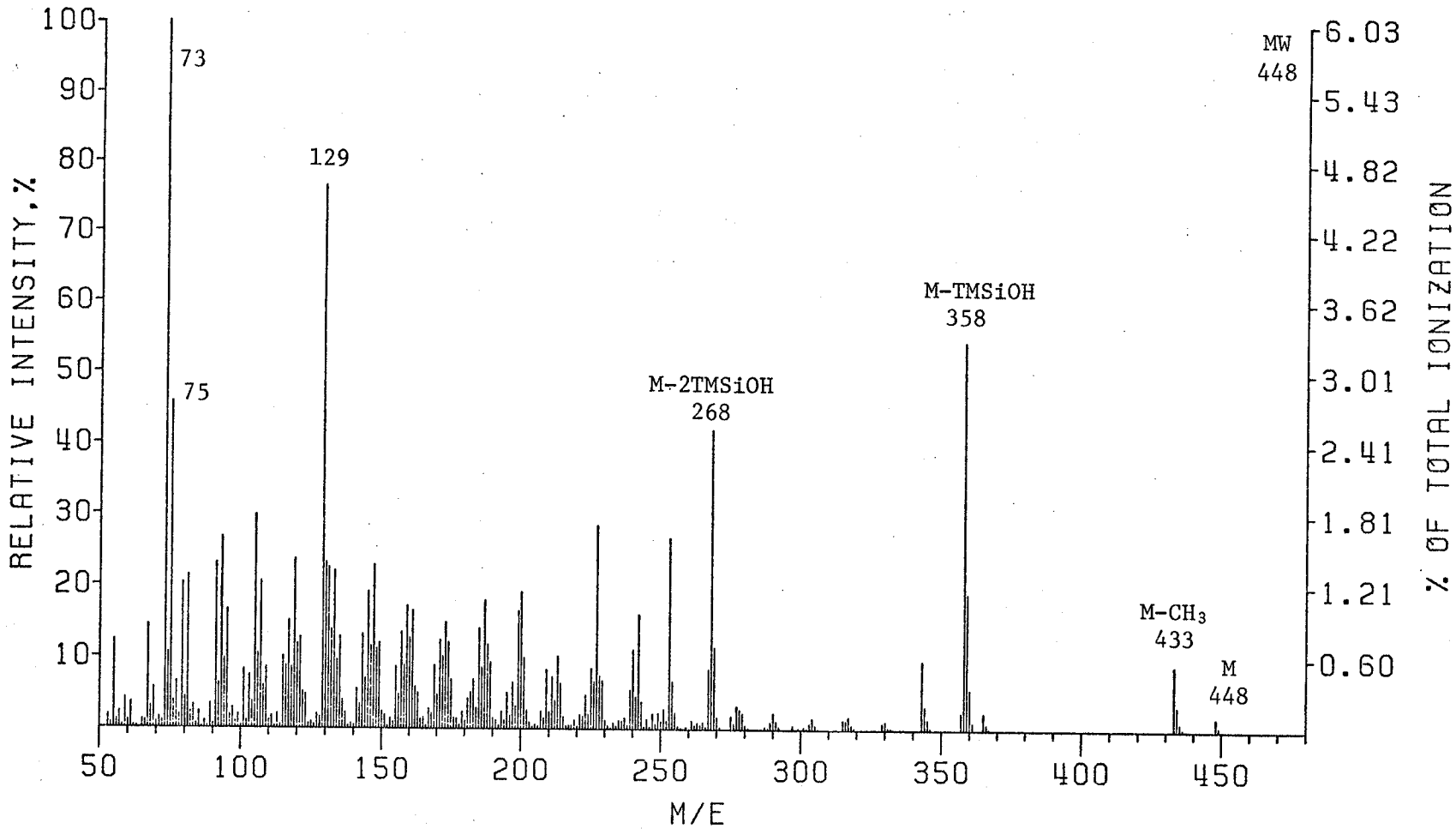


Figure 138. Mass spectrum of TMSi derivative of the 6α,17β-diol metabolite (XXVe) recorded on Finnigan MS at 70 eV.

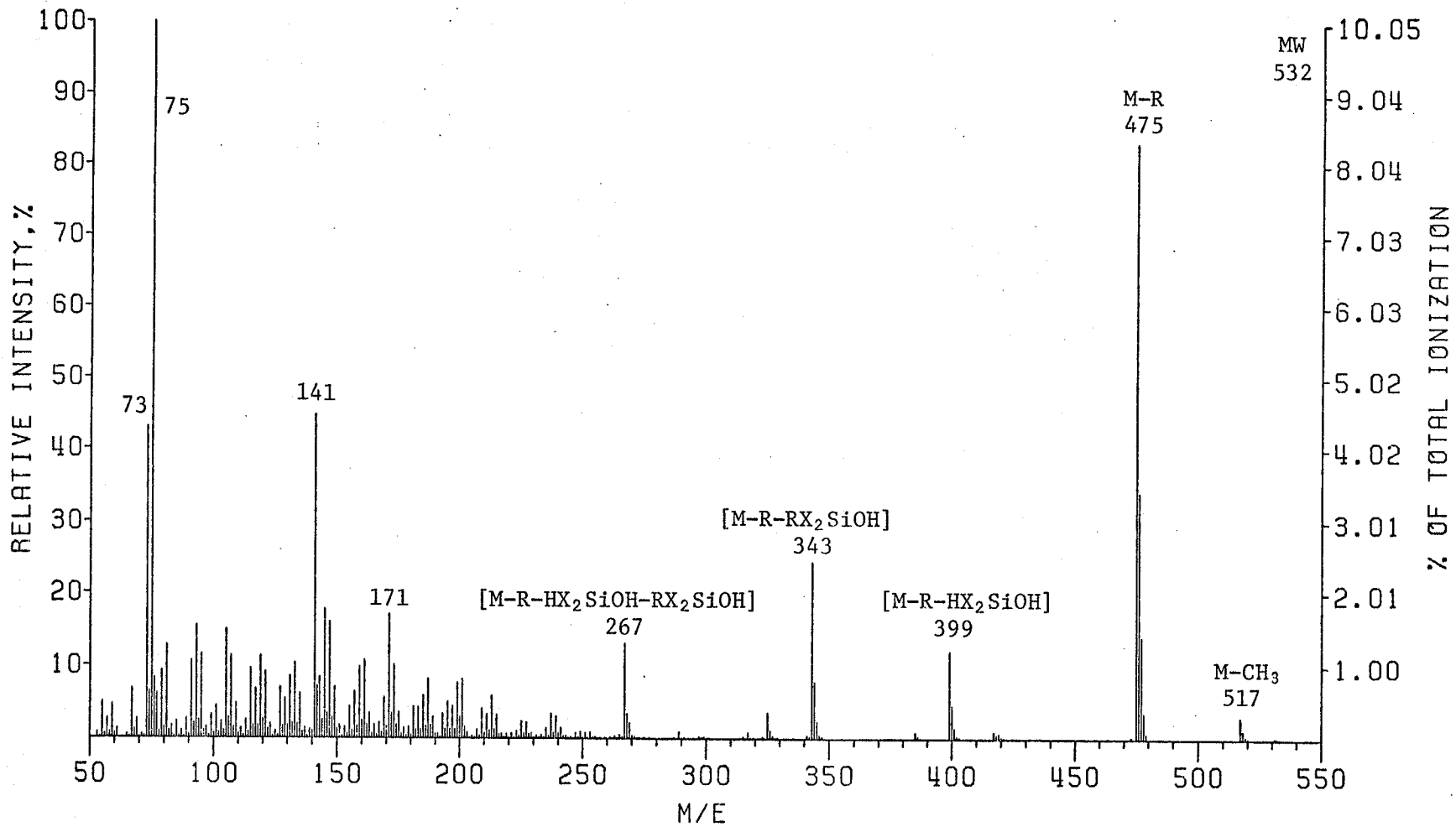


Figure 139. Mass spectrum of TBDMSi derivative of the 4α,17α-diol metabolite (XXIVb) recorded on Finnigan MS at 70 eV.

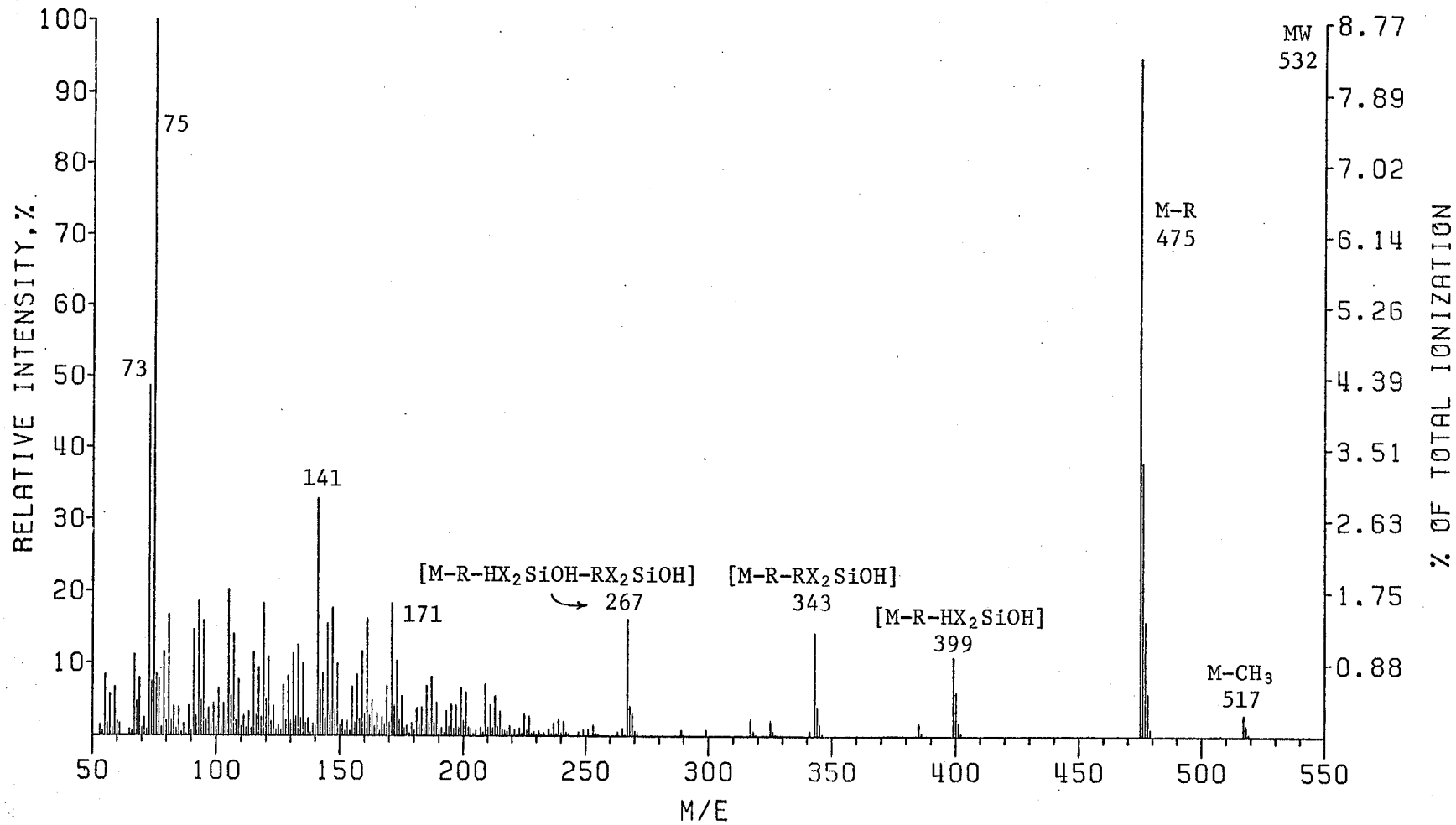


Figure 140. Mass spectrum of TBDMSi derivative of the 4α,17β-diol metabolite (XXVIb) recorded on Finnigan MS at 70 eV.

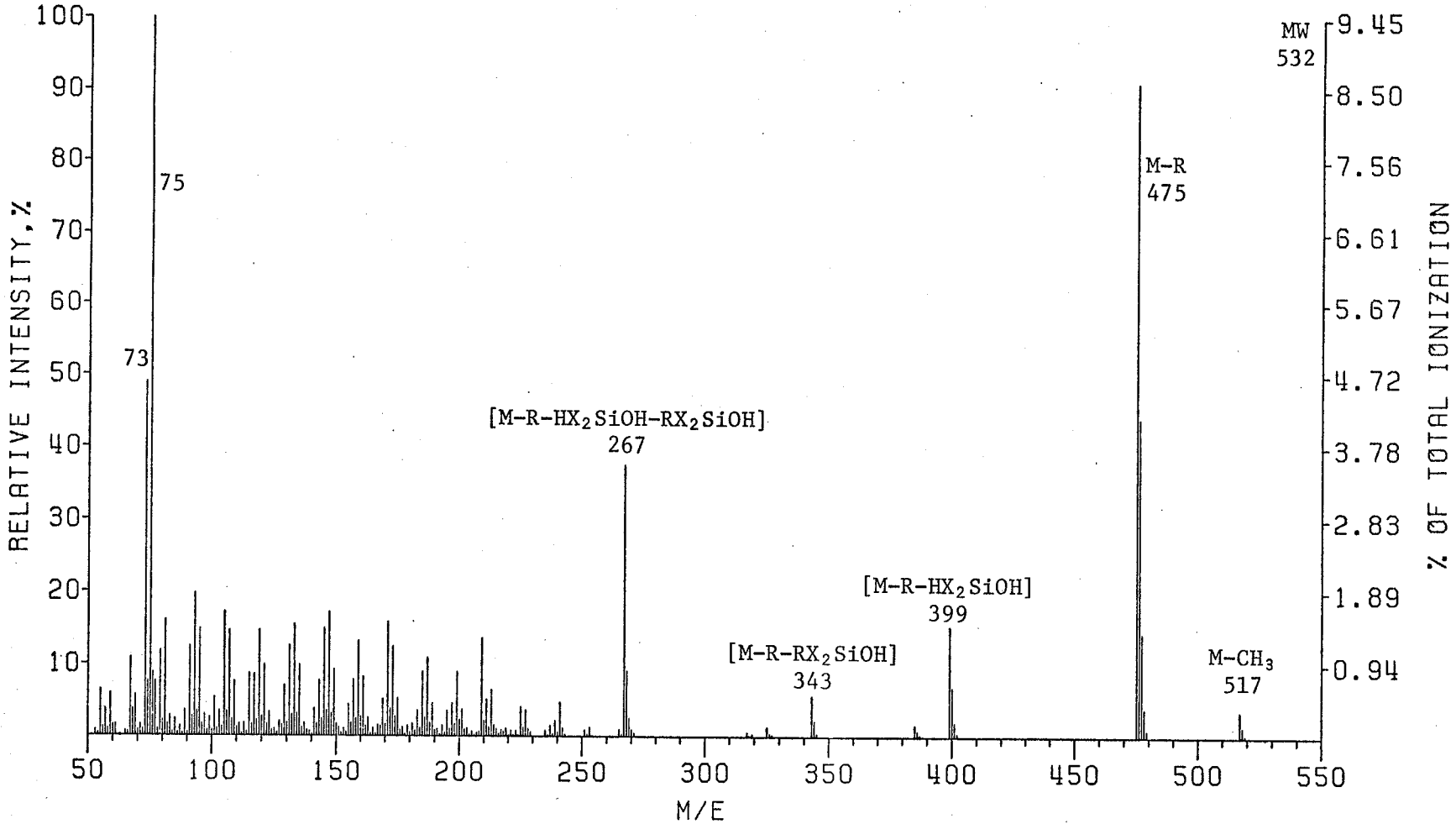


Figure 141. Mass spectrum of TBDMSi derivative of the 6α,17β-diol metabolite (XXVb) recorded on Finnigan MS at 70 eV.

TABLE XXXII. Partial mass spectra of silyl ether derivatives of di-hydroxy steroid metabolites.

Derivative:	TMSi				TBDMSi			
Diol: (Ion type)$^+$	m/e	4α,17α XXIVe	4α,17β XXVIe	6α,17β XXVe	m/e	4α,17α XXIVb	4α,17β XXVIb	6α,17β XXVb
M	448	13.4	12.5	1.8	532	0.16	-	-
$M-CH_3$	433	8.6	8.9	9.1	517	3.0	2.8	3.5
M-R	a	a	a	a	475	83	95	91
$M-RX_2SiOH$	358	18.7	17.3	54	400	4.6[i]	5.9[i]	6.8[i]
$M-R-HX_2SiOH$	357	1.8	2.3	2.4	399	12.1	10.9	15.3
$M-CH_3-RX_2SiOH$	343	5.4	4.1	9.6	385	0.87	1.5	1.6
$M-R-RX_2SiOH$	b	b	b	b	343	24	14.2	5.6
$M-R-RX_2SiOH-H_2O$	325	0.49	0.38	-	325	3.5	2.0	1.3
$M-2RX_2SiOH$	268	13.4	12.2	42	268	3.4[i]	3.9[i]	9.1[i]
$M-R-HX_2SiOH-RX_2SiOH$	267	2.7	1.8	8.4	267	13.1	16.0	37
$M-CH_3-2RX_2SiOH$	253	10.7	9.5	27	253	0.92	1.3	1.2
$C_{15}H_{22}$	202	31	32	2.7	202	1.7[i]	1.2[i]	0.97[i]
$RX_2SiOC_5H_7$	156	97	98	4.9	198	1.3[i]	1.2[i]	1.6[i]
$X_2SiOC_5H_7$	141	65	65	5.6	141	45	33	3.8
$RX_2SiOC_3H_4$	129	72	76	77	171	17.0	18.2	15.8
X_2SiOH	75	50	47	46	75	100	100	100
X_2SiCH_3	73	100	100	100	73	43	49	49
RX_2Si	c	c	c	c	115	9.6	11.5	8.7
$(M-2R)^{2+}$	209	6.2	5.8	8.3	209	4.1	7.0	13.6
Other ions	242	5.4	5.1	16.0				
	227	9.6	9.3	28				
Base peak as $\%\Sigma_{50}$		6.72	6.97	6.03		10.05	8.77	9.45

[a] Same as $(M-CH_3)^+$

[b] Same as $(M-R-RX_2SiOH)^+$

[c] Same as $X_2SiCH_3^+$

[i] Uncorrected intensity. Contains significant contribution from ion type of lower mass.

continued . . .

TABLE XXXII. (continued)

Derivative:	TMTBSi				TMIPSi			
Diol:		4α,17α	4α,17β	6α,17β		4α,17α	4α,17β	6α,17β
(Ion type)$^+$	m/e	XXIVc	XXVIc	XXVc	m/e	XXIVd	XXVId	XXVd
M	584	0.38	0.32	0.22	556	0.74	0.65	0.45
M-CH_3	569	0.98	1.1	1.1	541	0.72	0.63	0.44
M-R	527	91	100	100	513	96	81	100
M-RX_2SiOH	426	8.2^i	11.0^i	7.9^i	412	9.3^i	13.4	17.7^i
M-R-HX_2SiOH	425	12.8	9.8	17.7	411	7.8	4.8	15.5
M-CH_3-RX_2SiOH	411	1.2	5.5	2.4	397	0.79	3.0	1.5
M-R-RX_2SiOH	369	38	19	8.9	369	36	20	12.9
M-R-RX_2SiOH-H_2O	351	5.3	3.3	1.5	351	5.4	2.7	1.7
M-$2RX_2SiOH$	268	7.5^i	6.9^i	9.7^i	268	8.9^i	9.8^i	15.9^i
M-R-HX_2SiOH-RX_2SiOH	267	25	22	39	267	20	22	45
M-CH_3-$2RX_2SiOH$	253	2.1	1.9	1.6	253	4.5	4.9	3.4
$C_{15}H_{22}$	202	2.6^i	1.9^i	0.94^i	202	3.8^i	3.2^i	1.4^i
$RX_2SiOC_5H_7$	224	0.58^i	0.36^i	0.29^i	210	2.5^i	1.8^i	0.73^i
$X_2SiOC_5H_7$	167	50	43	1.7	167	53	32	2.5
$RX_2SiOC_3H_4$	197	8.4	7.5	4.4	183	14.0	16.0	7.4
X_2SiOH	101	100	88	68	101	100	100	87
X_2SiCH_3	99	28	27	21	99	45	50	43
RX_2Si	141	7.6	7.3	3.9	127	19.6	21	12.1
$(M-2R)^{2+}$	235	3.9	7.0	10.7	235	5.6	6.0	13.3
Base peak as $\%\Sigma_{50}$		7.02	8.08	10.20		6.18	6.19	7.56

C. Summary

The preceding results have demonstrated that sterically crowded trialkylsilylation has a number of valuable features for the chromatography and mass spectrometry of biologically important compounds:

(a) Derivatives of compounds with primary and sterically unhindered secondary hydroxyls are easily prepared in quantitative yield under mild reaction conditions.

(b) The hydrolytic stability of SCTASi ether derivatives allows easy handling, quantitative analysis by liquid chromatography, and isolation of stable, pure standards for reference or quantitative work.

(c) The low polarity of SCTASi derivatives eliminates tailing and non-quantitative elution problems encountered in the liquid chromatography of polar compounds and allows the use of low polarity, volatile mobile phases. Also, silylation can be useful for "group separations" of different classes of compounds (e.g., hydroxysteroids from keto-hydroxysteroids).

(d) Most SCTASi derivatives have sufficient volatility and thermal stability for quantitative gas chromatography and mass spectrometry.

(e) SCTASi derivatives can yield improved separations of isomers and mixtures by gas chromatography (relative to underivatized and/or trimethylsilylated compounds).

(f) The use of an homologous series of SCTASi derivatives for GC and the measurement of δI values can yield structural information, such as the number of reactive hydroxyls in a compound.

(g) The mass spectra of SCTASi derivatives have an abundant $[M-R]^+$ fragment ion useful for determining molecular weights and for selected ion recording work.

(h) Mass spectral fragmentations are directed by the SCTASi group, usually via siliconium ion rearrangements. Since the latter are dependent upon the steric accessibility of silyl groups and electron dense functions, they often lead to structurally and stereochemically informative fragment ions, and characteristically different spectra for isomeric compounds.

(i) An homologous series of SCTASi groups allows the labelling of silyl substituents for the elucidation of mass spectral fragmentation pathways. This avoids the use of expensive deuterium labelled silyl groups.

(j) Mixed derivatizations are relatively easy to perform and allow a detailed elucidation of mass spectral fragmentation mechanisms. Mixed TMSi/SCTASi derivatives are especially useful in this regard since they allow the "isolation" of individual siliconium ion species and their daughter ions.

Of course there are a few disadvantages attached to the use of SCTASi derivatives for GC and MS:

(a) Sterically hindered hydroxyls are difficult to silylate. (Mixed derivatization schemes may be a solution to this problem.)

(b) Due to the large retention increment effect for the addition of each SCTASi group, derivatives of polyhydroxy compounds may have excessively long retention times.

(c) The high mass of the SCTASi ether derivatives of polyhydroxy compounds could be a disadvantage when using some models of mass spectrometers with low mass limits. (On the other hand, it may be an advantage in GC/MS-SIR of some compounds, where the increased mass will place important fragment ions in a region free from GC bleed peaks.)

Overall, for the analysis of nucleosides, steroids, and other compounds of biological interest, SCTASi derivatives appear to be complementary to the widely used TMSi derivatives and may be vastly superior for certain applications.

VI. CONCLUSION

The results presented in this thesis have clearly indicated (a) that the techniques of gas phase analytical chemistry (derivatization, liquid and gas chromatography, and mass spectrometry) are valuable for the separation and characterization of synthetically important silyl derivatives of nucleosides, and (b) that sterically crowded trialkylsilylation is useful not only for the synthesis but also the chromatography and mass spectrometry of biologically important compounds.

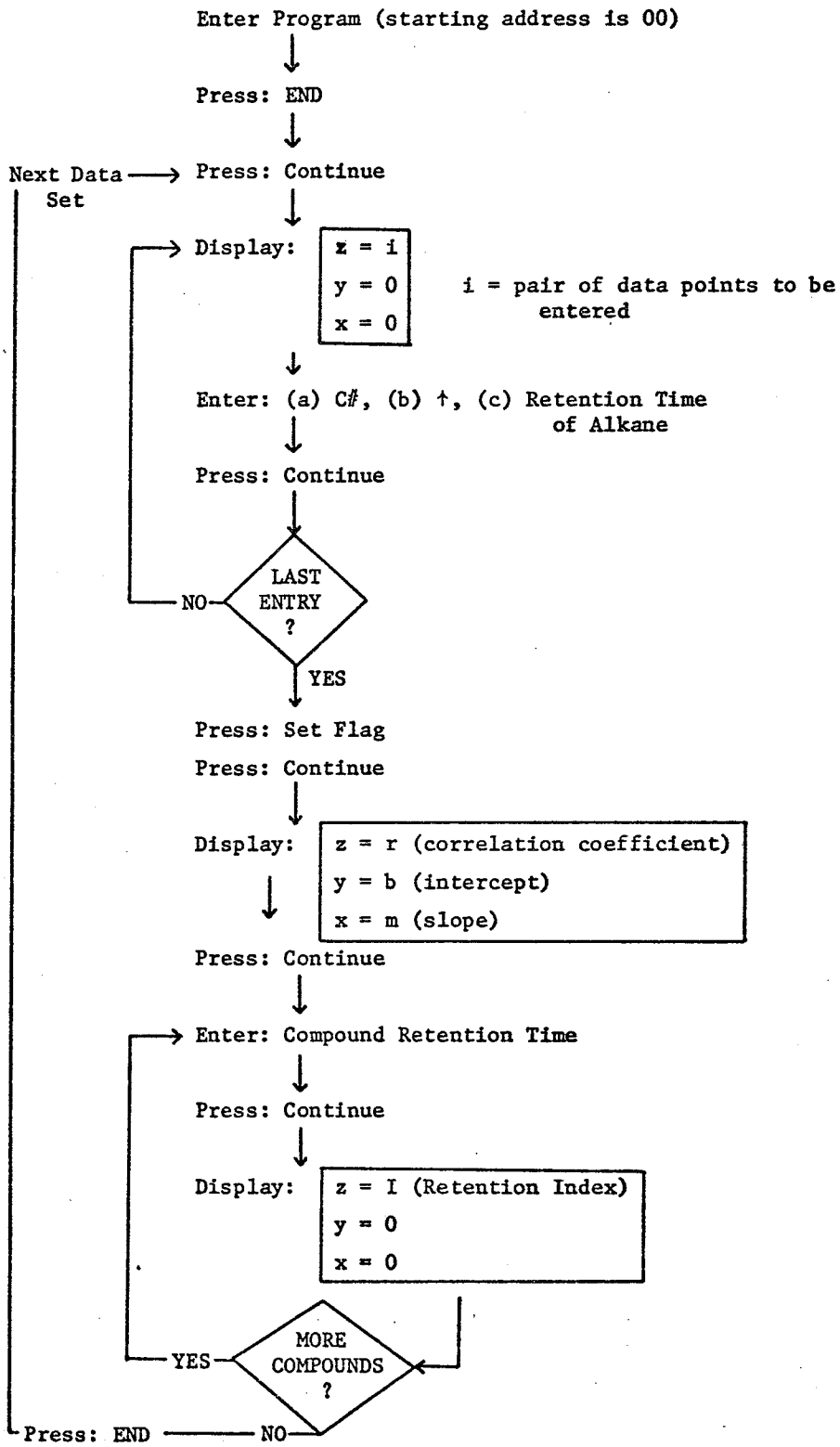
It is anticipated that both sterically crowded trialkylsilyl derivatives and gas phase analysis will play an important role in future research in synthetic and analytical fields. Therefore, it is hoped that some of the methods and results described in this thesis will stimulate continued investigation in these areas.

VII. APPENDIX

A. Program for the Hewlett-Packard 9100A calculator for the calculation of Kovats' isothermal retention indices (I values). A flow diagram for operation is given on the next page.

00	Clear	30	+	60	X	90	↑
01	x → ( )	31	y → ( )	61	a	91	STOP
02	d	32	c	62	X	92	LOG_{10} x
03	x → ( )	33	a	63	↓	93	↑
04	c	34	↑	64	−	94	b
05	x → ( )	35	1	65	y → ( )	95	−
06	b	36	+	66	c	96	e
07	1	37	y → ( )	67	b	97	÷
08	x → ( )	38	a	68	↑	98	0
09	a	39	0	69	f	99	↑
0a	Roll ↓	3a	↑	6a	↑	9a	GO TO ( )( )
0b	STOP	3b	GO TO ( )( )	6b	e	9b	9
0c	IF FLAG	3c	0	6c	X	9c	1
0d	4	3d	b	6d	a	9d	END
10	0	40	a	70	X		
11	LOG_{10}x	41	↑	71	↓		
12	x ⇄ y	42	1	72	−		
13	↑	43	−	73	d		
14	1	44	↓	74	↑		
15	0	45	x → ( )	75	↓		
16	0	46	a	76	$\sqrt{x}$		
17	X	47	e	77	÷		
18	↓	48	↑	78	c		
19	ACC +	49	a	79	$\sqrt{x}$		
1a	↑	4a	÷	7a	÷		
1b	X	4b	y ⇄ ( )	7b	d		
1c	x ⇄ y	4c	f	7c	Roll ↑		
1d	y ⇄ ( )	4d	÷	7d	x ⇄ y		
20	d	50	y → ( )	80	÷		
21	+	51	e	81	y ⇄ ( )		
22	y ⇄ ( )	52	X	82	e		
23	d	53	e	83	e		
24	↓	54	X	84	X		
25	X	55	d	85	f		
26	b	56	x ⇄ y	86	x ⇄ y		
27	+	57	−	87	−		
28	y → ( )	58	y → ( )	88	e		
29	b	59	d	89	STOP		
2a	Roll ↑	5a	c	8a	y → ( )		
2b	↑	5b	↑	8b	b		
2c	X	5c	f	8c	0		
2d	c	5d	↑	8d	↑		

Appendix A (continued)



B. Fortran IV program for plotting mass spectra. (An improved version of that reported by D.C.K.Lin (Ph.D. Thesis, University of Manitoba, 1972).)

```
C  MASSPLOT 12/4/76
C
C     PROGRAM TO CALCULATE AND PLOT A NORMALIZED MASS SPECTRUM
C     MAGNIFICATION AND SCALE EXPANSION FEATURES AVAILABLE
C
C  INPUT PARAMETERS:
C
C    CARD #1. FORMAT: (2I1,I4,10A4)
C
C        PARAMETER 1: K = 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 CORRECTED FOR MASS DISCRIMINATION
C          2 - 1015 QUADRUPOLE, SPECTRUM CORRECTED FOR MASS DISCRIM.
C          3 - 1015 QUADRUPOLE, ACTUAL SPECTRUM
C        PARAMETER 2: L = INTEGER DENOTING FORMAT OF DATA INPUT
C          1 - FORMAT 1, SEE CARD #5
C          2 - FORMAT 2, SEE CARD #5
C          3 - FORMAT 3, SEE CARD #5
C        PARAMETER 3: ID = 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
C    CARD #2. FORMAT: (1F10.4)
C
C        PARAMETER: SEF = SCALE EXPANSION FACTOR
C
C    CARD #3. FORMAT: (1F10.4)
C
C        THIS CARD TO BE INCLUDED ONLY WITH FORMATS 2 AND 3
C        PARAMETER : DSTART = THE FIRST MASS NO. TO BE READ IN FROM
C              COL.1 OF THE FIRST DATA CARD.
C
C    CARD #4. FORMAT: (8F10.4)
C
C        PARAMETER 1: XSCALE = MASS SCALE IN UNITS OF MASS NUMBER/CM.
C        PARAMETER 2: XSTART = MASS NUMBER AT WHICH PLOT IS TO BEGIN.
C              THIS MAY BE: 0,10,20,30,40,50;THEN:100,150,ETC.
C        PARAMETER 3: XSTOP = MASS NUMBER AT WHICH PLOT IS TO END.
C        PARAMETER 4: YHGHT = HEIGHT OF THE PLOT IN CM. (MAX=19.1)
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: XEROX = NUMBER OF PLOTS OF THE SAME SPECTRUM.
C
C    CARD#5-ONWARDS: DATA CARDS - IN FORMAT 1,2 OR  3:
C
C FORMAT 1: (2F10.4) MASS NUMBER AND INTENSITY OF THE PEAKS IN THIS
C              SPECTRUM.DENOTE THE END OF THIS SERIES BY A BLANK CARD.
C EG.     16.0       10.0
C         20.1225    12.1531
C
C
C
C FORMAT 2: (I1,F9.2,7F10.2)
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 - 9 : INTENSITIES, IN ORDER OF MASS, OF EIGHT
C                MASSES FROM E.G. 33.0 TO 40.0, OR FROM 32.5 TO 36.0,
C                FOLLOWED BY 36.5 TO 40.0  EVEN IF THERE IS NO PEAK AT
C                THAT MASS. END OF DATA WHEN INTENSITY=9999.
C
C FORMAT 3: (8F10.2)
C                MASS NO. OBTD FROM POSN OF INTENSITY VALUE ON DATA CARD
C         PARAMETERS 1-8: INTENSITIES IN ORDER OF MASS, AT 8 MASS INTER-
C             VALS, EG. FROM 33. TO 40. (WHEN THERE IS NO PEAK AT A MASS,
C             LEAVE A BLANK). COMPLETION OF DATA IS DENOTED WHEN INTENSITY
C             =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
C
      INTEGER IBUF(4000),TITLE(10),AST(100)
      REAL MASSNO(400),INTEN(400),MSTART,MSTOP,MAGNFY,PKHT(8)
      DO 10 J=1,100
   10 AST(J)=11
      CALL PLOTS(IBUF,4000)
      CALL PLOT(6.0,-10.0,-3)
      CALL PLOT(0.0,1.78,-3)
C READ CARD #1
   20 READ (5,480,END=460) K,L,ID,TITLE
      IF (ID.LE.0) GO TO 470
C READ SCALE EXPANSION FACTOR (CARD #2)
      READ (5,485,END=460) SEF
C READ DSTART (CARD #3) FOR FORMATS 2 AND 3
      IF (L.GT.1) READ (5,485,END=460) DSTART
C READ PLOT PARAMETERS (CARD #4)
   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
C CONVERT TO INCHES FOR PLOTTER
      YHGHT = YHGHT/2.54
      XSCALE = XSCALE*2.54
      I=1
      IF (L.EQ.3) GO TO 60
      IF (L.EQ.2) GO TO 90
C FORMAT 1 INPUT:
   40 READ (5,495,END=460) MASSNO(I),INTEN(I)
      IF (MASSNO(I).LT.1.0E-10) GO TO 50
      I=I+1
      GO TO 40
   50 NOPKS=I-1
      GO TO 150
C FORMAT 2 INPUT:
   60 TMASS=DSTART-1.0
   70 READ (5,500,END=460) (PKHT(J),J=1,8)
      DO 80 J=1,8
      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).EQ.9999.) GOTO 140
      GO TO 70
C FORMAT 3 INPUT:
   90 TMASS=DSTART-1.0
  100 READ (5,510,END=460) M,(PKHT(J),J=1,8)
      XINC=1.0
```

```
01300          IF(M.EQ.1) XINC=0.5
01310      120 DO 130 J=1,8
01320          TMASS=TMASS+XINC
01330          IF (PKHT(J).LE.1.0E-10) GO TO 130
01340          INTEN(I)=PKHT(J)
01350          MASSNO(I)=TMASS
01360          I=I+1
01370      130 CONTINUE
01380          IF (INTEN(I-1).EQ.9999.) GO TO 140
01390          GO TO 100
01400    C
01410      140 NOPKS=I-2
01420      150 BIGPK=-1.0E49
01430          SUMINT=0.0
01440          IF (K.NE.2) GO TO 180
01450    C QUADRUPOLE MASS DISCRIMINATION CORRECTION
01460      160 DO 170 I=1,NOPKS
01470      170 INTEN(I)=INTEN(I)*MASSNO(I)
01480    C SUM PEAK HEIGHTS (SUMINT) AND SELECT BASE PEAK (BIGPK)
01490      180 DO 190 I=1,NOPKS
01500          TEMP=INTEN(I)
01510          SUMINT=SUMINT+TEMP
01520          IF (TEMP.GT.BIGPK) BIGPK=TEMP
01530      190 CONTINUE
01540      200 SCALE=100.0/BIGPK
01550          SUMINT=SUMINT*SCALE
01560    C CORRECT ANY MIXUP IN XSTART AND XSTOP
01570          IF (XSTOP-XSTART) 210,460,220
01580      210 TEMP=XSTART
01590          XSTART=XSTOP
01600          XSTOP=TEMP
01610      220 XSCAL2=1.0/XSCALE
01620          YSCAL2=YHGHT/BIGPK
01630          COPY=XEROX-0.1
01640      230 COPY=COPY-1.0
01650    C PRINT AND PLOT THE TITLE
01660          WRITE (6,520) TITLE
01670          IF (K.EQ.1) GO TO 250
01680          IF (K.EQ.2) GO TO 260
01690          IF (K.EQ.3) GO TO 250
01700      240 WRITE (6,530)
01710          GO TO 270
01720      250 WRITE (6,540)
01730          GO TO 270
01740      260 WRITE (6,550)
01750      270 WRITE (6,560)
01760          CALL SYMBOL(0.0,YHGHT+0.85,0.14,TITLE,0.0,40)
01770          IF(K.GT.0)GO TO 280
01780          CALL SYMBOL(0.0,YHGHT+0.6,0.14,44HHITACHI RMU-6D MAGNETIC SECTOR M
01790         &ASS SPECTRUM,0.0,44)
01800          GO TO 310
01810      280 CALL SYMBOL(0.0,YHGHT+0.6,0.14,41HFINNIGAN-1015 RF-QUADRUPOLE MASS
01820         & SPECTRUM,0.0,41)
01830          IF(K.NE.2)GO TO 310
01840          CALL SYMBOL(0.0,YHGHT+0.35,0.14,33HCORRECTED FOR MASS DISCRIMINATI
01850         &ON,0.0,33)
01860    C PLOT X-AXIS
01870      310 POSN=(XSTOP-XSTART)/XSCALE/2.0-0.21
01880          CALL SYMBOL(POSN,-0.65,0.14,3HM/E,0.0,3)
01890          CALL PLOT(0.0,0.0,3)
01900          X=XSTART-10.
01910          I=5
01920          IF(XSTART.LT.0.1.OR.XSTART.GT.49.9) GO TO 320
01930          I=IFIX(X/10.)
01940      320 X=X+10.
01950          TEMP=(X-XSTART)*XSCAL2
```

```
01960          CALL PLOT(TEMP,0.0,2)
01970          CALL PLOT(TEMP,-0.05,2)
01980          I=I+1
01990          IF (I.LT.5) GO TO 340
02000          CALL PLOT(TEMP,-0.15,2)
02010          POSN=0.18
02020          IF(X.LT.99.5)POSN=0.11
02030          IF(X.LT.0.1)POSN=0.07
02040          CALL NUMBER(TEMP-POSN,-0.35,0.14,X,0.0,-1)
02050          I=0
02060      340 CALL PLOT(TEMP,0.0,3)
02070          IF (X.LT.XSTOP) GO TO 320
02080    C PLOT RIGHT-HAND Y-AXIS
02090      350 TEMP=(XSTOP-XSTART)*XSCAL2+1.0
02100          POSN=YHGHT/2.0-1.47
02110          CALL SYMBOL(TEMP,POSN,0.14,21H% OF TOTAL IONIZATION,90.0,21)
02120          TEMP=TEMP-1.0
02130          CALL PLOT(TEMP,0.0,3)
02140          TEMP1=YHGHT/10.0
02150          Y=0.0
02160          X=0.0
02170          PCTG=1000.0/SUMINT/SEF
02180      360 Y=Y+TEMP1
02190          CALL PLOT(TEMP,Y,2)
02200          CALL PLOT(TEMP+0.05,Y,2)
02210          X=X+PCTG
02220          CALL NUMBER(TEMP+0.11,Y-0.07,0.14,X,0.0,2)
02230      370 CALL PLOT(TEMP,Y,3)
02240          IF (Y.LT.(YHGHT-0.1)) GO TO 360
02250    C PLOT LEFT-HAND Y-AXIS
02260          POSN=YHGHT/2.0-1.4
02270      380 IF(SEF.LT.1.01)GOTO 382
02280          CALL SYMBOL(-0.80,POSN,0.14,20HRELATIVE INTENSITY,%,90.0,20)
02290          GO TO 384
02300      382 CALL SYMBOL(-0.50,POSN,0.14,20HRELATIVE INTENSITY,%,90.0,20)
02310      384 CALL PLOT(0.0,0.0,3)
02320          Y=TEMP1
02330          X=0.0
02340      390 X=X+10.00/SEF
02350          CALL PLOT(0.0,Y,2)
02360          CALL PLOT(-0.05,Y,2)
02370          IF(SEF.LT.1.01) GO TO 392
02380          POSN=-0.60
02390          IF(X.LT.9.95)POSN=-0.46
02400          CALL NUMBER(POSN,Y-0.07,0.14,X,0.0,1)
02410          GO TO 394
02420      392 POSN=-0.31
02430          IF(X.GT.99.0)POSN=-0.45
02440          CALL NUMBER(POSN,Y-0.07,0.14,X,0.0,-1)
02450      394 CALL PLOT(0.0,Y,3)
02460          Y=Y+TEMP1
02470          IF (X.LT.(100.0/SEF-0.05)) GO TO 390
02480      400 CALL PLOT(0.0,0.0,3)
02490    C PLOT MASS SPEC PEAKS AND PRINT HISTOGRAM AND INTENSITY DATA
02500          DO 410 I=1,NOPKS
02510          TEMP=MASSNO(I)
02520          IF(TEMP.LE.XSTART)GO TO 410
02530          RELINT=INTEN(I)*SCALE
02540          ITEMP1=RELINT
02550          PCTG=RELINT/SUMINT*100.00
02560          WRITE (6,570) TEMP,RELINT,PCTG,(AST(J),J=1,ITEMP1)
02570          TEMP1=(TEMP-XSTART)*XSCAL2
02580          TEMP2=INTEN(I)*YSCAL2*SEF
02590          IF (TEMP.GT.MSTART.AND.TEMP.LT.MSTOP) TEMP2=TEMP2*MAGNFY
02600          IF(TEMP2.GT.(YHGHT+0.01))TEMP2=YHGHT+0.03
02610          CALL PLOT(TEMP1,0.0,3)
```

```
      CALL PLOT(TEMP1,TEMP2,2)
      IF(TEMP2.LT.(YHGHT+0.01))GOTO405
      CALL SYMBOL(TEMP1,TEMP2,0.07,6,0.0,-1)
      CALL NUMBER(TEMP1+0.05,TEMP2-0.07,0.07,RELINT,0.0,-1)
      POSN=0.19
      IF(RELINT.GT.99.4)POSN=0.26
      CALL SYMBOL(TEMP1+POSN,TEMP2-0.07,0.07,108,0.0,-1)
  405 CALL PLOT(TEMP1,0.0,3)
  410 CONTINUE
      IF (MSTOP.LT.0.0) GO TO 450
C PLOT MAGNIFICATION INDICATORS
  420 TEMP=(MSTART-XSTART)*XSCAL2
      YHT2=YHGHT/2.0
      CALL PLOT(TEMP,0.0,3)
      CALL PLOT(TEMP+0.25,YHT2,2)
      CALL PLOT(TEMP+0.50,YHT2,2)
      CALL SYMBOL(TEMP+0.50,YHT2,0.07,6,270.0,-1)
      CALL SYMBOL(TEMP+0.55,YHT2,0.14,61,0.0,-1)
      CALL NUMBER(TEMP+0.70,YHT2,0.14,MAGNFY,0.0,-1)
      IF (ABS(MSTOP-XSTOP).LT.1.0E-05) GO TO 450
  430 TEMP=(MSTOP-XSTART)*XSCAL2
      CALL PLOT(TEMP-0.50,YHT2,3)
      CALL SYMBOL(TEMP-0.50,YHT2,0.07,6,90.0,-1)
      CALL PLOT(TEMP-0.25,YHT2,2)
      CALL PLOT(TEMP,0.0,2)
  450 CALL PLOT((XSTOP-XSTART)*XSCAL2+6.0,0.0,-3)
C REPEATS FOR EXTRA COPIES, DISCRIMINATION CORRECTION AND SEF=1.0
      IF (COPY.GT.0.0) GO TO 230
      IF (K.NE.1) GO TO 455
      K=K+1
      GO TO 150
  455 IF(SEF.LT.1.01)GOTO 20
      SEF=1.0
      COPY=XEROX-0.1
      GO TO 230
  460 WRITE (6,580)
  470 WRITE (6,590)
      CALL PLOT(0.0,0.0,999)
      CALL EXIT
  480 FORMAT (2I1,I4,10A4)
  485 FORMAT (F10.4)
  490 FORMAT (8F10.4)
  495 FORMAT (2F10.4)
  500 FORMAT (8F10.2)
  510 FORMAT (I1,F9.2,7F10.2)
  520 FORMAT (1H1,//1H  ,15X,10A4)
  530 FORMAT (1H  ,15X,'SPECTRUM RECORDED ON HITACHI RMU-6D MAGNETIC SEC
     &TOR MS')
  540 FORMAT (1H  ,15X,'SPECTRUM RECORDED ON FINNIGAN 1015 RF-QUADRUPOLE
     & MS')
  550 FORMAT (1H  ,15X,'SPECTRUM RECORDED ON FINNIGAN 1015 RF-QUADRUPOLE
     & MS AND CORRECTED FOR MASS DISCRIMINATION')
  560 FORMAT (1H  ,//1H  ,' MASS    INTENSITY    %',/1H  ,'NUMBER   (NML
     &IZED)   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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