STEREOCHEMICALLY CONTROLLED ELIMINATION OF HMe_2SiOH IN THE ELECTRON IONIZATION MASS SPECTRA OF *t*-BUTYLDIMETHYLSILYL ETHER DERIVATIVES OF 17ξ-METHYL-5ξ-ANDROSTANE-3ξ,17ξ-DIOLS: AN ISOTOPIC LABELING STUDY

BY

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BY

HELENA MAJGIER - BARANOWSKA

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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ABSTRACT

In the electron ionization mass spectra of *t*-butyldimethylsilyl ether derivatives of the eight 17ξ-methyl-5ξ-androstane-3ξ,17ξ-diols, there is a stereochemically controlled elimination of HMe_2SiOH from the $[M - t\text{-}Bu]^+$ and $[M - t\text{-}Bu - H_2O]^+$ ions. The main part of this thesis describes double hydrogen rearrangement involved in this elimination and also the single hydrogen transfer in elimination of H_2O from the $[M - t\text{-}Bu]^+$ ion. Also described are formation of $[(CH_3)_2SiOH]^+$ ions and elimination of $Me_2Si{=}O$ from *t*-butyldimethylsilyl ethers derivatives of the examined steroids.

The origin of the transferred hydrogens and interpretation of the fragmentation mechanisms were assisted by synthesis of more than forty mono- or poly-deuterated analogues of 17ξ-methyl-5ξ-androstane-3ξ,17ξ-diols, with the label at positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 16, and 20. These syntheses are described. The gas-chromatographic properties of the 3ξ-TBDMSi ether derivatives of the eight 17ξ-methyl-5ξ-androstane-3ξ,17ξ-diols on a DB-5 capillary column were used to monitor the syntheses, the deuterium content being determined mass spectrometrically.

Positive-ion EI mass spectra (70 eV) of the 3ξ-TBDMSi-ether derivatives are reported. Decompositions of metastable or collisionally-activated ions and exact mass measurements were employed to support proposed fragmentation pathways.

A mechanism for formation of the $[CH_3COOSi(CH_3)_2]^+$ ion from 3ξ-OSiDMTB-11α-acetyloxy-17β-hydroxy-17α-methyl-5ξ-androstane is proposed. This invokes transfer of the 11α-acetyloxy group to positively charged silicon after the *t*-butyl radical has been eliminated.

A novel fragmentation mechanism, i.e. transfer of tertiary hydride to positively charged silicon, and of a hydrogen radical to 3-oxygen, is the principal mode of the double hydrogen transfer in $HSiMe_2OH$ elimination. The role of the newly formed tertiary planar carbocation, its influence on the geometry (stereochemistry) of

intermediate ions and on the fragmentation pathways is discussed. To test the feasibility of the hydrogen transfers, the interatomic distances in the transition states were estimated from Dreiding models and molecular mechanics calculations.

A novel structure for the m/z 75 ion, $[H(CH_3)SiOCH_3]^+$ is proposed. A fragmentation mechanism for $Me_2Si{=}O$ elimination from the [M - *t*-Bu - H_2O]$^+$ ion is suggested.

Mechanisms for water elimination from the conformationally rigid ring D of [M - *t*-Bu]$^+$ and molecular ions are proposed. A significant correlation between the abundance of the [M - *t*-Bu - H_2O]$^+$ fragment ion and the configuration at C-17 is evident. The ratios of the abundance of the [M - *t*-Bu - H_2O]$^+$ ion to that of the [M - *t*-Bu]$^+$ ion differentiate the 17α-OH and 17β-OH epimers of mono-3ξ-OSiDMTB derivatives, irrespective of C-3 and C-5 configurations, the extent of water elimination being greater for the 17α-OH steroids.

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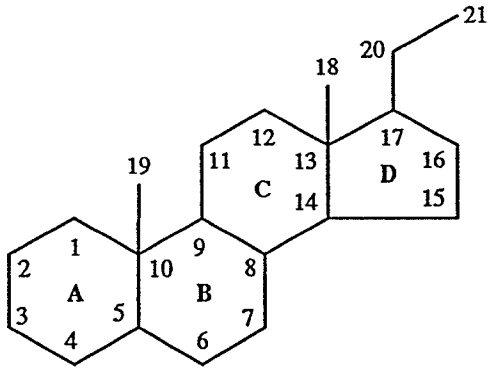
STEROIDS

(Structural, positional, and conformational terminology. Chemical abbreviations and symbols)

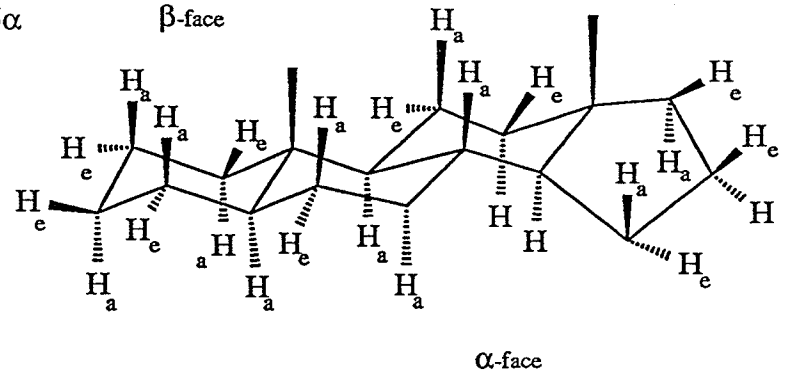
A steroid molecule is characterized by a basic carbon skeleton consisting of three six-membered rings and one five-membered ring, Scheme i-1. The rings are denoted as A, B, C, and D. The particular carbon atoms, starting from ring A, are numbered as in Scheme i-1.



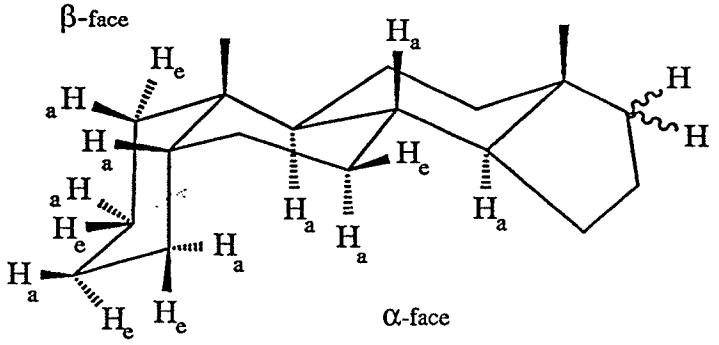
Scheme i-1. Basic steroid structure with standard atomic numbering and ring designations.

The steroid molecule is three dimensional. The space below and above the steroid skeleton is denoted as the α or β face, respectively, Scheme i-2. Atoms or groups are attached to a steroid skeleton by spatially directed bonds. The stereochemistry of hydrogen atoms, or substituents, is shown as α (a broken line) or β (a full line) and depends on their orientation, i.e. whether it is on face α or β. The stereochemistry of rings A and B is specified by showing the orientation of the hydrogen atoms attached at C-5 (5α or 5β). The unspecified orientation of a substituent is written as ξ- (xi), for example, as at C-17, Scheme i-2 (b). The hydrogen atoms or substituents perpendicular to the mean plane of a ring are denoted as axial (H_a), those in the mean plane are denoted as equatorial (H_e).

(a) 5α

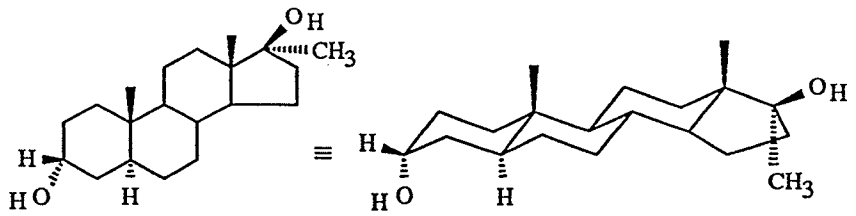


(b) 5β

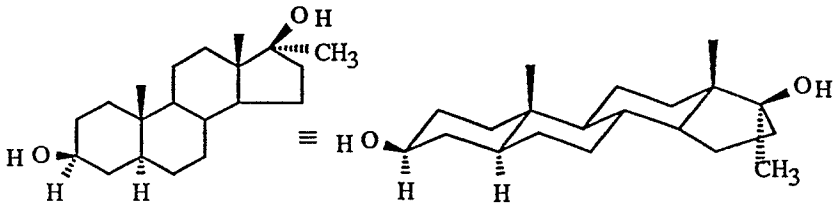


Scheme i-2. Structural and positional assignment of hydrogen atoms in the steroid skeleton. (The 5-H is axial to ring A and equatorial to ring B.)

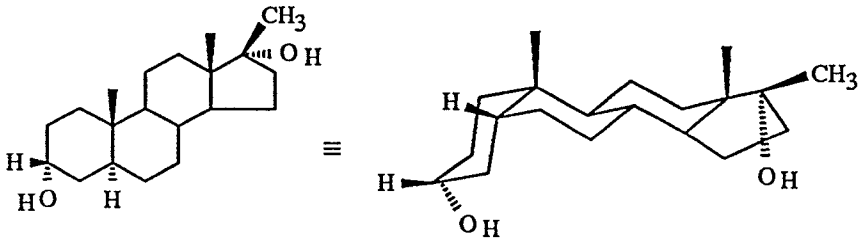
A steroid molecule can be represented as flat or three dimensional in a structural diagram. The four 17β-methyl-5ξ-androstane-3ξ,17β-diol isomers, as examples, are represented in Scheme i-3.



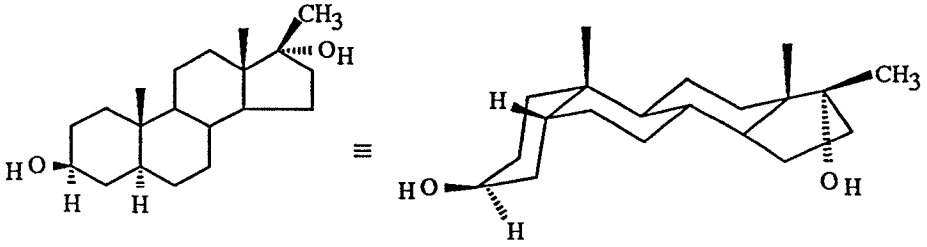
17α-methyl-5α-androstane-3α,17β-diol (5α-A,3β-OH,17β-OH-17α-CH_3)
(A = androstane; rings A, B, and C have chair conformations)



17α-methyl-5α-androstane-3β,17β-diol (5α-A,3β-OH,17β-OH-17α-CH_3)



17β-methyl-5β-androstane-3α,17α-diol (5β-A,3α-OH,17α-OH-17β-CH_3) (rings A, B and C have chair conformations)



17β-methyl-5β-androstane-3β,17α-diol (5β-A,3β-OH,17α-OH-17β-CH_3)

Scheme i-3. Structures of steroid alcohols.

GLOSSARY

(Abbreviations, symbols, and definitions of terms)

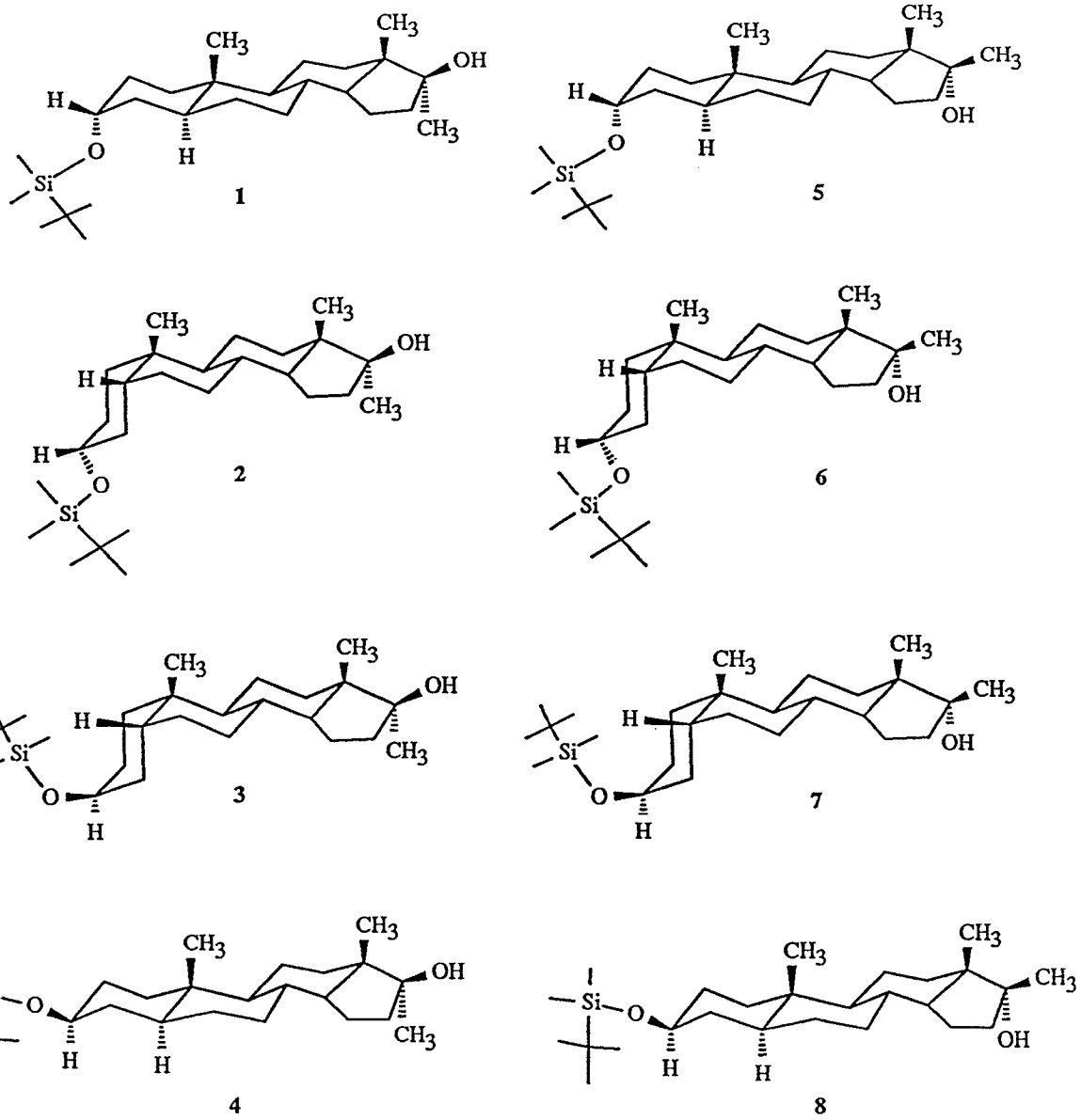
The International Union of Pure and Applied Chemistry (IUPAC) (G. P. Moss, *Pure & Appl. Chem.*, **61**, 1783 (1989)) and the Editorial Board of the Organic Mass Spectrometry journals (A. Maccoll, *Org. Mass Spectrom.*, **14**, 1 (1979)) standardized the symbols and nomenclature for mass spectrometry. The nomenclature used in this thesis is based on their monographs.

(i) Abbreviations

TMS	trimethylsilyl
TBDMSi	*tert*-butyldimethylsilyl (sometimes written SiDMTB, if attached to oxygen, i.e. OSiDMTB)
ClDMSi	chlorodimethylsilyl
IPDMSi	isopropyldimethyl
EDMSi	ethyldimethylsilyl

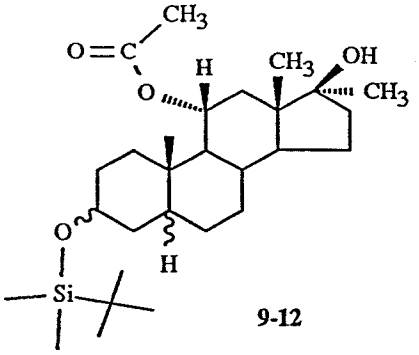
(ii) Symbol

tert-butyl

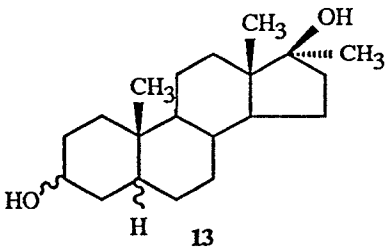


1: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane
2: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane
3: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane
4: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane
5: 3α-OSiDMTB-17α-hydroxy-17β-methyl-5α-androstane
6: 3α-OSiDMTB-17α-hydroxy-17β-methyl-5β-androstane
7: 3β-OSiDMTB-17α-hydroxy-17β-methyl-5β-androstane
8: 3β-OSiDMTB-17α-hydroxy-17β-methyl-5α-androstane

Scheme i-4. TBDMSi-ether derivatives of 17ξ-methyl-5ξ-androstane-3ξ,17ξ-diols.



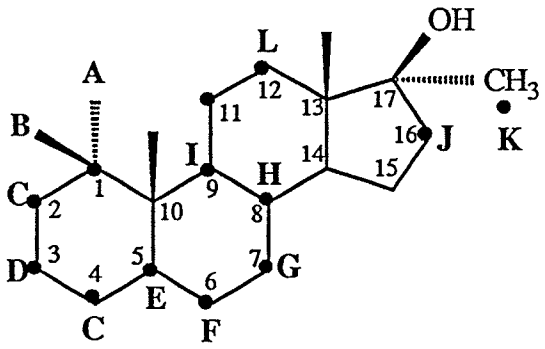
9-12



13

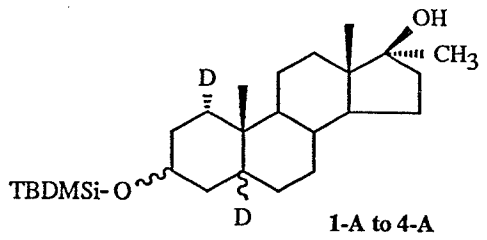
9: 3α-OSiDMTB-11α-acetyloxy-17β-hydroxy-17α-methyl-5α-androstane
10: 3α-OSiDMTB-11α-acetyloxy-17β-hydroxy-17α-methyl-5β-androstane
11: 3β-OSiDMTB-11α-acetyloxy-17β-hydroxy-17α-methyl-5β-androstane
12: 3β-OSiDMTB-11α-acetyloxy-17β-hydroxy-17α-methyl-5α-androstane
13: 17α-methyl-5ξ-androstane-3ξ,17β-diol (one iosomer)

Scheme i-5. 3ξ-TBDMSi-ether 11α-acetyloxy derivatives of 17β-hydroxy-17α-methyl-5ξ-androstane **(9-12)** and a representative example of the four isomers of 17β-methyl-5ξ-androstane-3ξ,17β-diols **(13)**



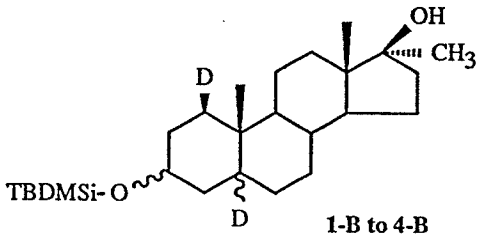
Denotation of labels:
A 1α,5ξ
B 1β,5ξ
C 2,2,4,4
D 3ξ
E 5ξ
F 6ξ
G 7ξ
H 6,6,8β
I 5ξ,9α,11α
J 16,16
K 17α
L 9α,12

Scheme i-6. Denotation of deuterium label(s) in sites of the steroid skeleton of the examined compounds.



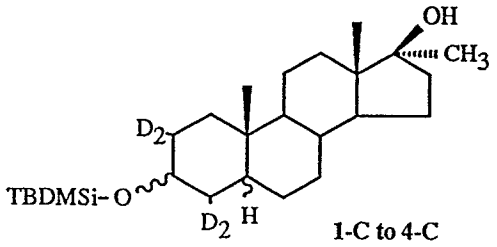
1-A to 4-A

1-A: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-1α,5α-d_2
2-A: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-1α,5β-d_2
3-A: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-1β,5β-d_2
4-A: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-1β,5α-d_2



1-B to 4-B

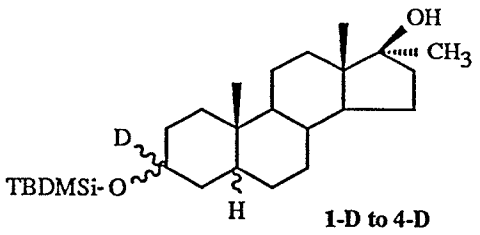
1-B: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-1α,5α-d_2
2-B: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-1α,5β-d_2
3-B: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-1β,5β-d_2
4-B: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-1β,5α-d_2



1-C to 4-C

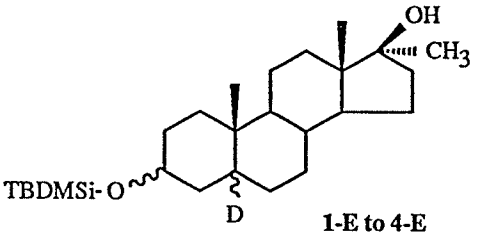
1-C: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-2,2,4,4-d_4
2-C: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-2,2,4,4-d_4
3-C: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-2,2,4,4-d_4
4-C: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-2,2,4,4-d_4

Scheme i-7. Deuterium-labeled analogues of compounds **1-8** from **Scheme i-4.**



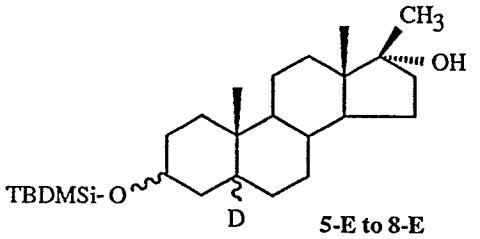
1-D to 4-D

1-D: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-3β-d_1
2-D: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-3β-d_1
3-D: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-3α-d_1
4-D: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-3α-d_1



1-E to 4-E

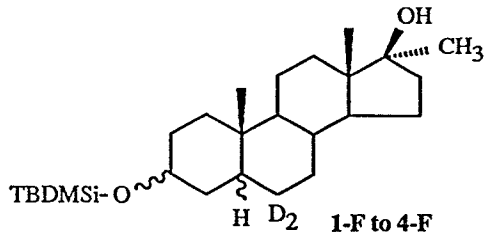
1-E: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-5α-d_1
2-E: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-5β-d_1
3-E: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-5β-d_1
4-E: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-5α-d_1



5-E to 8-E

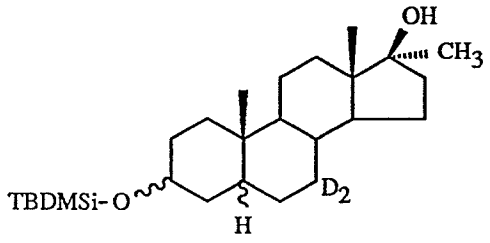
5-E: 3α-OSiDMTB-17α-hydroxy-17β-methyl-5α-androstane-5α-d_1
6-E: 3α-OSiDMTB-17α-hydroxy-17β-methyl-5β-androstane-5β-d_1
7-E: 3β-OSiDMTB-17α-hydroxy-17β-methyl-5β-androstane-5β-d_1
8-E: 3β-OSiDMTB-17α-hydroxy-17β-methyl-5α-androstane-5α-d_1

Scheme i-7. Deuterium-labeled analogues of compounds **1-8** from **Scheme i-4.**



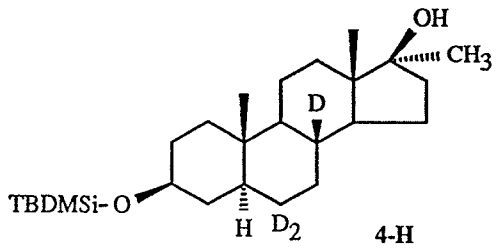
1-F to 4-F

1-F: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-6,6-d_2
2-F: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-6,6-d_2
3-F: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-6,6-d_2
4-F: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-6,6-d_2



1-G to 4-G

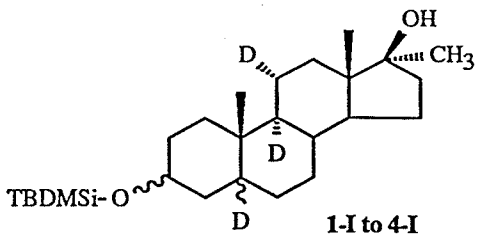
1-G: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-7,7-d_2
2-G: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-7,7-d_2
3-G: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-7,7-d_2
4-G: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-7,7-d_2



4-H

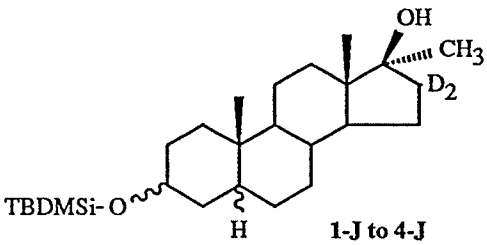
4-H: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-6,6,8β-d_3

Scheme i-7. Deuterium-labeled analogues of compounds **1-8** from **Scheme i-4.**



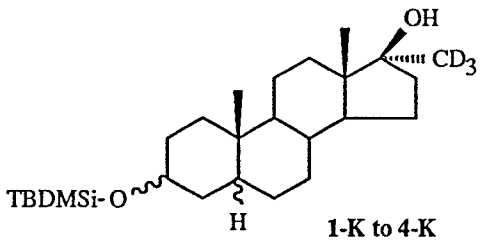
1-I to 4-I

1-I: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-5α,9α,11α-d_3
2-I: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-5β,9α,11α-d_3
3-I: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-5β,9α,11α-d_3
4-I: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-5α,9α,11α-d_3



1-J to 4-J

1-J: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-16,16-d_2
2-J: 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-16,16-d_2
3-J: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-16,16-d_2
4-J: 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-16,16-d_2



1-K to 4-K

1-K: 3α-OSiDMTB-17β-hydroxy-17α-methyl-d_3-5α-androstane
2-K: 3α-OSiDMTB-17β-hydroxy-17α-methyl-d_3-5β-androstane
3-K: 3β-OSiDMTB-17β-hydroxy-17α-methyl-d_3-5β-androstane
4-K: 3β-OSiDMTB-17β-hydroxy-17α-methyl-d_3-5α-androstane

Scheme i-7. Deuterium-labeled analogues of compounds **1-8** from **Scheme i-4.**

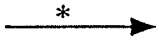
(ii) Abbreviations

EIMS	electron ionization mass spectrometry
CIMS	chemical ionization mass spectrometry
CAMS	collisional activation mass spectrometry
GC/MS	gas chromatography and mass spectrometry: the letters preceding MS can also be used separately.
u	dalton or mass unit ($^{12}C = 12$ daltons), formerly "amu"
e	magnitude of the electronic charge
m	the mass number of an ion
z	the charge number of an ion
m/z	the mass-to-charge ratio, formerly "m/e"
IE	ionization energy, formerly "ionization potential"
AE	appearance energy, formerly "appearance potential"
RA, %RA	The relative abundance of a peak as a percentage of the abundance of the base peak
M_r	Mol. mass relative molecular mass

(ii) Symbols

B	magnetic field (or magnetic sector)
E	electric field (or electric sector)
$M^{+\cdot}$ or $[M]^{+\cdot}$	molecular radical cation
F^+ or $[F]^+$	positive even electron fragment ion
$F^{+\cdot}$ or $[F]^{+\cdot}$	positive odd electron fragment ion
m*	apparent m/z value of a metastable peak
$\longrightarrow$	indication of a two electron shift

indication of a one electron shift

 process confirmed by the observation of a metastable peak

(iii) Definitions

Mass Spectrometer. An instrument used for the recording of separated ion beams by means of dedicated electronic devices; more suited for the measurment of isotopic abundance.

Molecular ion. The ion formed by removal of one electron from a molecule (positive) or the addition of one electron to a molecule (negative). The molecular ion represents the ionized molecule containing only the isotopes of greatest natural abundance.

Fragment ion. An ion formed by the cleavage of one or more bonds in the molecular ion. It may be an even electron ion (ion produced by removing an electron from a radical) or an odd electron ion (ion producing by removing an electron from a molecule).

Rearrangement ion. An ion formed by the rearrangement process of some ion species, including the molecular ion.

Parent ion. The precursor to a given fragment ion, not necessarily the molecular ion.

Daughter ion. Any ion generated by fragmentations as opposed to direct ionization.

α-Cleavage. The cleavage of a bond adjacent to an electron deficient group.

β-Cleavage. The cleavage of a bond β (one bond removed) to a given atom.

Allylic cleavage. The cleavage of a bond one removed from a double bond.

Percentage Total Ionization. The abundance of an individual ion compared with the

sum of the abundance of all the ions in a specified mass range or of a specified ion type.

Resolution: 10% valley definition. Let two peaks of equal height in a mass spectrum at masses m and m+Δm be separated by a valley which at its lowest point is equal to 10 per cent of the height of either peak. The resolution can then be described as m/Δm at a 10% valley definition. For example, when two masses (100.000, 100.005) are separated by a 10 per cent valley, the resolution of the instrument is 100.000/0.005, i.e. 20,000.

I. INTRODUCTION

Mass spectrometry has found wide utility in identifying and elucidating the structure of many classes of organic and biologically-active compounds[1]. Owing to their role in biochemistry and medicine, steroids were among the first natural products to be studied[2-4]. Reed and coworkers[2] concentrated on the use of low-voltage EI spectra for the determination of the molecular weight, and of the size of the side chain in sterol and triterpene types. They also listed the most important fragments in the high voltage EI spectra of a few members of these classes. Friedland *et al*[3] focused on the principal fragmentation processes of steroid alcohols having heterogeneous substituent groups. Mass spectrometry has been used for the determination of molecular weights and fragmentation patterns of bile acids[4,5]. A review of all the mass spectral studies reported to date on steroids will not be attempted here. For a comprehensive study of the major fragmentation routes and numerous types of fragmentation patterns found in different steroids, the reader is referred to several reviews[1,6-11]. Instead, discussion will focus upon studies which are of direct concern to the stereochemistry of water elimination from steroids having labile hydroxyl group(s) as well as to stereoselective silanol eliminations from silyl derivatives of steroid alcohols.

Elimination of H_2O from steroid alcohols.

Stereochemical requirements for water elimination from steroid alcohols have received considerable attention. Biemann and Seibel[12], in their pioneering study, reported that the orientation of the hydroxyl group in 3-hydroxy androstanes can be determined by mass spectrometry. Thus, a 3-axial hydroxy group was lost as water more readily than a 3-equatorial hydroxy group. The relative abundance ratio $[M - H_2O]^{+\cdot}/[M]^{+\cdot}$ was the criterion chosen for differentiation between epimers. This ratio for the epimer with an axial 3α-hydroxyl group was higher than for the epimer with an equatorial 3β-hydroxyl group. This communication sparked attempts by others to use

mass spectra to define the stereochemistry of steroid alcohols.

Wulfson *et al*[13], Ananchenko *et al*[14], and Zaretskii *et al*[15-16] studied the effect of the position of the hydroxyl group on fragmentation pathways in the mass spectra of tertiary and secondary alcohols. Studies undertaken on two pairs of epimeric 3-methoxy-17ξ-alkyl-1,3,5(10),8-D-*homo*estratetraen-17ξ-ols[16] and their 17ξ-CH_3-d_3-analogues, supported Biemann and Seidel's idea that the epimers with the axial 17α-hydroxy group underwent dehydration upon electron impact more readily than did their 17β-hydroxy epimers. Deuterium labeling established a contribution from 1,2 elimination of water[17], which normally occurs rarely upon electron impact, and was thus strongly criticized by Pandit[18], who suggested that it resulted from thermal decomposition. In the case of 17β-CD_3-analogues[16], the M^+ ions resulting from dehydration of the epimer with the 17α-hydroxy group gave rise to fragment ions having an *endo*cyclic double bond. On the other hand, elimination of water from the epimer with the 17β-hydroxy group resulted in ions with both *endo*- and *exo*-cyclic double bonds. The presence of ions with an *endo*- or *exo*-cyclic double bond, in the mass spectra of tertiary alcohols, was proposed for differentiation of the 17ξ-hydroxy epimers. A similar mechanism of dehydration was proposed for 3-methoxy-17ξ-methyl--1,3,5(10)-estratrien-17ξ-ol[19], in which the 17α- and 17β-substituents possess pseudoaxial and pseudoequatorial character in ring D. The elimination of a 17-hydroxy group, together with one of the pseudoaxial hydrogens from ring D, was suggested by these authors as additional proof of its axial configuration.

The studies of Wulfson *et al*[20] on 14α- and 14β-hydroxy-D-*homo*estrones labeled with deuterium, although with poor deuterium incorporation, demonstrated greater loss of HOD when 14ξ-hydroxy and 9ξ-H were spacially accessible, i.e. *cis*-diaxial, with respect to each other. The same compounds, deuterated at C-8, showed H_2O rather than DOH elimination[20].

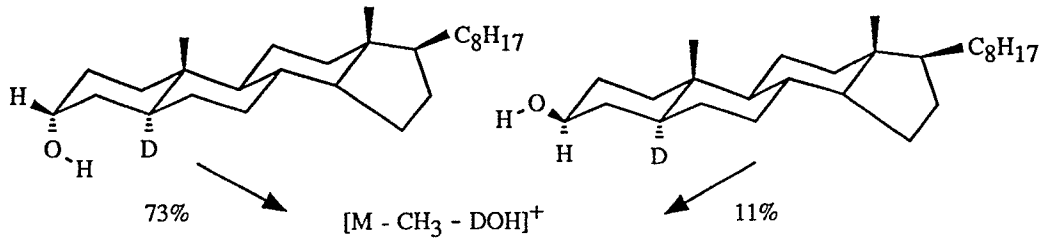
The epimeric pairs of secondary steroid alcohols, 11ξ-hydroxy- and

16ξ-hydroxy-progesterone, provided other molecular sets demonstrating these proximity effects[16]. Both 11α-hydroxy- and 16β-hydroxy- progesterone show little loss of water; the 11α-oxygen cannot approach the secondary hydrogens at C-1, and the 1,4 related hydrogen (14α-H) is not available. The 16β-hydroxy group may abstract hydrogen only from the 18-methyl group. In contrast, for 11β-hydroxy- and 16α-hydroxy-progesterone loss of water was significant, and eliminations of the C-8 and C-14 tertiary hydrogens, respectively, were suggested[21].

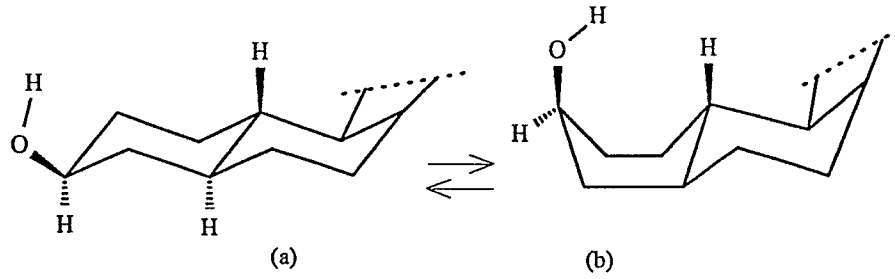
Early success in the observation of stereoisomeric dependence of the mass spectra of steroid alcohols led to the conclusion that "The results obtained permit a new approach to the determination of the configuration of steroid and possibly other cyclic alcohols by means of mass spectrometry"[15]. It was also noticed that the abundance ratio $[M - H_2O]^{+\cdot}/M^{+\cdot}$ was greater than unity for the axial, and lower than unity for the equatorial, hydroxy isomers (ring A)[20,22]. This ratio has been suggested as a criterion for the configurational assignment of the hydroxy group, even when only one isomer is available. This criterion was used in the determination of the configuration of 14α- and 14β-hydroxy-*homo*testosterone as well as of 11-hydroxy- and 16-hydroxy-progesterone[20]. This rule has also been extended to ring D, for which elimination of H_2O proceeds more easily from the 17α-hydroxy isomer (considered to be axial) than from the β-hydroxy isomer[20, 22]. This generalization was shown to be unreliable and a comparison of the ratio $[M - H_2O]^{+\cdot}/M^{+\cdot}$ for the epimers rather than its ratio (>1 or <1) was recommended[23].

Karliner, Budzikiewicz, and Djerassi[24] were the first to uncover the stereospecificity of the EI induced elimination of water from C-3 epimers of cholestan-3-ol. A deuterium-labeling study revealed that 3α-hydroxy-5α-cholestane lost water from $[M - CH_3]^+$ ions by abstraction of a 1α- or 5α-hydrogen. An activated tertiary 5α-hydrogen is sterically accessible in a 1,3 relationship to the 3α-hydroxyl group. This case is noteworthy in that elimination of water occurs only *after* loss of a

methyl radical from the molecular ion, i.e. from an even-electron ion. Hydrogen elimination from the corresponding 3β-hydroxy analog, in which the hydroxy group is in the equatorial position, tended to be more random and appeared to involve ring cleavage before loss of water. The percentages shown in Scheme 1 are the fraction of water loss occurring as HOD. The ease of formation of cyclic transition states for H_2O elimination for axial alcohols appears to be the reason for the higher $[M - H_2O]^{+\cdot}$ abundance in the spectra of these isomers.



Scheme 1. Water elimination from an even-electron ion (direct inlet, 70 eV)[24].



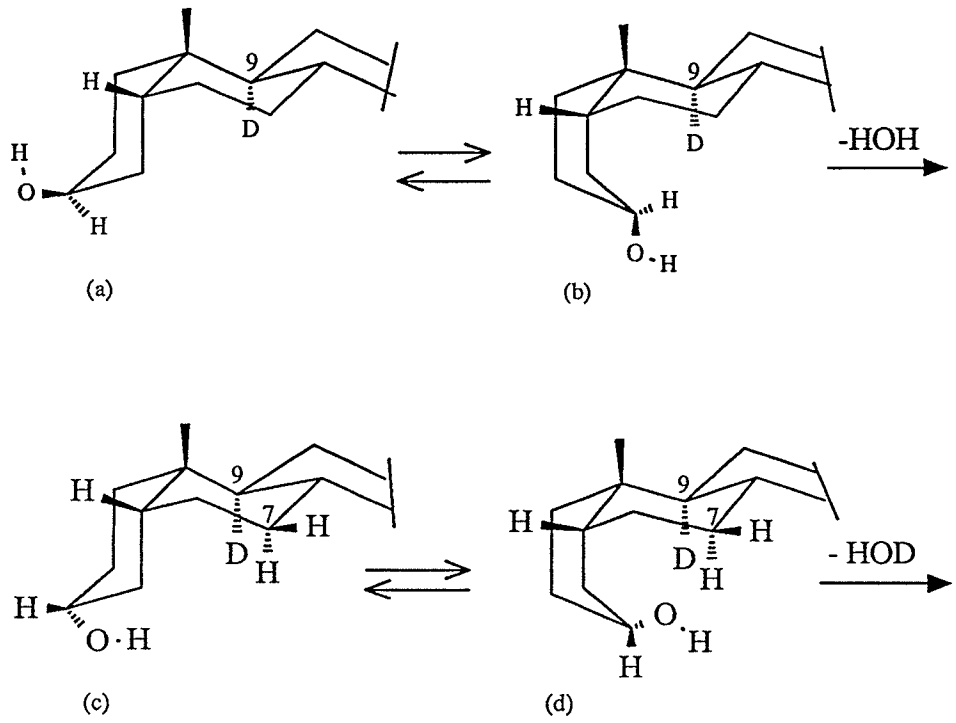
Scheme 2. Proposed water elimination from the boat form of a 3β-19-nor steroid[25].

There is little difference between the abundances of $[M - H_2O]^{+\cdot}$ ions in the mass spectra of 3α- and 3β-hydroxysteroids that have 17β-C_8H_{17}, 17β-hydroxy or 17-oxo

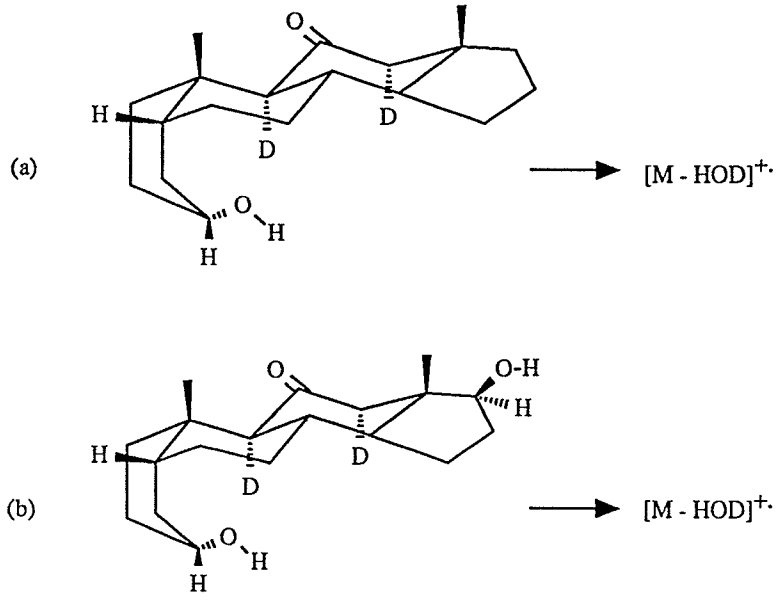
substituents[11]. However, the difference in water elimination becomes pronounced for their 19-nor analogues. Thus, Egger and Spiteller[25] reported that the tendency for 3-hydroxy steroids to eliminate water depends on their stereochemistry. In the mass spectra of 3β- and 3α-hydroxy-19-nor steroids, they observed that the 3β-alcohol underwent more facile dehydration than did the 3α-epimer, which suggested that a *cis* 1,4-elimination, in which the 3β-hydroxy group could be brought to within a short distance of the 10β-H in the boat conformation, took place, (see Scheme 2).

Egger and Spiteller[25] also noticed that the geometry of the junction of rings A and B has a strong effect on the dehydration of 3-hydroxy steroids. This process occurred to a much greater extent for the *cis*-A/B fused steroid alcohols than for the *trans*-isomers, and the preference was explained by the availabilty of the 9α-hydrogen atom in the cyclic transition state of the appropriate conformation, which is only possible for the *cis*-A/B fused isomer. The greater ease of water elimination from the 3α-hydroxy steroid compared to the 3β-hydroxy steroid of the 5β-series can be explained by conformational changes of ring A. Thus, as shown in Scheme 3, the chair conformation (a) may be converted to the boat conformation (b), and the C-3 hydroxy group changes from axial to equatorial with still poor proximity to the deuterium[25]. In the case of an initially equatorial 3α-hydroxy group (c), the reverse is true, and when it becomes axial (d), it is now closer to the 9α-D and C-7 hydrogens[25, 26]. The same dehydration mechanism was proposed by Elliot[27] for facile water elimination from 5β-H,3α-OH bile acids. The adoption of a boat conformation of ring A, as suggested by Egger and Spiteller[25], has also been supported by deuteration of 5β-cholan-3α-ol[28] at C-9.

Klein and Djerassi[26] found that the 3α-hydroxy group of 5β-cholane abstracts the 9α-deuterium in the course of dehydration, after ring A has changed to the boat structure, Scheme 3(c,d). A different mechanism must operate for the corresponding decomposition of 5β-cholan-3β-ol-9α-d_1 because only H_2O is ejected, Scheme 3(a,b). They concluded that the spatial separation of the hydroxyl group from an active hydro-



Scheme 3. EI-induced elimination of H_2O from cis-A/B fused steroids: (a,b), 3β-OH[26, 29]; (c,d) 3α-OH[25].

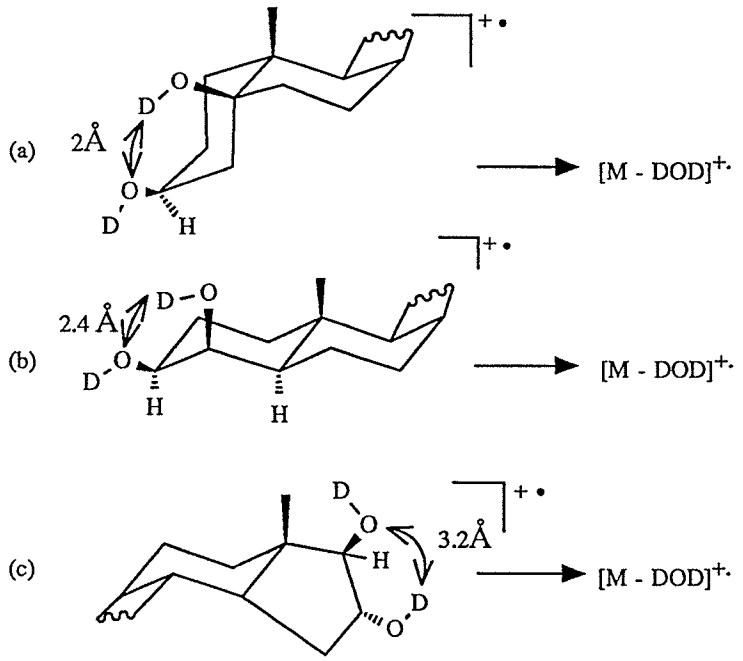


Scheme 4. EI-induced elimination of HOD from (a) 3α-hydroxy-5β-androstan-11-one-9,12α-d_2 and (b) 5β-androstan-3α,17β-diol-11-one-9,12α-d_2[30].

gen governs the extent of water elimination. A Dreiding model shows that the 9α-H atom may be brought to within 0.4 Å of the 3α-oxygen atom in the boat conformation of ring A. Similarly, Ende and Spiteller[28], investigating water elimination reactions of 3α-hydroxy-5β-androstan-11-one-9,12α-d_2 and 5β-androstan-3α,17β-diol-11-one--9,12α-d_2[2], showed stereospecificity in abstraction of the 9α-D by the 3α-hydroxy group (Scheme 4). For both compounds HOD, and not H_2O, was eliminated from both molecular and fragment ions (i.e. to give $[M - HOD]^{+\cdot}$ and $[M - (HOD + C_4H_6)]^{+\cdot}$. Their findings also imply that the secondary 17β-OH group is able to abstract an activated 12α-D (Scheme 4(b)).

An apt demonstration of this regioselectivity for the elimination of water was found in studies of 3β,12α,17β-trihydroxy-5α-androstane and its 12β-hydroxy isomer[29]. Although both isomers contained a pseudoaxial 17α-deuterium, the former sterol eliminates DOH, while the 12β-hydroxy epimer does not. This pseudoaxial, tertiary, abstractable 17α-hydrogen with reduced bond energy[30] is 1,3-related to the axial 12α-hydroxy group (four-membered ring for H transfer). On the other hand, the mass spectrum of the tertiary steroid alcohol, 17α-hydroxyprogesterone-11,11,12,12-d_4, showed that its dehydration process is quite different[31]. In this case, the pseudoaxial tertiary 17α-hydroxy group was eliminated solely as H_2O. Thus, all deuterium atoms at C-11 and C-12 were retained in the ion.

Evidence for the EI-induced steric sensitivity of 3-hydroxy steroids and related fused ring compounds towards water elimination comes from findings of Fenselau and Robinson[32] that diols capable of internal H-bonding in solution, deuterium-labeled as in Scheme 5, exhibit EI-induced loss of deuterium oxide. The direct interaction of two OD groups was inferred for (a) 3β,5β-dihydroxy-5β-cholestane, (b) 3β,4β-dihydroxy-5α-cholestane, and (c), and 16α,17β-dihydroxy-4-androsten-3-one. The estimated O-D...O distances, for a, b, and c within which D_2O loss occurred, were 2.0, 2.4, and 3.2 Å, respectively.

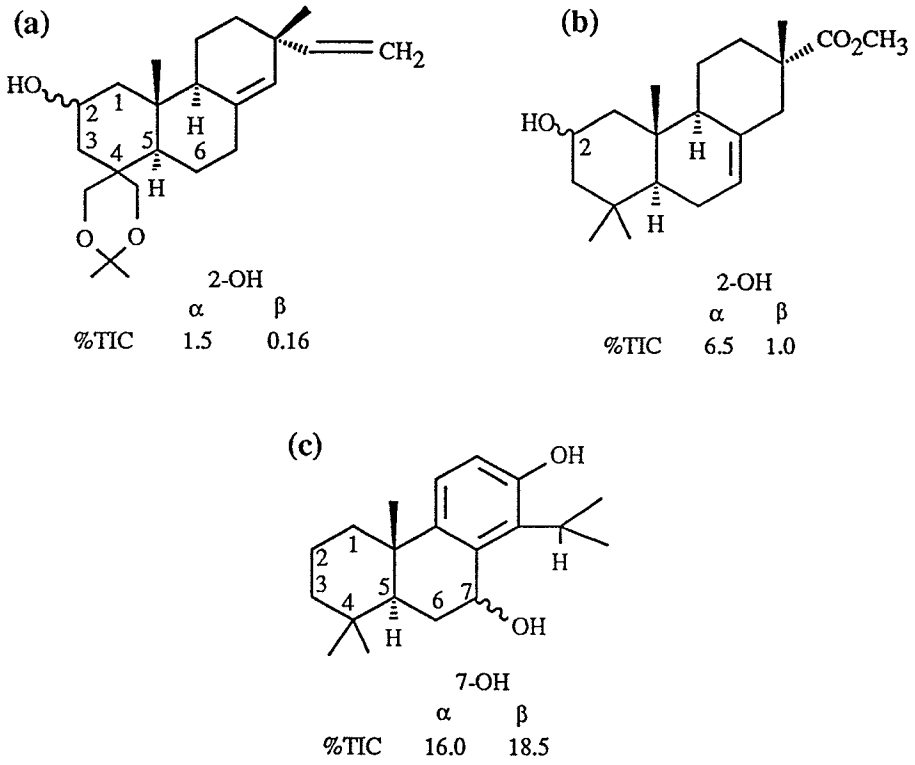


Scheme 5. EI-induced elimination of dideuterium oxide, demonstrating dependency on proximity of two hydroxy groups[28].

Studies on bile acids[27] provide a useful test for prediction of the propensity for EI induced elimination of water. Thus, Sjövall[27] pointed out that molecular ions are of low relative abundance for 3α-, 7α-, and 12α-hydroxy-5β-cholestanes; this is also true for the 7α-, 7β-, and 12α-ols of the 5α-series, but abundant molecular ions were noted for the 3β- and 7β-ols of the 5β-series, and for the 3α-ol of the 5α-series. Green[27] indicated that, for bile acids, the 6α-hydroxyl in the twist-boat configuration of ring B (with rings A and C having chair conformations) is within 1.6 Å of the C-1 and C-3 hydrogens. Moreover, these hydrogens are 1,4 related (six-membered ring for H transfer) to the hydroxyl group and they can be abstracted. The 1,4 relationship for the elimination of 7α-OH and 4α-H was considered to explain the loss of water from 5α-cholanic acid.

Enzell, Wahlberg, and Gunnarsson[34] noted that, in a series of sixteen pairs of

diterpene epimers (Scheme 6), if an axial 2-OH group can approach within 1.6 Å of the C-5 tertiary hydrogen atom in the boat conformation of ring A of compounds or (a) and (b), or, if any hydroxyl group of (c) is within 1.4 Å of the tertiary benzylic hydrogen on the pendant isopropyl group, then high stereospecificity for EI-induced loss of water is observed. In general, an increase in EI-induced loss of water is observed when a tertiary hydrogen is 1,3 related to a hydroxy group.



Scheme 6. Water elimination (%TIC) from $M^{+\cdot}$ ion of diterpene epimers. (T = 270^0C, direct inlet)

Fragmentation patterns of stereoisomers are a function of their steric and energetic differences. Because conformational energy differences are small, to detect differences between stereoisomers, the internal energy of nascent ions should be

minimized[22]. Therefore mass spectrometry of positive ions or negative ions generated by chemical ionization (CI) was suggested as a promising method for identification of stereoisomers because CI is a softer ionization technique than is EI. By selecting the appropriate reagent gas, temperature, and pressure, the internal energy of the molecular ion can be altered. Simplification of mass spectra by CI is well established for many compounds.

Michnowicz and Munson[35] examined the methane and H_2 positive CI mass spectra of several hydroxy steroids. The major fragment ions were formed by loss of water from $[M + H]^+$. The 5α- and 5β-androstan-3-one-17β-ol epimers could be distinguished by marked differences in the abundances of $[M + H]^+$, $[M + H - H_2O]^+$, and $[M + H - 2H_2O]^+$ ions in their CI spectra generated with the more energetic reactant gas H_2. The 5α-epimer showed smaller losses of one and two molecules of water than did the 5β-epimer. The difference was explained by chair-to-boat interconversion of ring A, in which the distance between 3-OH and 9α-H was much shorter in the 5β than in the 5α-epimer. The spectra of 5α-androstane-3α-ol and 3β-ol were essentially the same.

Beloeil *et al*[36] studied the OH^- negative chemical ionization (NCI) mass spectra of 17ξ-R-5α-androstan-17ξ-ol (R = H, C_2H or C_2H_3) and 17ξ-R-5α,14β-androstan-14β,17ξ-diols (R = CH_3, C_2H, C_2H_5 or C_2H_3). All spectra contained abundant characteristic deprotonated molecules $[M - H]^-$, which underwent competitive loss of water and either (i) an exocyclic hydrocarbon (RH) for tertiary alcohols, or (ii) molecular hydrogen (H_2) for secondary alcohols. No stereochemical effect for hydroxy groups in the C-17 position was observed. On the other hand, the diols exhibit a very marked difference between the spectra of their stereoisomers; only the trans 14β,17α-diols eliminate a hydrocarbon molecule to a significant extent. Deuterium labeling of the alcohol groups revealed that intramolecular interactions between 14β-OH and 17β-OH groups play a major role in stabilizing the resulting $[M - H - RH]^-$ fragment anion.

Prome *et al*[37] examined the methane and ammonia positive CI and OH^- negative

CI mass spectra of a series of four stereoisomeric 5ξ-androstan-3-one-17ξ-diols and eight 5ξ-androstan-3ξ,17ξ-diols using a reverse-geometry, double-focusing mass spectrometer. When CH_4 was employed as the reactant gas, the spectra of the diols were dominated by $[M + H - H_2O]^+$ and $[M + H - 2H_2O]^+$ ions. The abundances of these two species were greatly dependent on the stereochemistry at C-17; the value of the $[M + H - 2H_2O]^+$/[M + H - $H_2O]^+$ abundance ratio was much larger for the 17α-epimers. Similar behavior was observed when NH_3 was used as the reactant gas. The higher abundance of [M + H - $H_2O]^+$ ions for the 17α-hydroxy series was attributed to closer proximity of the axial hydroxy group to other ring D hydrogen atoms as compared to the equatorial epimer. The same stereochemical effect on dehydration (preferentially for the 17α-hydroxy series) was observed in the mass-analyzed ion kinetic energy (MIKE) spectra of the [M + $NH_4]^+$ ions formed, under NH_3/CI conditions, from the diols[37]. Under OH^-/NCI conditions, the abundant $[M - H - H_2O]^-$ ions indicated that the loss of water was once again favored in the 17α-series.

The conformational assignment of epimeric hydroxy steroids, differing in the orientation of the hydroxy group, is very often obscured by thermal dehydration of molecules prior to ionization. The occurrence of dehydration is supported by the presence of an $[M - 54]^{+\cdot}$ ion, which was suggested to be formed by retro-Diels-Alder (RDA) decomposition of the molecular ion, which has 2-unsaturation, of the molecule formed by thermal dehydration of the hydroxy steroid[38]. Therefore, fragmentations of metastable $M^{+\cdot}$ ions of hydroxy steroids were proposed as more suitable for study of the mechanism of water[38] elimination. Measurements of translational energy released during the loss of water, and collision-induced decomposition (CID) mass-analysed ion kinetic energy (MIKE) spectrometry, were employed to determine the configuration of the secondary steroid alcohols[38-40]. In certain cases, namely, for α,β-unsaturated 3-keto steroids with a hydroxy group in rings C or D, it was found that configurational assignment is possible even if only one of the epimers is available[39]. It was also found

that the mechanism of water elimination from $[M - CH_3]^+$ and $[M - \text{ring D}]^{+\cdot}$ ions of epimeric 3-hydroxy 5α- and 5β steroids differs from that of the molecular ion[38]. Differences between the dehydration mechanisms of the metastable $M^{+\cdot}$ and $[M - CH_3]^+$ ions of the 5α-H,3ξ-OH isomers were suggested to be connected with the flexibility of ring A in $M^{+\cdot}$. As previously reported by Karliner *et al*[24], labeling data support the idea that water elimination from the metastable $[M - CH_3]^+$ ion occurs by 1,3 elimination of the 3α-OH group, together with the 5α-H atom (75%) and 1α-H atom (16%). On the other hand, for the metastable $M^{+\cdot}$ ion, ~20% of the hydrogen participating in dehydration came from the 5α-position. The metastable $[M - \text{ring D} - H]^+$ ion lost water by 1,3 elimination of 3α-OH and 5α-H (40%), and of 3α-OH and 1α-H (7%). In the 5β-series[40], elimination of water from metastable $M^{+\cdot}$, $[M - \text{ring D} - H]^+$, and $[M - \text{ring D}]^{+\cdot}$ ions of the 3α-hydroxy steroid occurred as a loss of the 3α-OH group together with a hydrogen atom, about 40% of which came from the 9α-position. The observed dehydration was explained by conformational changes of ring A from a chair to a boat form. This mechanism is in agreement with the results reported earlier by Klein and Djerassi[26].

Elimination of alkylsilanol from silyl ether derivatives of steroids.

In certain cases, the formation of derivatives has been recommended in order to permit stereochemical differentiation of steroid alcohols by mass spectrometry, especially in combination with gas chromatography. It was shown that methyl ethers or acetates of 3-hydroxy steroids exhibit behaviour similar to that of the corresponding alcohols. Thus, methyl ethers and acetates eliminate CH_3OH[41] and CH_3COOH[42], respectively, preferentially from the axial isomer. Silyl ether derivatives are also employed. Before dealing with the problem of stereochemical assignments with the aid of silyl derivatives, a short introduction to generally used silyl ethers is necessary.

By modification of the silyl group, a number of substituted silyl derivatives have been introduced for characterization of biological compounds by gas phase analytical methods. Some examples of these silyl groups are : **alkyldimethylsilyl**, RMe_2Si-, where R = hydrogen[54-57], ethyl[58], *n*-propyl[58], isopropyl[59], *tert*-butyl[60-71], allyl[66,72], vinyl[73], methoxy[75], ethoxy[75], chloromethyl[76-79], bromomethyl[77,80,81], iodomethyl[77,82,83], trifluoropropyl[76], heptafluoropentyl[76], pentafluorophenyl[76-79], phenyl[88] or benzyl[88]; **trialkylsilyl**, R_3Si-, where R = ethyl, *n*-propyl, *n*-butyl, or n-hexyl[89-91]; **alkoxyalkylsilyl**[83], $R^1OR^2R^3Si$- , where $R^1= R^2= CH_3$ and $R^3= C_2H_5$; $R^1= CH_3$ and $R^2= R^3= C_2H_5$; and **sterically crowded trialkylsilyl**[92,93] (SCATSi) represented by *cyclo*-tetramethylene-*tert*-butyl- (TMTBSi) and *cyclo*-tetramethylene-*iso*-propylsilyl (TMIPSi). All of these reagents have been synthesized for specific purposes.

As first introduced, chloromethyldimethylsilyl ethers ($ClCH_2DMSi$) were used for electron capture detection gas chromatography[77,80]. The mass spectra of the XCH_2Me_2Si-ethers[81] of steroids (X = halogen) have a weak molecular ion and a characteristic, abundant ion at $[M - CH_2X]^+$. As the halogen decreases in electronegativity, the silicon-CH_2X bond becomes stronger, and loss of CH_2X from $M^{+\cdot}$ becomes less likely[81]. For mono-hydroxy steroids, elimination of $XCH_2Si(CH_3)_2OH$ from the molecular ion is a characteristic fragmentation. The elimination of dimethylsilanol, $HSiMe_2OH$, from $[M - ClCH_2]^+$ ions also occurred but, at that time, was not recognized[81].

Trimethylsilyl ether derivatives (TMSi) were the first to find wide application, not only in gas phase studies[43], but also in organic chemistry[44, 45]. They were introduced to separate complex biological mixtures on low-resolution packed GC columns[44, 45, 89, 94, 95]. TMSi-ethers of steroid alcohols, and their use in gas phase analytical studies, were first reported by Luukkainen *at al*[43]. TMSi-ethers have excellent volatility and thermal stability for GC/MS analysis, and are easily prepared[44, 45]. However, they suffer from the disadvantage of being very sensitive to protic media, and this limits their use in

analytical and synthetic chemistry; TMS ethers are 10^4 times less stable to hydrolysis than *t*-butyldimethylsilyl (TBDMSi) ethers[46]. Furthermore, resolution of isomers as TMSi-ether derivatives on a GC column is difficult. Their mass spectra have abundant $[M - TMSiOH]^{+\cdot}$, $[M - CH_3]^+$, and molecular ions.

To improve resolution, homologous trialkylsilyl ethers, such as triethylsilyl- (TESi), tri-*n*-propylsilyl- (TnPSi), and tri-*n*-butylsilyl- (TnBSi), were used to increase retention increment shifts for compounds containing two or more hydroxy groups so that they could be separated from monohydroxy compounds[90-92, 96]. In general, molecular ions were weak or absent in their mass spectra, which showed a prominent ion due to cleavage of a silicon-alkyl group bond. The higher mass region of the spectra contained a series of relatively abundant ions produced by elimination of C_nH_{2n} fragments from the silyl groups. Elimination of dialkylsilanol, $HSiR^1_2OH$ (R^1 = alkyl), from the $[M - R]^+$ ions was observed to be the major mode of fragmentation, which parallels loss of trimethylsilanol (TMSiOH) observed for the TMS derivatives. Samples containing two silyl substituents had abundant doubly charged ions due to cleavage of an alkyl group from both silicon centers[89, 96].

Searching for more hydrolytically stable silyl derivatives, Corey *et al*[47-49] proposed the isopropyldimethylsilyl (IPDMSi) and TBDMSi ethers as useful protecting groups in prostaglandin synthesis. In general, the TBDMSi ethers were found to be stable to aqueous or alcoholic base under the normal conditions for acetate saponification, hydrogenolysis (H_2/Pd), mild chemical reductions (Zn/CH_3COOH), and chromium trioxide/pyridine oxidation techniques[49-53]. The alkyldimethylsilyl substituents, *tert*-butyldimethylsilyl (TBDMSi)[60-71], isopropyldimethylsilyl (IPDMSi)[59], ethyldimethylsilyl (EDMSi)[58], and *n*-propyldimethylsilyl (PDMSi)[58] were introduced as protecting groups for steroid analysis.

The allyldimethylsilyl (ADMSi)[66, 72] derivatives were synthesized to provide derivatives more hydrolytically stable than TMS ethers but more volatile than TBDMSi

ethers. A further useful feature of ADMSi derivatives is the ability of the allyl substituent to stabilize the molecular ion[77].

The mass spectra of organodimethylsilyl derivatives of steroids showed intense, weak, or absent, molecular ions, with an abundant $[M - R]^+$ ion (R = ethyl, propyl, *t*-butyl, allyl). The relative abundance of the $[M - R]^+$ ion increased in the order: EDMSi < PDMSi << TBDMSi, and ADMSi < TBDMSi. The $[M - t\text{-Bu}]^+$ and $[M - \text{allyl}]^+$ ions were recommended for single ion monitoring[62, 66, 69], due to their high abundance. Further decomposition of the $[M - R]^+$ ions gave characteristic, very abundant $[M - R - HSi(CH_3)_2OH]^+$ ions.

In contrast to most alkyldimethylsilyl derivatives, vinyldimethylsilyl[73, 74] (VDMSi) and dimethoxymethylsilyl[75] derivatives combined the stability of the TBDMSi-ethers with the fragmentation behavior of the TMS-ethers. Loss of VDMSiOH (102 u) from $M^{+\cdot}$ and the formation of diagnostic silicon-containing ions were dominant in the VDMSi spectra, paralleling the fragmentation behavior of the TMS derivatives. The vinyl group was not lost, unlike the large alkyl groups of alkylsilyl derivatives. This was attributed to the stability of the Si-vinyl bond, enhanced by donation of the π-electrons to the d orbitals of silicon[74]. However, the vinyl group was eliminated to a small extent as ethylene[74] (28 u) by hydrogen migration from the steroid skeleton in a manner analogous to the loss of ethane[96] (30 u) and propene (42 u) from triethylsilyl and allyldimethylsilyl[66] derivatives, respectively. For all cases, the origin of the migrating hydrogens was not determined. Fragmentation of the $[M - CH_3]^+$ ion of the VDMSi derivatives by loss of methylvinylsilanol (102 u) was slightly more important than the corresponding loss of dimethylsilanol (76 u) in the spectra of the TMS derivatives[67, 74].

The sterically crowded trialkylsilyl[84, 85] (SCATSi) derivatives were found to be resistant to hydrolysis. Their mass spectra show an abundant $[M - R]^+$ ion while molecular ions were weak or absent. This ion was often the base peak and the precursor for the remaining silicon-containing fragments[67, 93].

In general, the fragmentation patterns of silyl ethers have been classified into two types[99, 100]. In the first type, exemplified by TMS derivatives, abundant, diagnostic $M^{+\cdot}$ and $[M - CH_3]^+$ ions, are formed; additionally TMS groups are eliminated from the molecular ion as trimethylsilanol, to give $[M - TMSiOH]^+$ ions. These fragmentations are greatly favoured and structurally informative. For steroid alcohol derivatives the expelled CH_3 group can originate either from the TMSi group or from one of the angular positions. Thus, both M^+ and $[M - CH_3]^+$ ions are potential precursors for loss of TMSiOH. However, the $[M - CH_3]^+$ ion, in which the methyl group has been lost from the TMS substituent, can solely be a precursor of $[M - CH_3 - HSi(CH_3)_2OH]^+$ ions observed in some spectra[60, 66, 67, 73, 101, 102]. Their abundance was very sensitive to the structure of the examined steroid. The type of fragmentation leading to $[M - RSiMe_2OH]^{+\cdot}$ (R = alkyl) is given by the following ether derivatives: TMSi, VDMSi, methoxydimethylsilyl, and dimethoxymethylsilyl.

The second type of fragmentation leading to $[M - R - HSiMe_2OH]^+$ (R = alkyl) is shown by most derivatives containing alkyl groups larger than methyl attached to the silicon atom. The major fragmentation route is initiated by elimination of the alkyl group, to form $[M - R]^+$ ions, followed by loss of dimethylsilanol (HMe_2SiOH), formed by a double hydrogen rearrangement, rather than by loss of trialkylsilanol (RMe_2SiOH) from $M^{+\cdot}$. This fragmentation pattern results in simple spectra containing abundant $[M - R]^+$ ions suitable for single ion monitoring[62, 63, 66]. This class is represented by the following ether derivatives: TBDMSi, trialkylsilyl, ethyldimethylsilyl, tri-isopropylsilyl, di-isopropylmethylsilyl, cyclotetramethylene-isopropylsilyl, cyclotetramethylene-*t*-butylsilyl, methoxydiethylsilyl, methoxydimethylsilyl, and allydimethylsilyl.

From the point of view of reactivity of a silyl reagent, it was found that steric factors were very important in controlling the rate and extent of the silylation reaction. For example, for the hydroxy group, the order of reaction rate of the silyl reagents is primary > secondary > tertiary[40, 41]. For unhindered hydroxyl groups the approximate

order of reactivity of the organodimethylsilyl reagents is: TMSi > ADMSi > EDMSi > PDMSi >> TBDMSi. Futhermore, the TMS group has similar geometry to the *t*-butyl group, but the TBDMSi group is larger. Trialkylsilyl and alkyldimethylsilyl groups are also bulkier than the TMS group so that the steric effect is magnified[96]. For example, to derivatize the tertiary 17α-OH group of 5β-pregnan-3α,17α,20α-triol, heating for 10 hr at 100°C with ethyldimethylsilylimidazole was required for complete reaction, compared to heating for 2 hr at 100°C with trimethylsilylimidazole (TMSIm[97]). The allyldimethylchlorosilyl reagent[62] was not so subject to a steric effect and reacted readily even with hindered hydroxyl groups under mild conditions. TBDMSiCl was unreactive even under forcing conditions[62]. Ballhorn *et al*[98] reported successful derivatization of the tertiary 17β-hydroxy group of 17α-methyl-17β-hydroxy-5α-androstan-3-one by the bulky TBDMSiCl reagent at elevated temperature (40° for 2hr), but the reported partial mass spectrum implies silylation of the 3-enol rather than the 17β-hydroxy group. The presence of the molecular ion (25% of base peak) is suspicious. There is no reason for stabilization of $M^{+\cdot}$, unless a double bond is present in the vicinity of the derivatized group.)

A survey of the reported spectra of TBDMSi ethers of saturated keto-steroids having a secondary hydroxyl at C-3 or C-17[62,63,67,69], showed the absence of $M^{+\cdot}$. In contrast, abundant molecular ions were observed for TBDMSi ethers of enolized testosterone[63], methyltestosterone[63], 20β-hydroxy-4-pregnen-3-one[63], progesterone[98], 4-androsten-3-one[67], and 3-OSiDMTB-1,3,5,(10)estratrien-17-one[62]. These data provide evidence that 2- or 3-unsaturation (i.e. next to the 3-OSiDMTB substituent) is required to stabilize $M^{+\cdot}$. Therefore, the formation of 17β-OSiDMTB-17α-methyl-5α-androstan-3-one postulated above [98] must be considered unreliable.

The application of mass spectrometry to structure elucidation of steroid alcohols as their chlorodimethylsilyl (ClDMSi) derivatives was noted over 18 years ago[81], i.e. to identify the stereochemistry of the 3-hydroxy group and of the A/B ring junction. For

example, formation and elimination of $ClCH_2Me_2SiOH$ from the molecular ion of epietiocholanone was ascribed to the presence of the 5β-hydrogen. Similarly, formation of the m/z 271 ion ([M - $ClCH_2Me_2SiOH$ - H]$^+$, now known to be [M - $ClCH_2$ - $HSiMe_2OH$]$^+$) is favoured in epimers with an axial substituent.

Attempts have been made to utilize trimethylsilyl (TMS) etherification for differentiation of stereoisomeric steroid alcohols[103-105]. Low energy electron ionization measurements have been found to give rise to sufficient differences between the mass spectra of TMS ethers of epimeric estratriols to allow their differentation by mass spectrometry coupled with gas chromatography[105]. Detailed studies with specifically-labeled TMS^{18}O-ethers of several 5α-H steroidal diols show that the elimination of trimethylsilanol (90 u) takes place more readily from 3α-ethers than from 3β-epimers[104]. This leads to more abundant [M - 90]$^{+\cdot}$ and [M - 2x90]$^{+\cdot}$ ions in the mass spectrum of the di-TMS ether of 5α-androstane-3α,17β-diol than in that of the 3β-isomeric di-TMS ether. For the 5β-H,3ξ-OTMS pair of isomers, however, identical abundances of the two relevant ions were reported[104,106]. The elucidation of stereochemistry of substituents can often be determined from rearrangement processes occurring, for example, in the mass spectra of TMS-ethers of steroidal phosphates[107], diols[103, 107] and triols[108, 109], and other hydroxy steroids[95, 107, 110-118]. The presence of two or more trimethylsilyl groups formed from di- or poly-hydroxy compounds often results in the formation of ions of m/z 147, containing two silicon atoms, formed by rearrangement, to give the structure:

$$(CH_3)_3Si\text{-}O\text{-}Si^+(CH_3)_2 \text{ <---> } (CH_3)_3Si\text{-}O^+{=}Si(CH_3)_2$$

Its formation is sensitive to the stereospecificity of trimethylsilyl group interactions[103, 119, 120]. It has been established as a progenitor of the [$(CH_3)_3Si$]$^+$ ion [95,121],

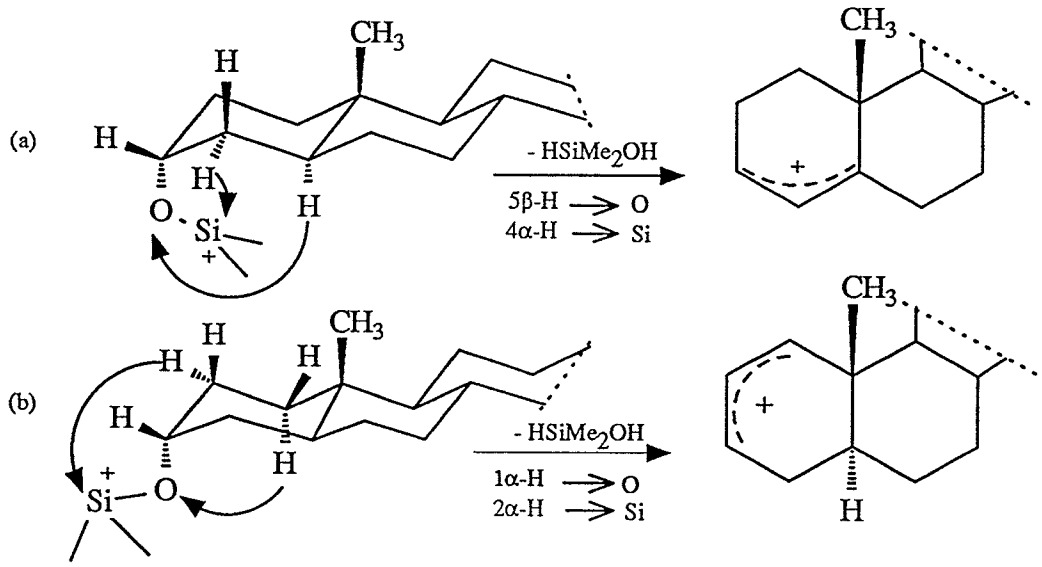
tert-Butyldimethylsilyl ethers were found to be very useful derivatives for steroid

3ξ-alcohol differentiation[67, 69,71,101]. A general fragmentation was loss of 76 u ($HSiMe_2OH$) from the $[M - t\text{-Bu}]^+$ ions, with the steroid skeleton remaining intact[62, 67, 101]. It is interesting to note that only one proposal has been offered as a rationalization for this characteristic fragmentation[67, 101]. This is presented in Schemes 7-9 for three of the isomers of 3ξ-OSiDMTB-5ξ-androstanes. Multicentre transition states were proposed to explain the double hydrogen migration required in the elimination of dimethylsilanol. Conformation and stereochemistry, as well as the position of the silyloxy substituent, were suggested to be important for the stereospecific $HSiMe_2OH$ elimination.

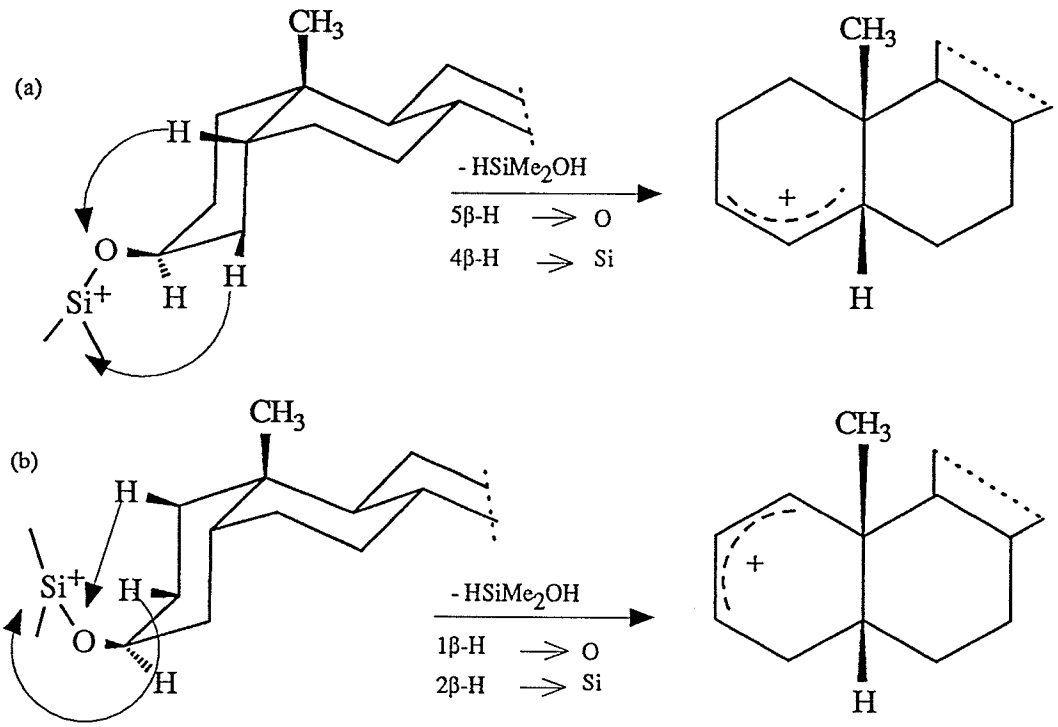
This mechanism was based on a desire to involve rearrangement of two hydrogen atoms when one of them originated from the C-5 position, as accepted by independent considerations[70]. In the first mechanism, Scheme 7(a), the axial C-5 hydrogen migrates to oxygen and the equatorial C-4 hydrogen to silicon, while ring A remains in a chair form, to generate an allylic cation. The second mechanism, Scheme 7(b), involves simultaneous transfer of hydrogens from C-1 (axial) and C-2 (equatorial) to the Me_2Si-O charged site to give, again, an allylic cation.

Similarly, multicentre transition states (a) and (b), Scheme 8, were proposed to explain the abundant $[M - t\text{-Bu} - HSi(CH_3)_2OH]^+$ ions from TBDMSi-ethers of steroids having the 3β-OTBDMSi-5β-H structure. These implied simultaneous rearrangement of a pair of hydrogen atoms, in which an axial hydrogen (5β-H or 1β-H) was transferred to oxygen and an equatorial hydrogen (4β-H or 2β-H) to silicon. Ring A was assumed to retain the chair conformation.

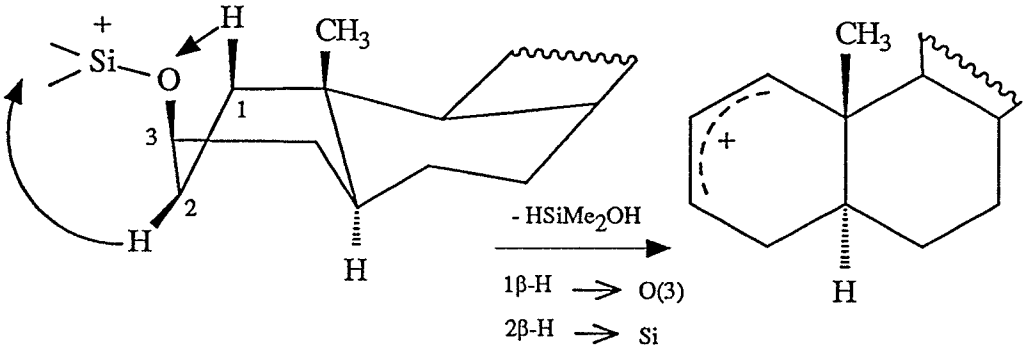
To explain the formation of the $[M - t\text{-Bu} - HSi(CH_3)_2OH]^+$ ions in mass spectra of 3β-silyloxy-5α-steroids, Quilliam and Westmore[67] assumed the chair → boat transformation of ring A, in which the 1β-H migrates to oxygen and the 2β-H to silicon, Scheme 9. Deuterium labeling[122] of 3ξ-OSiDMTB-5α-androstanes at C-2 and C-4 showed dimethylsilanol elimination solely as $HSiMe_2OH$. Even though this result does



Scheme 7. Proposed $HSiMe_2OH$ elimination from the [M - *t*-Bu]$^+$ ion of 3α-OSiDMTB-5β-androstane[67, 101].



Scheme 8. Proposed $HSiMe_2OH$ elimination from the [M - *t*-Bu]$^+$ ion of 3β-OSiDMTB-5β-androstane[67,101].



Scheme 9. Proposed $HSiMe_2OH$ elimination from [M - *t*-Bu]$^+$ ion of 3β--OSiDMTB-5α-androstane[67,101].

not confirm the proposed rearrangement mechanisms[67,101], involvement of H-5 remains anopen question.

Gaskell *et al*[69] investigated the mass spectrometric fragmentation of TBDMSi ethers of eight 5ξ-androstan-3ξ,17ξ-diols, with particular reference to the influence of stereochemistry on competing fragmentation pathways. They observed four competing pathways, each characterized by losses of alkylsilanols [$H(CH_3)_2SiOH$, $(CH_3)_3SiOH$, $H(C_4H_9)CH_3SiOH$ or $C_4H_9(CH_3)_2SiOH$] from a common [M - C_4H_9]$^+$ precursor. The reported fragmentations involved losses of *t*-butylmethylsilanol [$H(C_4H_9)CH_3SiOH$] and trimethylsilanol [$(CH_3)_3SiOH$]. However, they did not attempt to confirm which hydrogens or methyl groups rearranged to the silicenium residue. Similarly, losses of 90 u from the [M - *t*-Bu]$^+$ ion of 20α-OSiDMTB-4-pregnen-3-one were reported, but not discussed, by Quilliam and Westmore[67]. Pronounced differences in the daughter and parent ion spectra obtained by linked scanning complemented the conventional mass spectra in the differentiation of the isomeric structures[69]. Gaskell *et al*[69] concluded that, for bis-TBDMSi ether derivatives of 5α-androstane-diols, $HSiMe_2OH$ elimination is

favored by 3β- and 17β-OSiDMTB stereoisomers. Moreover, among TBDMSi-ethers of 5β-androstanediols, 17α-OTBDMSi stereochemistry appeared to promote loss of $(CH_3)_3SiOH$ and $H(C_4H_9)CH_3SiOH$ from $[M - t\text{-Bu}]^+$ ions.

Gaskell and Pike[70] also examined *bis*-TBDMSi-ether derivatives of eight isomeric 5ξ-androstane-3ξ,17ξ-diols. Fragmentation was strongly directed by the derivatized functional groups. For derivatives of 3-hydroxy-androstanolones loss of dimethylsilanol, $HSiMe_2OH$, from the $[M - t\text{-Bu}]^+$ ion was evidently favoured by the proximity of the 3-alkylsilyl function and the 5-hydrogen. Thus, the $[M - t\text{-Bu} - HSiMe_2OH]^+$ ions were abundant in the spectra of compounds having the 5α-H,3α-OSiDMTB, and 5β-H,3β-OSiDMTB structures. Additionally, for compounds having a secondary 17-OSiDMTB substituent, losses of trimethylsilanol (TMSiOH) were observed.

Harvey and Vouros[123] investigated the fragmentation patterns of some TMS ether derivatives of 6-hydroxy and 3,6-dihydroxy steroids, together with deuterium and ^{18}O-labeled analogs. They noticed that the presence of axial OTMS groups gave prominent ions produced by loss of TMSiOH, attributed to greater hydrogen accessibility to oxygen, while molecular ions were of low abundance. However, no correlation was found between the abundance of the characteristic ions, $[M - 2x90 - CH_3]^+$ and $[M - 131]^+$, and the stereochemistry at C_3, C_5, or C_6.

Harvey[74], examining the mass spectra of alkoxyethylmethyl-($R^1OSiEtMe$-) and alkoxydiethyl- (R^1OSiEt_2-) ethers of 3ξ-OH-5ξ-androstan-17-ones, found that elimination of the ethyl radical, to form very abundant $[M - C_2H_5]^+$ ions, was strongly favoured. Fragmentation of these ions paralleled that of the $[M - alkyl]^+$ ions in the spectra of other alkylsilyl derivatives with alkyl groups larger than methyl. Loss of alkoxyalkylsilanol ($HR^1OSiMeOH$ or $HR^1OSiEtOH$) from $[M - C_2H_5]^+$ ions of the four isomeric 3ξ-hydroxy-5ξ-androstan-17-ones was again favoured. The abundances of $[M - C_2H_5 - HR^1SiMeOH]^+$ or $[M - C_2H_5 - HR^1SiEtOH]^+$ ions were dependent on the

stereochemistry of the steroid derivatives, i.e. they were much higher in the spectra of steroids containing an axial silyloxy substituent.

All spectra of TBDMSi-ether derivatives of 3-hydroxy-17-one and 17-hydroxy-3-one androstanes, and 20-hydroxy-3-one pregnanes, showed the presence of characteristic $[M - 149]^+$ ions. Similarly, $ClCH_2DMSi$-[81] and *i*-PrDMSi-ether[59] derivatives of monohydroxy- or dihydroxy-keto-5β-steroids, with the keto group at C-3, C-17 or C-20, gave abundant $[M - 141]^+$ and $[M - 135]^+$ ion analogues respectively. Chapman and Bailey[81] assigned the $[M - 141]^+$ ion for $ClCH_2DMSi$-ethers specifically as $[M - OH - ClCH_2DMSiOH]^+$. Lisboa and Ganchow[71] described the $[M - 149]^+$ fragment ion as $[M - (134 + 15)^+]$ for mono-TBDMSi ethers, while Phillipou *et al.*[62], assigned it as $[M - 57 - 92]^+$, reporting it to be characteristic of steroids possessing a saturated ring carbonyl. Thus, it is a prominent ion for the silyl derivatives of the following hydroxyketo-5β-steroids: 3α-OR-5β-androstan-17-one where R = TBDMSi[71] (80% RA), $ClCH_2DMSi$[81] (68% RA), and *i*-PrDMSi[59] (69% RA); 17α-OSiDMTB-5β-androstan-3-one (25% RA) and 17β-OSiDMTB-5β-androstan-3-one (15% RA).

The fragmentation patterns of TBDMSi ethers seem to be similar to those of $ClCH_2DMSi$ or *i*-PrDMSi ethers, as judged from the spectra of 3α-hydroxy-5β-androstan-17-one as TBDMSi-, $ClCH_2DMSi$[79], and *i*-PrDMSi[59] ethers. The $[M - 133]^+$ and $[M - 149]^+$ ions in the spectra of TBDMSi-ethers[71] correspond, respectively, to $[M - 125]^+$ and $[M - 141]^+$ ions in the spectra of the analogous $ClCH_2DMSi$-ethers[79] or to $[M - 119]^+$ and $[M - 135]^+$ ions in the spectra of the *i*-PrDMSi[59] ethers. The formation mechanisms of the $[M - 135]^+$, $[M - 141]^+$, and $[M - 149]^+$ ions were neither explained nor suggested. Similarly, a survey of the available spectra showed this ion to be abundant for the following hydroxyketo- or keto-5α-steroids, with or without unsaturation in the steroid skeleton; 3α-OSiDMTB-5α-androstan-11,17-dione[81] (100% RA), 3α-OSiDMCH_2Cl-17-hydroxy-5α-androstan-11-one[81] (19% RA), 3β-OSiDMTB-5β-pregn-16-en-20-one[71] (14.5% RA), 3β-OSiDMCH_2Cl-5-androstene[81] (23% RA),

3β-OSiDMTB-5-androstene[101] (20% RA).

In this thesis I have examined EI mass spectra of the *t*-butyldimethylsilyl derivatives of the eight 17ξ-methyl-5ξ-androstane-3ξ,17ξ-diols, shown in Scheme i-4, with the following objectives:

(a) to investigate the origin of the hydrogens transferred in $HSiMe_2OH$ elimination from the $[M - t\text{-}Bu]^+$ and $[M - t\text{-}Bu - H_2O]^+$ ions,

(b) to clarify the nature of the dependence of $HSiMe_2OH$ and water elimination from the $[M - t\text{-}Bu]^+$ ions on stereochemistry of intermediate ions,

(c) to determine the origin, structure, and mechanism of formation of the $[Me_2SiOH]^+$ ion,

(d) to clarify the mode of formation of the $[M - 149]^+$ ion.

The results of these studies should lead to useful generalizations in structural, stereochemical, and conformational investigations of steroids. For these studies of reactions involving single or double hydrogen rearrangement, it was necessary to synthesize steroid alcohols labeled with deuterium at many sites of the steroid skeleton, as shown in Scheme i-6.

The reasons for concentration on the *t*-butyldimethylsilyl derivatives of 17ξ-methyl-5ξ-androstane-3ξ,17ξ-diols were as follows: (a) a set of eight 17ξ-methyl-5ξ-androstane-3ξ,17ξ-diols was available; (b) fragmentation of *t*-butyldimethylsilyl derivatives of the 3-hydroxy steroids was expected to be directed by *t*-butyl radical elimination from $M^{+\cdot}$ to produce abundant ions corresponding to $[M - t\text{-}Bu]^+$, $[Me_2SiOH]^+$, and $[M - t\text{-}Bu - HSiMe_2OH]^+$.

Additionally, the mass spectra of compounds **9-12** (Scheme i-5) revealed unexpected fragmentations, i.e. rearrangement of the 11α-acetyloxy group to silicon and production of the $[CH_3COOSi(CH_3)_2]^+$ ion. These led to proposals for novel mechanisms of hydride transfer to silicon after *t*-butyl radical elimination.

II. E X P E R I M E N T A L

II.A. Instruments and techniques.

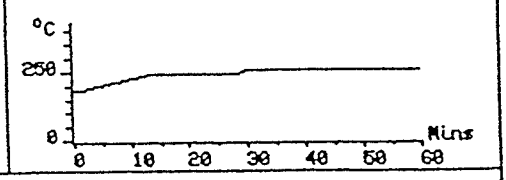
II.A.1. Mass spectrometry and gas chromatography.

Low resolution, positive-ion EI mass spectra were recorded at a background pressure of 10^{-9} Torr (10^{-7} Pa) on a VG-7070E-HF double-focusing mass spectrometer[124] (Nier-Johnson geometry) equipped with a DEC Micro PDP-11/73 data system (Digital Equipment Corporation, Maynard, Mass). The following operating parameters were used: accelerating voltage, 6KV; resolution, 1000; electron beam energy, 70 V (except where noted); trap current, 200 μA; ion source temperature 200^{0}C; scan rate, 7.0 sec/decade. Perfluorokeresone (PFK), used as a mass calibrant for mass spectra, was admitted into the ion source through a heated septum inlet. Sample introduction was accomplished by injection into a heated GC port (250^0 C) and, via a capillary column, transmitted directly to the ion source. Gas chromatography (GC) was carried out on a Hewlett Packard 5890 gas chromatograph equipped with a DB-5 glass capillary column of length 30 m, filled with polydiphenyldimethylsiloxane (non polar). The column temp-

Table 1. Conditions of the GC system.

SYSTEM: HB (GCC) Update Parameters 13-FEB-89 Page 1 of 2

Temperature Ramp Parameters			Acquisition Parameters	
TE1 Temperature 1	(°C)	100	INO Instrument number	1
TI1 Time 1	(mins)	1.50	AQT Acquisition type	SCN
RT1 Rate 1	(°C/min)	5.0	AQS Acquisition system	LOGC
TE2 Temperature 2	(°C)	240	DAT Datafile name	A:B
TI2 Time 2	(mins)	15.00		
RT2 Rate 2	(°C/min)	10.0		
TE3 Temperature 3	(°C)	250		
TI3 Time 3	(mins)	30.00		
RT3 Rate 3	(°C/min)	0.0		
TE4 Temperature 4	(°C)	0		
TI4 Time 4	(mins)	0.00		
RT4 Rate 4	(°C/min)	0.0		
TE5 Temperature 5	(°C)	0		
TI5 Time 5	(mins)	0.00		



?=help 'Q=quit RETURN=next 'G=go ESC=previous "name"=select 'X=exit <=page CTRL/A=abort >=page 'H=hardcopy 'C=create

erature was programmed as shown in Table 1. Thus, the initial temperature, T_1 = 180°, was held constant for 1.5 min (solvent delay period), and then the temperature was increased at rate(1) = 5°C/min until it reached t_2= 240°C, where it was held constant for 15 min. The temperature was then raised to 250°C in 1 min and held constant for 30 min. The total time of the experiment was set for 58.5 min maximum. The flow of helium carrier gas was 30 mL/min.

Decompositions of metastable and collisionally activated ions in the mass spectrometer were studied by linked scanning of the electric and magnetic sectors. In brief, when a metastable ion precursor, P^+, of mass m_1, fragments in the field-free region between the acceleration region and the electric sector (FFR1) into a daughter ion, D^+, of mass m_2 and a neutral molecule N of mass $(m_1 - m_2)$:

$$\underset{(m_1)}{P^+} \text{ -------> } \underset{(m_2)}{D^+} + \underset{(m_1 - m_2)}{N}$$

the translational energy of P^+ is zeV and of D^+ is (m_2/m_1)zeV, where V is the ion accelerating voltage and z is the number of electronic charges on the ion. In order to transmit D^+ through the electrostatic sector the electrostatic field strength must be decreased by a factor (m_2/m_1) of that needed to pass M^+. When the magnetic field is set to focus P^+ ions:

$$(m_1v_1^2)/r = Bzev_1 \qquad \text{or} \qquad B = (m_1v_1)/zer$$

where v_1 is the velocity of P^+ and r is the radius of curvature of the ion beam in the magnetic field of strength B. The D^+ ion will have the same velocity, v_1, as M^+ but a smaller mass, m_2. In order to focus D^+, the magnetic field strength must be changed to $(m_2v_1)/zer$, a factor of (m_2/m_1) of that for P^+. Since both magnetic and electrostatic field

strengths are reduced by the same amount, they can be scanned down together at a fixed ratio of B/E, which will be determined by the mass of the parent ion[124, 125]. Thus, a linked scan at constant B/E gives daughters of a selected parent ion. Alternatively, the magnetic field may be set to focus a "normal" daughter ion of mass m_2 formed in the ion source. The D^+ ion has translational energy $T = zeV = 1/2\ m_2v^2$ and momentum $m_2v = (2m_2zeV)^{1/2}$. To transmit the same ion D^+, formed in FFR1 from a precursor (m_1), the electric sector voltage must be changed from E to $E' = (m_2/m_1)E$. The D^+ ion after decomposition in FFR1, possesses a momentum m_2/m_1 of that of $P^+ = (m_2/m_1)(2m_1zeV)^{1/2} = (m_2/m_1)^{1/2}(2m_2zeV)^{1/2}$. Therefore, to focus D^+ ions on the detector, B must be changed simultaneously to $B(m_2/m_1)^{1/2}$. Thus, observation of precursor ions, P^+, for a chosen daughter ion D^+ requires scanning such that B^2/E is constant.

Linked scanning reduces the sensitivity of the mass spectrometer by a factor of approximately one hundred due to the need for simultaneous scanning of both sectors. Often, parent ions are so stable, and the ion currents of their daughter ions so low, that the acquisition of a daughter ion spectrum is difficult. For this reason, parent ions are often "activated" by collisions with an inert gas[124]. A collision between the inert gas and a parent ion gives the ion a little extra energy, which is enough to promote fragmentation but not so much as to defeat the purpose of activation. Thus collision-induced dissociation (CID) was implemented to increase the abundance of daughter ions by introducing helium gas into a collision cell located in FFR1.

II.A.2. NMR spectrometry.

Deuterium (^2H) and carbon-13 magnetic resonance spectra were recorded on a BRUKER AM300 spectrometer. Deuterium magnetic resonance spectra were recorded using chloroform as solvent and tetramethylsilane (TMS-d_9)) as the internal standard. Carbon-13 magnetic resonance spectra were recorded using the polarization transfer spectroscopy technique[126, 127] and chloroform-d_1 as a solvent.

II.B. Syntheses of Silyl Derivatives of Steroid Alcohols and their Deuterium-Labeled Analogues.

II.B.1. Materials.

Eight isomers of 17ξ-methyl-5ξ-androstane-3ξ,17ξ-diols, synthesized by Jackson[128], were obtained from Dr. J. F. Templeton (Faculty of Pharmacy, University of Manitoba). Some steroids, namely 17β-hydroxy-17α-methyl-4-androsten-3-one (Searle Chemicals, Inc.), 17β-hydroxy-17α-methyl-5α-androstan-3-one (Sigma, Chemical Co., St. Louis), 17β-hydroxy-17α-methyl-5β-androstan-3-one and 17α-hydroxy-17α-methyl-1,4-androstadiene were also kindly donated by Dr. J. F. Templeton. Of the steroids used in the syntheses, 17α-methyl-5-androsten-3β,17β-diol and 17β-hydroxy-17β-methyl--4,9(11)-androstadien-3-one were purchased from Steraloids Inc. (Wilton, Mass). The solvents tetrahydrofuran, 1,4-dioxane, and benzene were distilled over sodium before use, and then stored over molecular sieve (4Å). *tert*-Butyldimethylsilyl chloride (TBDMSiCl) was purchased from Sigma Chem. Co. St. Louis MO. Sodium borodeuteride was obtained from Aldrich Chem., Co., Milwaukee, Wisc. Methyl alcohol-OD (99% atom D) and deuterium gas (99.5 atom % D) were purchased from MSD Isotope Division of Merck Frost Canada Inc. Montreal. Catalysts were kindly donated by Drs. J. F. Templeton (5% Rh/C), L. Kruczynski ($(C_6H_5)_3P)_3RhCl$), and J. Charlton (10% Pd/C, 5% Pt/C, and PtO_2 (Adams' catalyst)). Thin-layer chromatography (TLC) was carried out on silica-coated flexible plates, PE SIL G/UV_{254}, Whatman Ltd., England (donated by Dr. J. Charlton) or on 0.25 mm thick silica gel plates, UNIPLATE, Analtech, Inc. (donated by Dr. J. F. Templeton) using 50% ethyl acetate/hexane as the liquid phase. Components on the TLC plates were made visible by irradiating the plate with an ultraviolet lamp, and/or by immersion in an 8% v/v solution of sulfuric acid/ethanol, followed by heating at approximately 100°C on a hot plate for 5 to 10

minutes. Column chromatography was carried out on alumina (Firma activity II aluminum oxide for chromatographic absorption analysis, BDH Chemicals Ltd.).

II.B.2 General synthetic procedures.

Procedure A. Deuterium or hydrogen incorporation by reduction of ketones with sodium borodeuteride or borohydride in methanol or ethanol.

To the steroid ketone, dissolved in methanol or ethanol, was added $NaBH_4$[129] or $NaBD_4$[130] (for proportions see the particular procedure, Section II.D.3). After refluxing the solution in an atmosphere of nitrogen gas for 1 hr and evaporating the solvent *in vacuo*, dilute hydrochloric acid (1M) was added to the dry residue and the steroid was then extracted with ether. The ether extract was worked up by washing with water, saturated sodium bicarbonate, water, saturated sodium chloride, dried over anhydrous $MgSO_4$ or Na_2SO_4, and then the solvent was evaporated under reduced pressure to give the steroid residue.

Procedure B. Preparation of mono-TBDMSi ether derivatives[47-49].

The conventional procedure for the preparation of TBDMSi-ethers involves reaction of steroid alcohols with *t*-butyldimethylchlorosilane (TBDMSiCl) in the presence of imidazole (Im), as a catalyst, in dimethylformamide (DMF).

The standard silylation reagent for this reaction was prepared by adding DMF (2mL) to TBDMSiCl (0.300 mg, 1.992 mmol) and imidazole (0.272 g, 3.994 mmol), i.e. 1M chlorosilane and 2M imidazole concentrations. This solution was stored in a teflon-lined, septum-capped hypo-vial under a drying material (anhydr. $CaSO_4$) or in glass vials with polyethylene caps.

Derivatization of steroid alcohols was performed in dry teflon-lined septum-capped 0.3 mL vials (screw-top "Reacti-vials" from Pierce Chemical Co.). The

standard reagent (50 μL) was added to 0.1 - 0.2 mg of steroid and the reaction mixture was allowed to stand at room temperature; reaction was complete in 1 hr. The resulting solution (1 μL - 10 μL) was diluted with methylene chloride (0.5 mL) prior to GC/MS analysis.

Procedure C. Jones oxidation[134].

A 0.5-5% solution of the steroid alcohol in acetone (distilled over $KMnO_4$) was treated dropwise with Jones reagent until a permanent orange color persisted. After 10-30 minutes, methanol, to decompose an excess of oxidant, and saturated $NaHCO_3$ solution, to neutralize acid, were added. Acetone was removed at reduced pressure and the residue was extracted with ether. The ether layer was washed with water, saturated sodium chloride, dried over Na_2SO_4, and evaporated under reduced pressure to yield the product ketone.

Procedure D. Hydrogenation (deuteration) by heterogeneous (Pd/C[130], Pt/C[130], PtO_2[130], Rh/C[139]) or homogeneous [$(C_6H_5)_3P]_3RhCl$[135] catalysts.

The hydrogenation (deuteration) apparatus, used in this study, was designed as depicted in Fig.1. In a typical experiment, if no pretreatment was done (pretreatment procedure - solvent and catalyst were saturated with deuterium gas for a period of time prior to adding steriod), a heterogeneous or homogeneous catalyst was added to the reaction flask containing solvent and steroid. The hydrogenation system was alternately evacuated, flushed with nitrogen (3x), and hydrogen or deuterium gas (3x), after which time the magnetic stirrer was turned on. With the H_2/D_2 pressure at *ca* 2 atm. (*ca* 70 cm Hg above atmospheric pressure) the mixture was vigorously stirred. Usually, the reduction reaction was performed at room temperature for sufficient time to complete the reaction. The progress of deuteration was monitored periodically by withdrawing a small sample of solution with a syringe and analyzing it by TLC or by GC/MS.

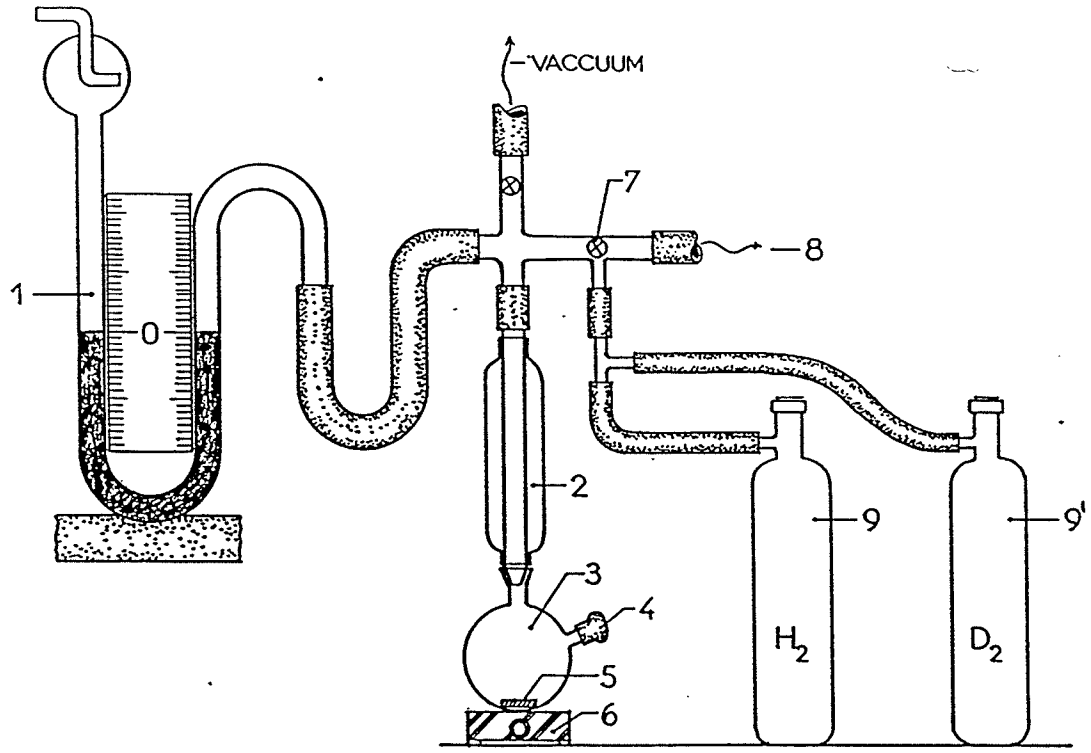


Figure 1. Hydrogenation/deuteration apparatus: **(1)** open-ended mercury manometer, **(2)** condensor, **(3)** reaction flask, **(4)** rubber septum, **(5)** magnetic bar, **(6)** magnetic stirrer, **(7)** three-way stopcock, **(8)** N_2 inlet, **(9/9′)**, H_2 or D_2 supply tank.

Procedure E. Hydrogen/deuterium exchange (back-equilibration) of steroid ketones under basic conditions[130].

The steroid ketone was dissolved in methanol, to which was added sufficient solid sodium hydroxide or potassium hydroxide to obtain a 2M solution. The solution was stirred for 1-4 hr in an atmosphere of nitrogen, cooled to room temperature, acidified with dilute hydrochloric acid (1M), and then the residue was extracted with ether. The organic layer was washed with water, saturated sodium bicarbonate, water, saturated

sodium chloride (brine), then dried over anhydrous $MgSO_4$ or Na_2SO_4. The solvent was then evaporated under reduced pressure to yield a residue containing the steroid ketone.

Procedure F. Deuterium incorporation into steroid ketones by equilibration under basic conditions[130].

Standard Reagent. The standard reagent (1M) was prepared by dissolving a clean piece of sodium (455 mg, 20 mmol) in cooled methanol-d_1 (15 mL). To this solution deuterium oxide (5 mL) was added.

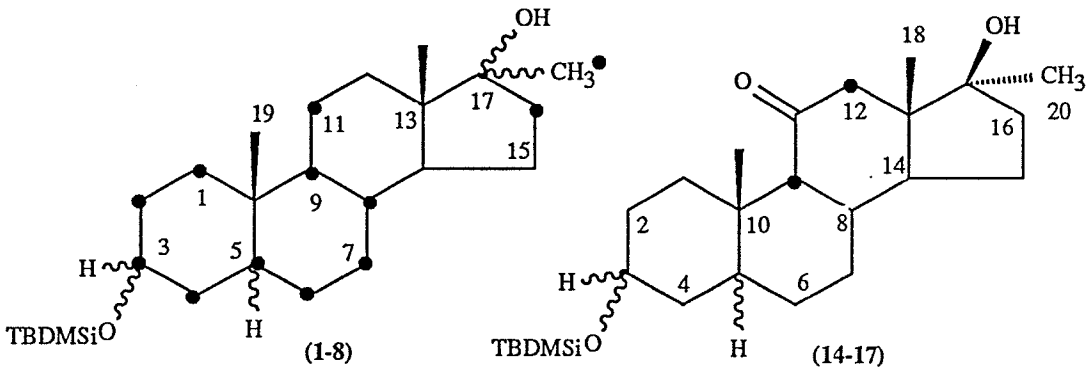
The steroid ketone was dissolved in a small amount of the above reagent and the mixture refluxed for at least 24 hr (unless otherwise stated). *Work up:* the mixture was neutralized with 5% DCl/D_2O, the solvent was evaporated under reduced pressure, and the residue was then extracted with ether. The extract was washed with cold water, saturated sodium chloride (brine), dried over $MgSO_4$, and then the solvent was evaporated to give a residue of the deuterated steroid ketone.

Procedure G. Acetylation of steroid alcohols[139].

To a 10% solution of steroid in dry pyridine was added acetic anhydride (one-half the volume of pyridine). After standing overnight, the reaction mixture was poured into ice-water and allowed to stand for 30 minutes. This mixture was acidified with 5% hydrochloric acid and extracted with ether. The organic layer was washed with water, 5% sodium bicarbonate solution, water, brine, and dried over $MgSO_4$ or Na_2SO_4. Evaporation of solvent *in vacuo* yielded a residue of the acetylated steroid alcohol.

II.B.3. Outline of Organic Syntheses.

In this section, syntheses of deuterium-labeled 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstane (**1-8**) and 17α-methyl-5ξ-androstan-11-one-3ξ,17β-diol (**14-17**) will be discussed.

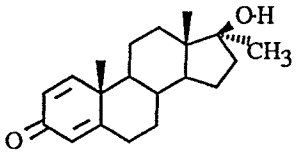
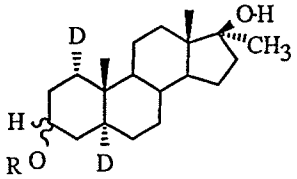
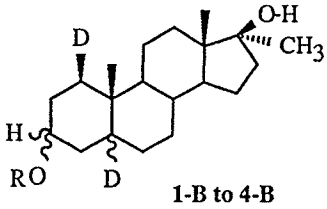
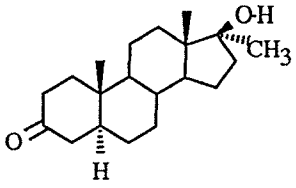
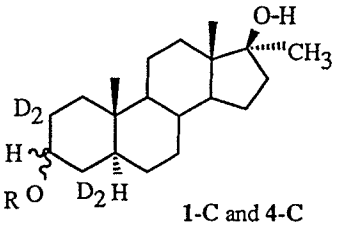
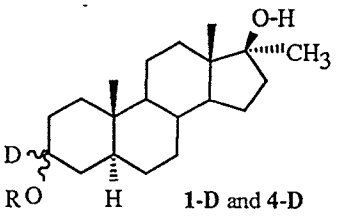


- Denotes a site of deuterium incorporation

During the course of this study deuterium was incorporated at all carbon atoms, except for 12, 14, 15, 18, and 19 in **1-8.** In the case of **14-17** only the 9 and 12 positions were deuterium labeled. A method for determination of deuterium incorporation is described in Appendix I. The extent of deuterium incorporation achieved, as determined from the mass spectra of the TBDMSi dervatives, is presented later in the Tables 12, 14, 16, and 18 as indicated in Table 2. Table 2 also gives information on specific syntheses, as now described. The starting materials were always the specific ketones, except for one case (entry 5).

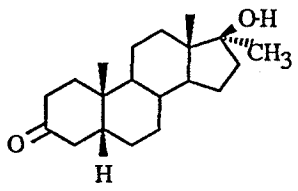
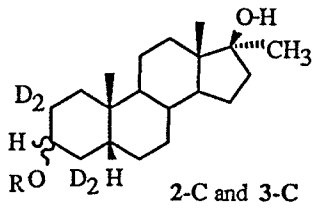
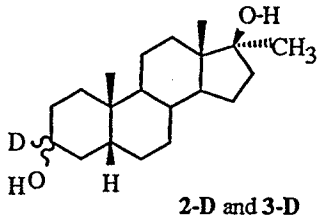
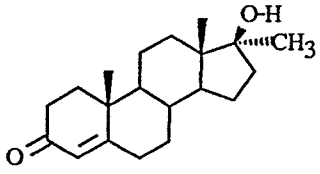
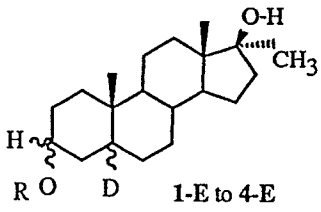
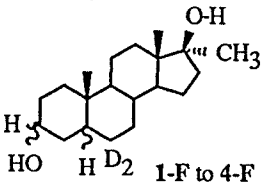
Introduction of deuterium atoms at the positions 2 and 4 to give **1-C and 2-C**, is outlined in **Scheme I.** After base-catalyzed equilibration of the starting ketone,

Table 2. The starting materials and reaction schemes for synthesis of labeled steroid derivatives, including Table numbers where the extent of deuterium incorporation is given. (R = TBDMSi)

Starting material	Final product	Labeled position(s)	Reaction Scheme
1. 	 **1-A and 4-A**	1α,5α-d_2 (Tables 12 and 18)	**VIII**
	 **1-B to 4-B**	1β,5ξ-d_2 (Tables 12, 14, 16 and 18)	**IX**
2. 	 **1-C and 4-C**	2,2,4,4-d_4 (i.e. 5α-H only) (Tables 12 and 18)	**I**
	 **1-D and 4-D**	3ξ-d_1 (i.e. 5α-H only) (Tables 12 and 18)	**III**

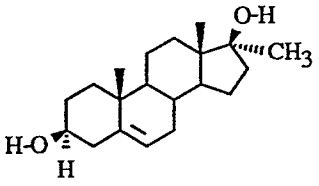
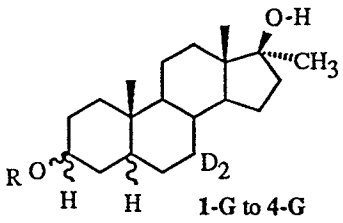
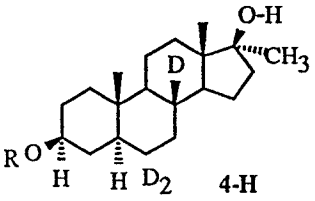
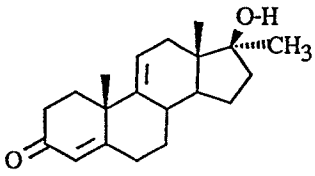
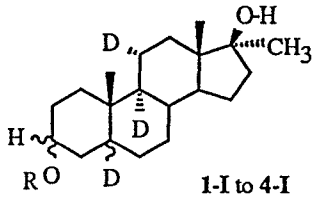
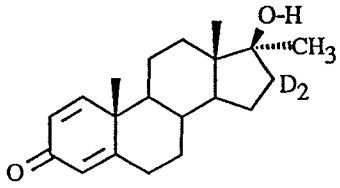
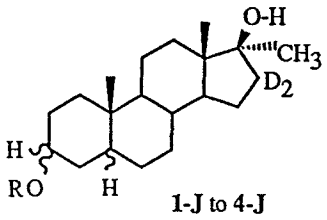
(cont'd)

Table 2 (continued)

Starting material	Final product	Labeled position(s)	Reaction Scheme
3. 	 2-C and 3-C	2,2,4,4-d_2	II
	(i.e. 5β-H only)	(Tables 14 and 16)	
	 2-D and 3-D	3ξ-d_1	IV
	(i.e. 5β-H only)	(Tables 14 and 16)	
4. 	 1-E to 4-E	5ξ-d_1	V
		(Tables 12, 14, 16 and 18)	
	 1-F to 4-F	6,6-d_2	X
		(Tables 12, 14, 16 and 18)	

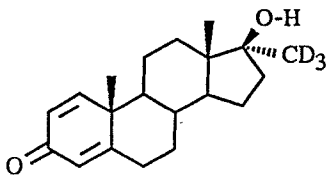
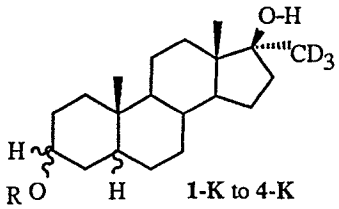
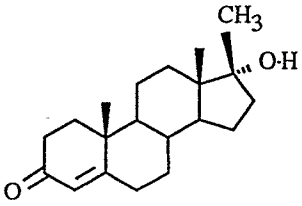
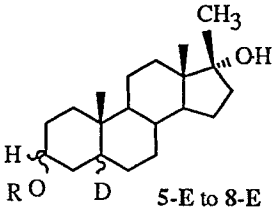
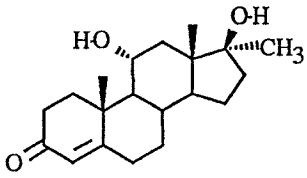
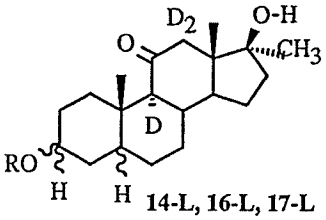
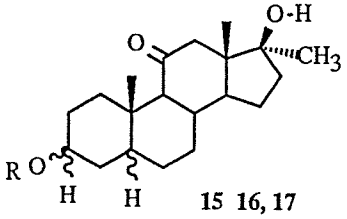
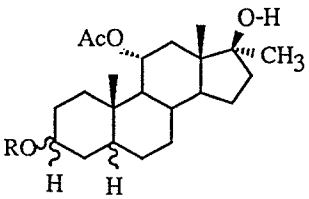
(cont'd)

Table 2 (continued)

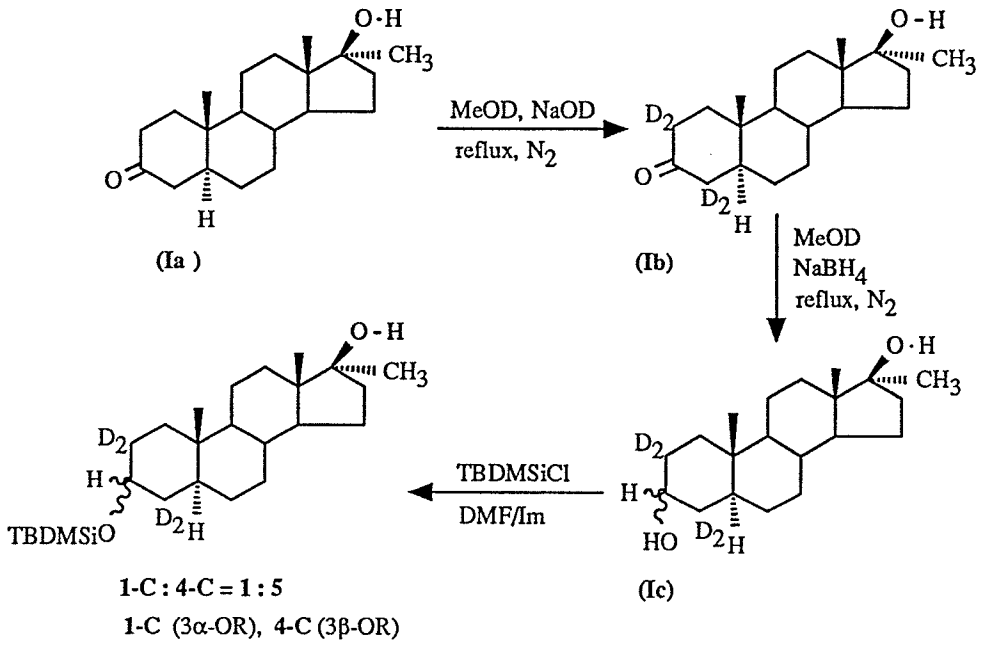
Starting material	Final product	Labeled position(s)	Reaction Scheme
5. 	 **1-G to 4-G**	7,7-d_2	**XII**
		(Tables 12, 14, 16 and 18)	
	 **4-H**	6,6,8β-d_3	**XI**
	5α-H only	(Table 18)	
6. 	 **1-I to 4-I**	5ξ,9α,11α-d_3	**XVI**
		(Tables 12, 14, 16 and 18)	
7. 	 **1-J to 4-J**	16,16-d_2[128]	**XVII**
		(Tables 12 14, 16 and 18)	

(cont'd)

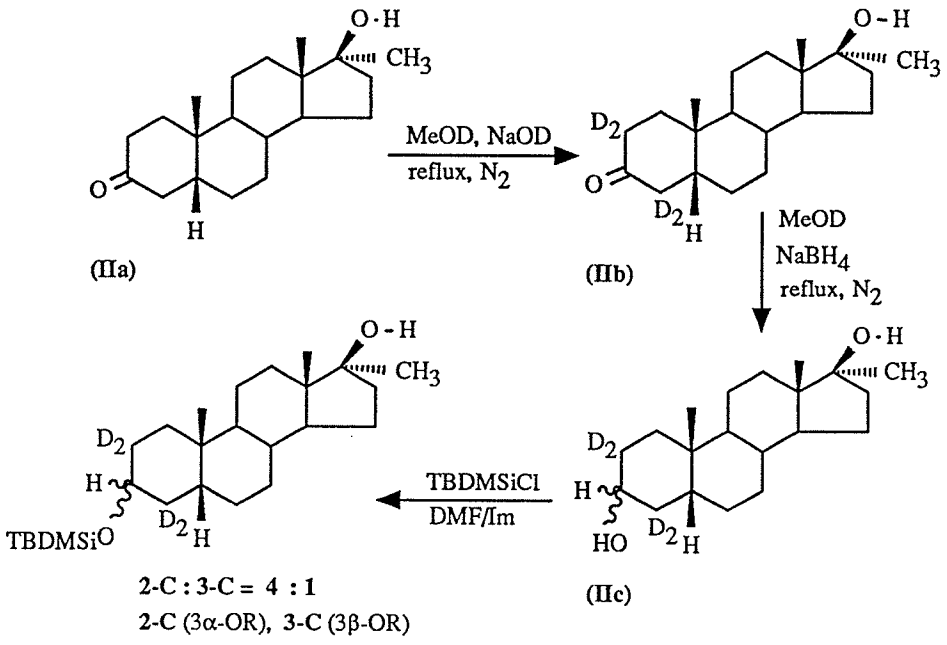
Table 2 (continued)

Starting material	Final product	Labeled position(s)	Reaction Scheme
8. 	 **1-K to 4-K**	17α-CD_3[128] (Tables 12, 14, 16 and 18)	**XVIII**
9. 	 **5-E to 8-E**	5ξ-d_1 (Table 30)	**VI**
10. 	 **14-L, 16-L, 17-L** (no 5β-H,3α-OH formed,**15-L**) no % deuterium determined	9α,12,12-d_3	**XIII**
	 **15 16, 17** (no 5α-H,3α-OH formed;**14**)	none	**XIV**
	 **9 - 12**	none	**XV**

Scheme I. Preparation of 3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-2,2,4,4-d_4 (**1-C**) and 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-2,2,4,4-d_4 (**4-C**)



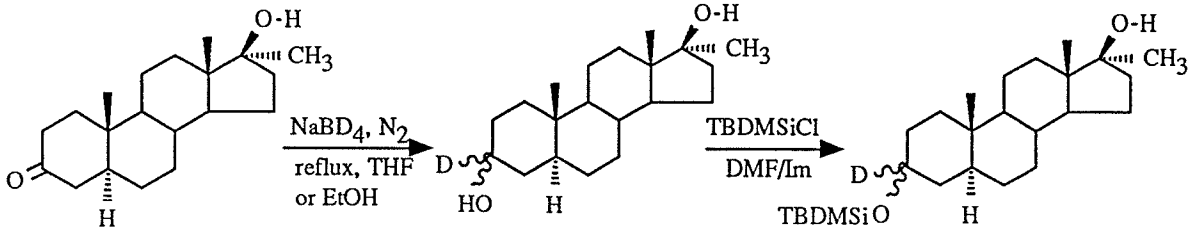
Scheme II. Preparation of 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-2,2,4,4-d_4 (**2-C**) and 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-2,2,4,4-d_4 (**3-C**).



17β-hydroxy-17α-methyl-5α-androstan-3-one **(Ia)**, in CH_3OD/D_2O solution, followed by reduction with sodium borohydride[129] in CH_3OD under nitrogen [131] yielded **Ic**. (CH_3OD was used to prevent deuterium, previously incorporated at the 2,2,4,4 positions, from back exchanging). After formation of the TBDMSi derivative, gas chromatography showed that the 3α-OH and 3β-OH sterols had been formed in a 1:5 ratio, respectively. The 3α-epimer was formed in slightly greater amount[129] when sodium borohydride, instead of lithium aluminum hydride, was used. Similarly, **Scheme II** shows the preparation of **2-D** and **3-D** from the 5β isomer, 17β-hydroxy-17α-methyl-5β-androstan-3-one **(IIa)**.

The availability of 17β-hydroxy-17α-methyl-5α-androstan-3-one **(IIIa)** and 17β-hydroxy-17α-methyl-5β-androstan-3-one **(IVa)** provided a convenient route to deuterium introduction at position C-3 by reduction with sodium borodeuteride in THF or ethanol as shown in **Schemes III** and **IV**.

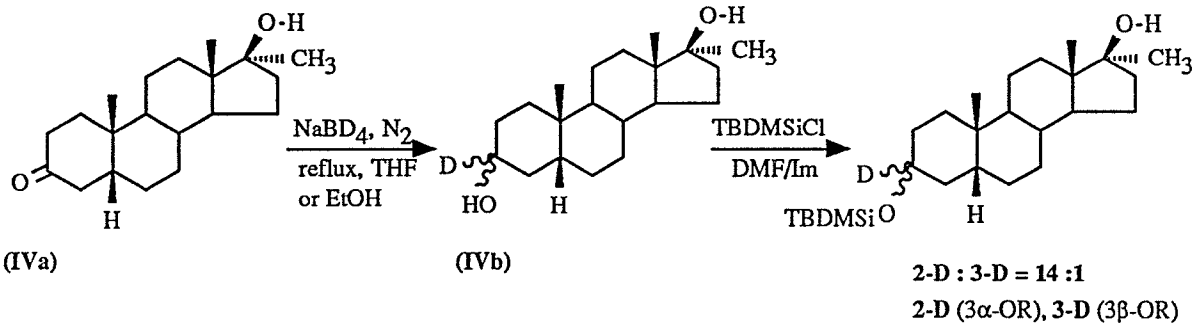
Scheme III. Preparation of 3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-3β-d_1 **(1-D)** and 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-3α-d_1 **(4-D)**.



(IVa) (IVb) **1-D : 4-D = 1 : 7**

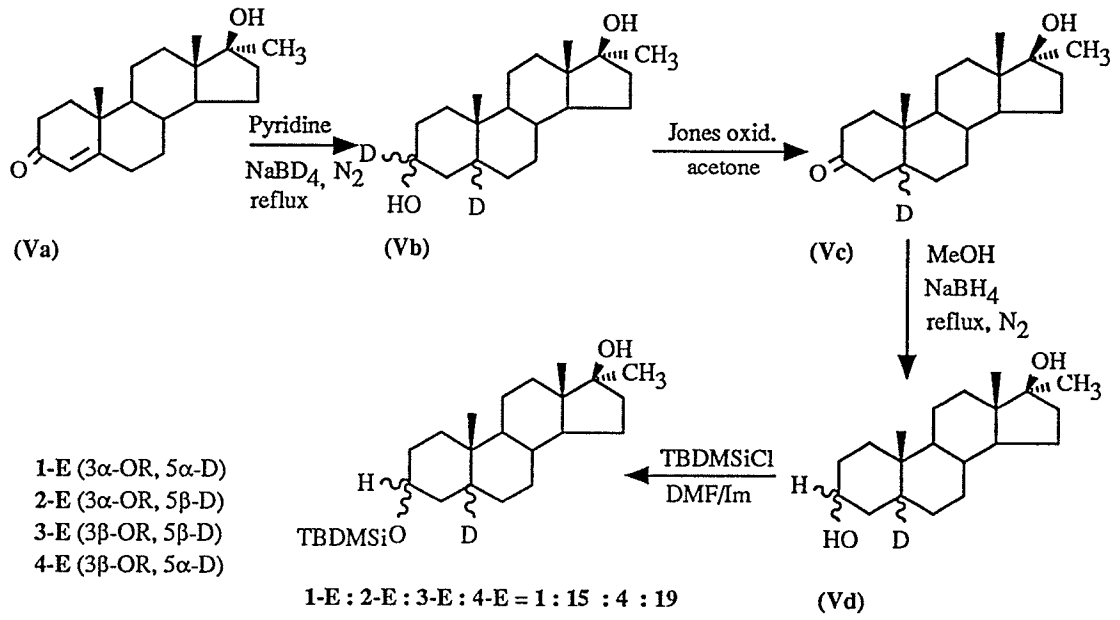
1-D (3α-OR), **4-D** (3β-OR)

Scheme IV. Preparation of 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-3β-d_1 **(2-D)** and 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-3α-d_1 **(3-D).**



One of the origins from which mass spectral hydrogen migration was suspected was the C-5 site, as observed in the elimination of water in the 5α-[24] or 5β-series, or as proposed by Chapman and Bailey[81], by Quilliam and Westmore[67], and Quilliam [101] for steroid silyl ethers. To test this hypothesis, all four isomers of 17α-methyl-5ξ--androstan-3ξ,17β-diol-5ξ-d_1 were obtained by a one step reduction of 17β-hydroxy-17α-methyl-4-androsten-3-one **(Va)** with an excess of sodium borodeuteride in anhydrous pyridine (**Scheme V**). To date, the reduction of 4-en-3-one steroids with $NaBD_4$, to introduce deuterium as 5-D, has not been reported. The stereochemical result obtained in this study opposes those obtained by Kupfer[132] and Dauben *et al.*[133], who reported that borohydride reduction of 4-ene-3-ones in pyridine resulted in predominant attack at the least hindered side (α face) of the ketone to give solely 3β-hydroxy-5α-H derivative. It is interesting to note that, in the current study, the reduction of **Va** in refluxing pyridine gave a mixture of the 3ξ-hydroxy-5ξ-derivatives, i.e. including attack at the more hindered β-face. To obtain derivatives with deuterium at the C-5 position only, deuterium incorporated at C-3 was removed by conversion of the alcohols **(Vb)** into ketones **(Vc)** by oxidizing the crude products **(Vb)** with Jones reagent[134]. The newly formed C-3 carbonyl group was then reduced with $NaBH_4$ in methanol. From the product mixture **(Vd)**, the TBDMSi ether derivatives, **1-E to 4-E**

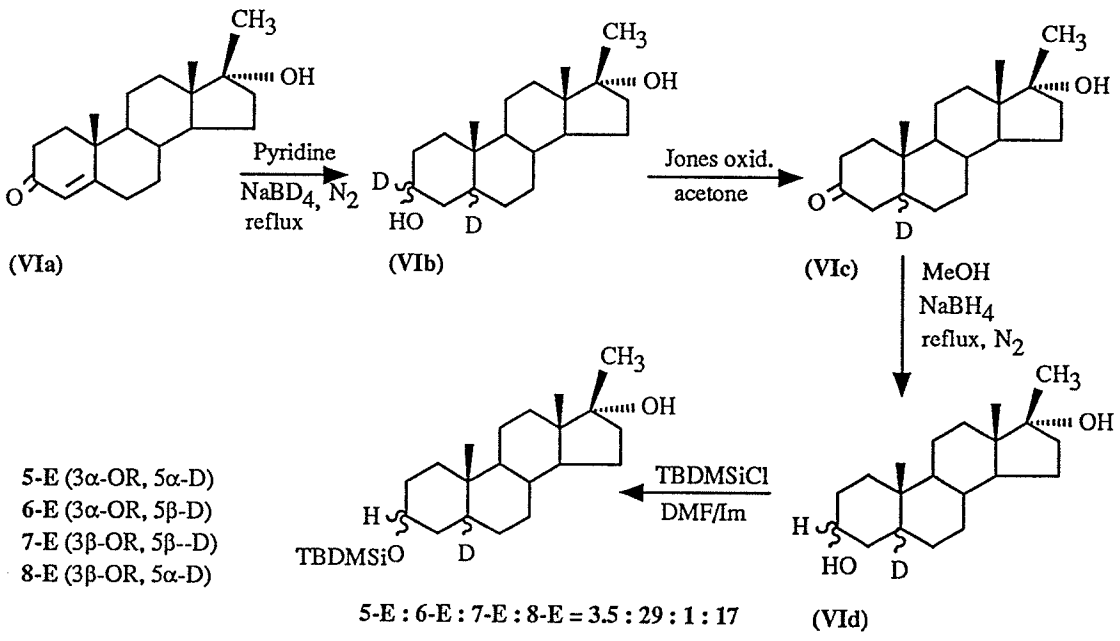
Scheme V. Preparation of all four isomers of 3ξ-OSiDMTB-17β-hydroxy--17α-methyl-5ξ-androstane-5ξ-d_1 **(1-E** to **4-E).**



1-E (3α-OR, 5α-D)
2-E (3α-OR, 5β-D)
3-E (3β-OR, 5β-D)
4-E (3β-OR, 5α-D)

1-E : 2-E : 3-E : 4-E = 1 : 15 : 4 : 19

Scheme VI. Preparation of all four isomers of 3ξ-OSiDMTB-17α-hydroxy--17β-methyl-5ξ-androstane-5ξ-d_1 **(5-E to 8-E).**



5-E (3α-OR, 5α-D)
6-E (3α-OR, 5β-D)
7-E (3β-OR, 5β--D)
8-E (3β-OR, 5α-D)

5-E : 6-E : 7-E : 8-E = 3.5 : 29 : 1 : 17

were prepared.

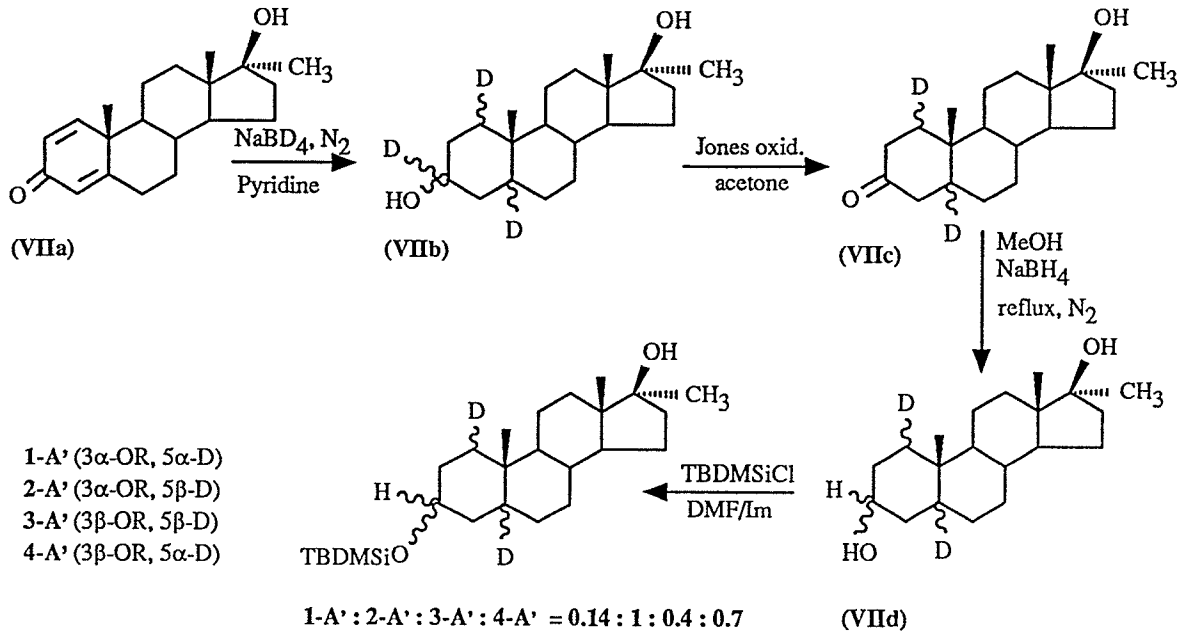
The same method was applied to the preparation of 3ξ-OSiDMTB-17α-hydroxy-17β-methyl-5ξ-androstanes-5ξ-d_1 **(5-E to 8-E, Scheme VI)** from the parent ketone 17α-hydroxy-17α-methyl-4-androsten-3-one (**VIa**).

In the 3α-OSiDMTB,5α-H and 3β-OSiDMTB,5β-H series, the distances between 1α-H (5α) or 1β-H (5β) and the oxygen atom at C-3 are small enough (2.53 Å or 2.62 Å, see section III.C.1. or III.C.3) to allow interaction and thus permit elimination of $HSi(CH_3)_2OH$ in the mass spectrum. Because of this, it was necessary to determine whether the 1α or 1β site was involved in the hydrogen transfer. Thus, it was necessary to prepare two independent compounds, with deuterium at the C-1α or C-1β positions.

Since reduction of the 4-en-3-one, with $NaBD_4$ in anhydrous pyridine to the saturated alcohols had been successful, this appeared to be a promising way to convert a 1,4-dien-3-one into a saturated steroid alcohol and in this way to incorporate deuterium atoms at the C-1 and C-5 positions, simultaneously. Thus, 17β-hydroxy-17α-methyl-1,4-androstadien-3-one (**VIIa**) was reduced with $NaBD_4$, in refluxing pyridine, (**Scheme VII**). In the product, **VIIb**, three deuterium atoms were incorporated as shown by GC/MS analysis. Jones oxidation of the crude **VIIb**, to eliminate the 3ξ-D atom, followed by reduction with $NaBH_4$ in methanol, and finally derivatization, gave the expected 1,5-d_2 products, **1-A'** to **4-A'**. Because the stereochemistry of the substituted deuterium atom at C-1, when reduced with $NaBD_4$ in pyridine, had never been determined our attention was directed toward stereochemically controlled deuteration.

Control of the stereochemistry of hydrogenation (deuteration) can be achieved by using homogeneous rhodium[135] or heterogeneous palladium[136,137] catalysts for reactions with 1,4-dien-3-ones. Thus, it has been reported that treatment of **VIIa** with deuterium gas, in the presence of tris(triphenylphosphine)rhodium chloride proceeds at the α face[133], leading to androst-4-ene-3,17-dione-1α,2α-d_2. By contrast, heterogeneous palladium-catalyzed tritiation[136] or deuteration[137] of this compound occurred from the

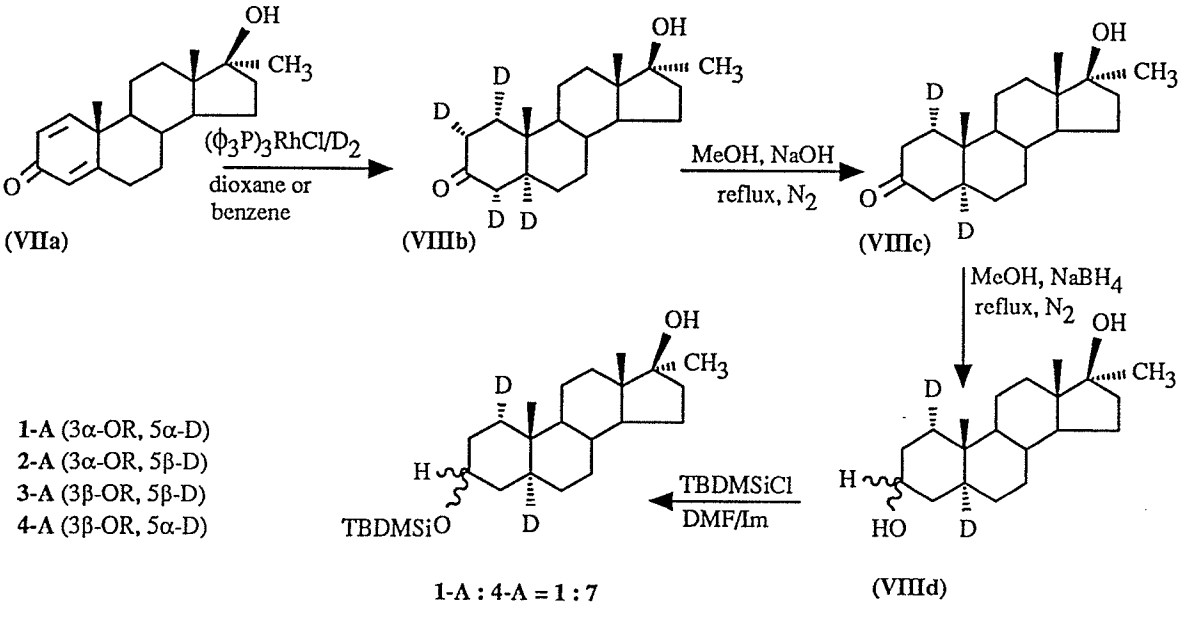
Scheme VII. Preparation of 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstanes-1ξ,5ξ-d_2 (**1-A'**, **2-A'**, **3-A'**, and **4-A'**).



1-A' (3α-OR, 5α-D)
2-A' (3α-OR, 5β-D)
3-A' (3β-OR, 5β-D)
4-A' (3β-OR, 5α-D)

1-A' : 2-A' : 3-A' : 4-A' = 0.14 : 1 : 0.4 : 0.7

Scheme VIII. Preparation of 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstanes-1α,5α-d_2 (**1-A** and **4-A**).



1-A (3α-OR, 5α-D)
2-A (3α-OR, 5β-D)
3-A (3β-OR, 5β-D)
4-A (3β-OR, 5α-D)

1-A : 4-A = 1 : 7

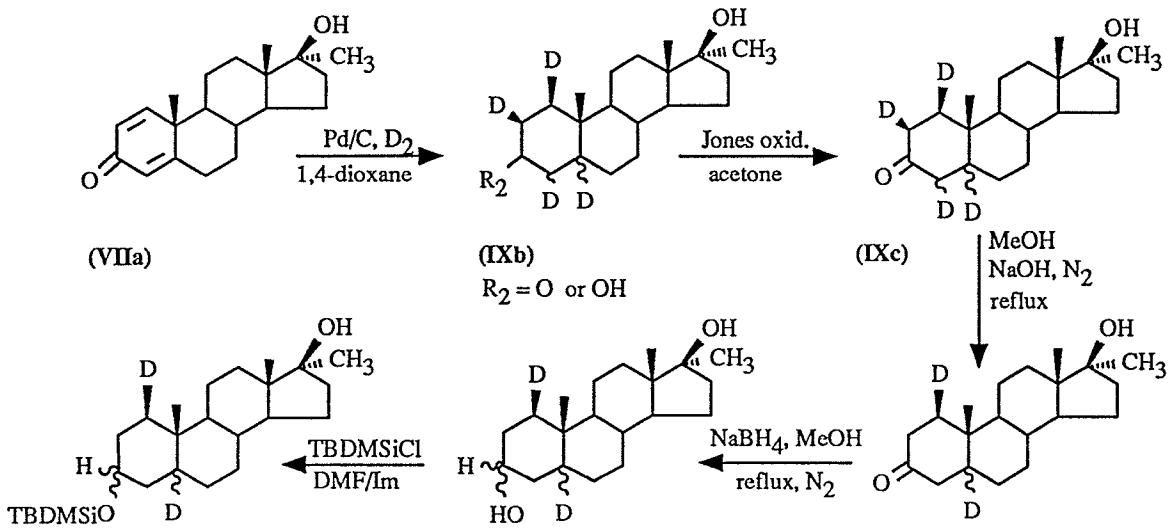
β-face and gave (after separation from other products) *ca* 15% of androst-4-ene-3,17--dione-1β-(t_1 or d_1). Based on these results, deuterium was inserted at the 1α and 1β positions, as now described.

In the presence of tris(triphenylphosphine)rhodium chloride, 17β-hydroxy--17α-methyl-1,4-androstadien-3-one, **VIIa**, was treated with deuterium gas in 1,4-dioxane/deuteriomethanol (10 : 1) for 10 days (**Scheme VIII**). Recrystallization of the product from methanol/methylene chloride gave only the 5α isomer, as determined by GC/MS. Base-catalyzed back-exchange of the 2α and 4α deuterium atoms, and subsequent reduction of the product ketone, **VIIIc**, with sodium borohydride in methanol, followed by derivatization gave the two 3ξ-epimers, **1-A** and **4-A**. However, treatement of **VIIa** with deuterium under the same conditions, for a longer period of time (twenty days), gave, additionally, a small amount of a saturated ketone having the 5β-H structure. Base catalyzed back-exchange of the mixture, followed by reduction with $NaBH_4$ and then derivatization, gave the four 5ξ-H,3ξ-OR isomers in the ratio 0.14 : 1 : 0.4 : 0.7, respectively.

Incorporation of deuterium at the 1β and 5ξ sites was achieved by treatment of 17β-hydroxy-17α-methyl-4-androsten-3-one (**VIIa**) with deuterium in 1,4-dioxane in the presence of a Pd/C catalyst[130] (**Scheme IX**). The crude deuterated product mixture, **IXb**, was oxidized by Jones reagent, followed by base-catalyzed back-exchange of two deuterium atoms on C-2 and C-4. Subsequent reduction of the ketone (**IXd**) with $NaBH_4$ in methanol, and derivatization of the product, gave the expected compounds, 3ξ--OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstanes-1β,5ξ-d_2, **1-B** to **4-B**.

The mass spectral studies to be described later show that the deuterium-labeled compounds described thus far do not allow all of the migrating deuterium to be accounted for. Thus, it was necessary to introduce labels onto further positions of the steroid skeleton to extend the study. At this stage of the experiments, we anticipated that

Scheme IX. Preparation of 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstane-1β,5ξ-d_2 **(1-B to 4-B).**



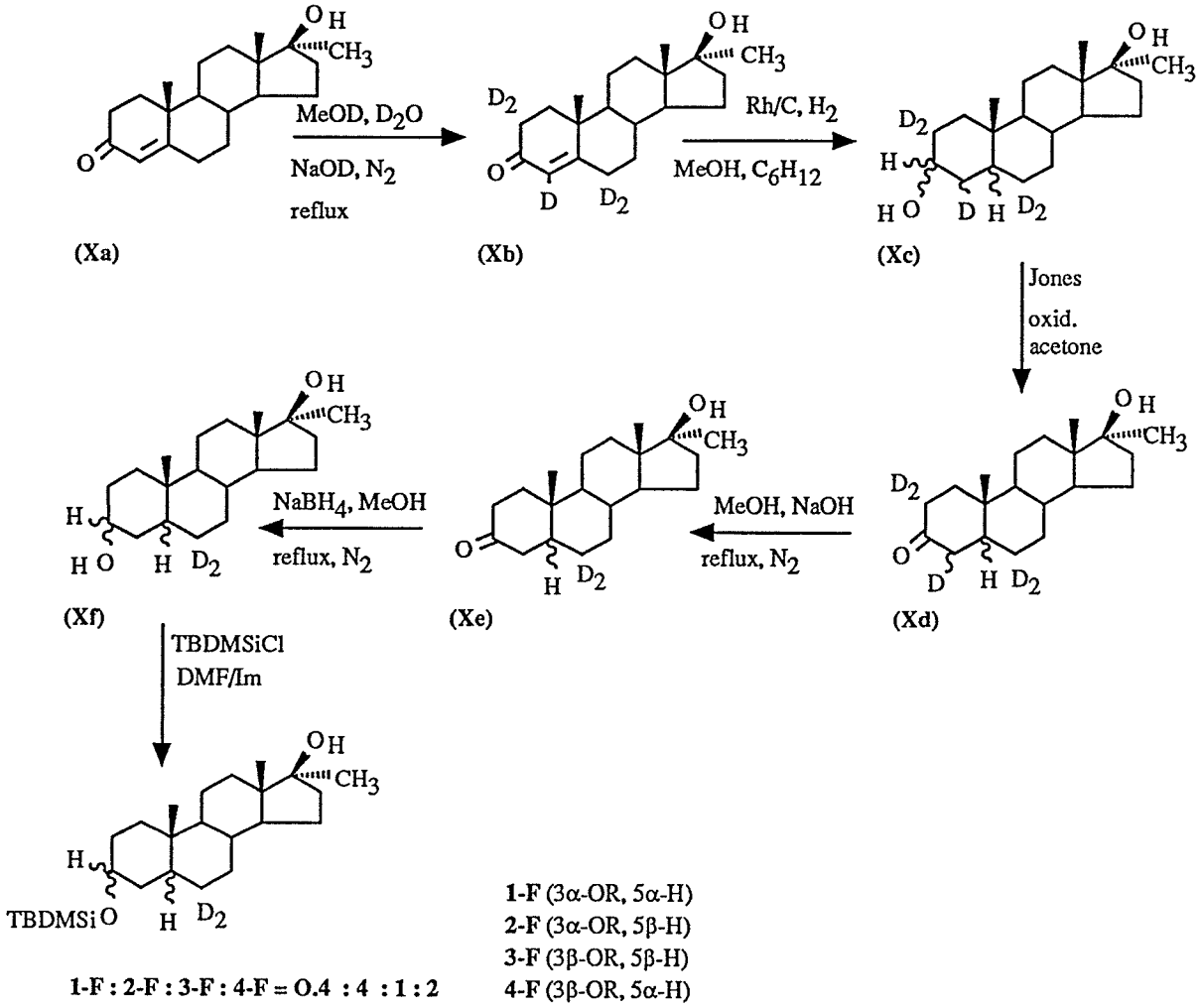
1-B : 2-B : 3-B : 4-B = 0.006 : 16 : 3 : 1

1-B (3α-OR, 5α-D)
2-B (3α-OR, 5β-D)
3-B (3β-OR, 5β-D)
4-B (3β-OR, 5α-D)

transfer of hydrogen might occur from C-9 or C-7 for **1**, from C-9 and/or C-14 for **2**, and from the 19-CH_3 group for **3.** However, we also decided to examine the C-6 position because it might be a close enough site for hydrogen transfer to occur.

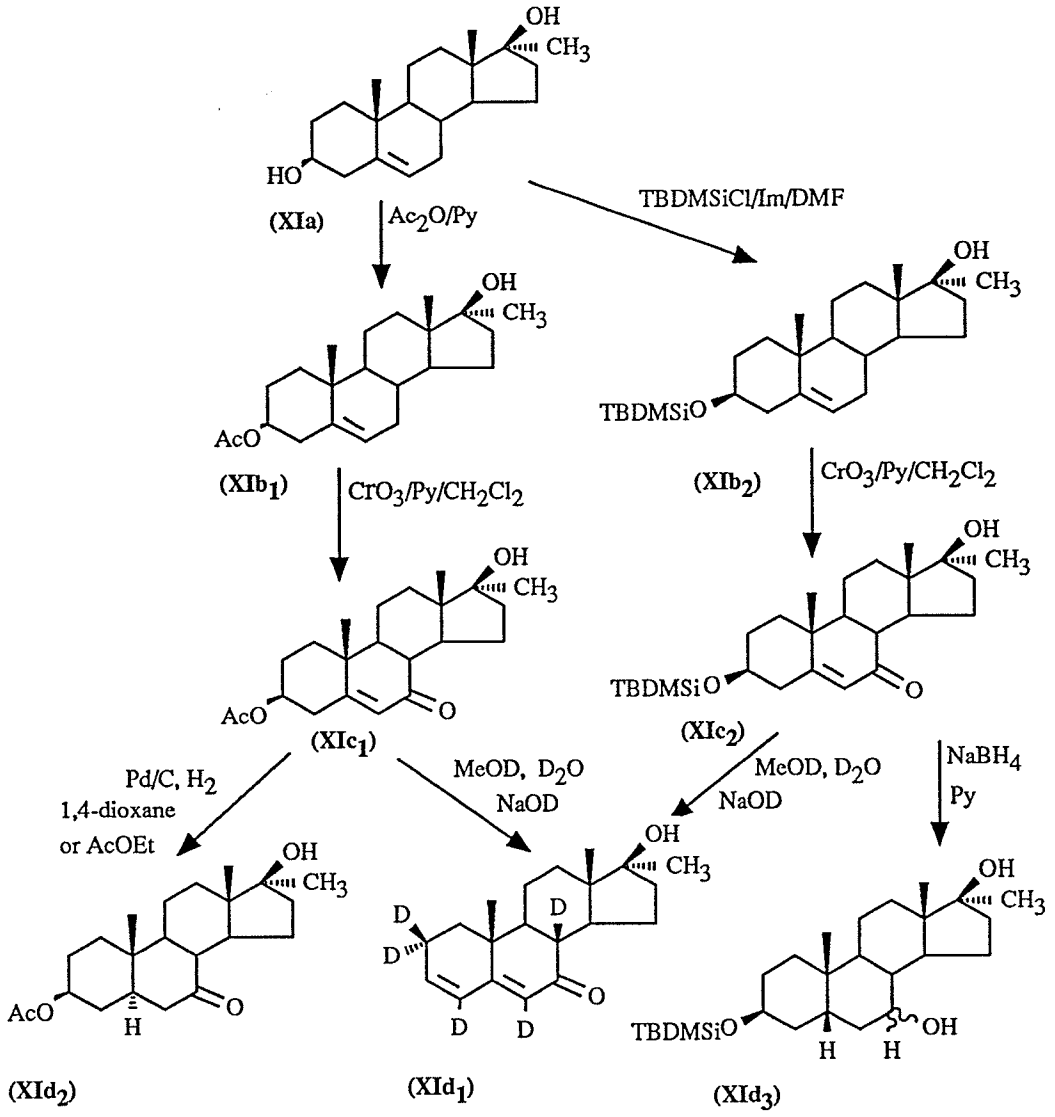
To introduce deuterium at C-6, advantage was taken of the fact that five hydrogens (i.e. 2,2,4,6,6) can be exchanged in 4-androsten-3-one[138]. Thus, in **Scheme X,** equilibration of 17α-methyltestesterone **Xa**, in basic methanol-d_1 gave the d_5-analogue **(Xb).** The double bond was then reduced with hydrogen in methanol/cyclohexane (1 : 4) in presence of 5% rhodium-on-charcoal[139] to give a mixture of the four 3ξ/5ξ isomers **Xc.** The desired 6,6-d_2 alcohols **(Xf)** were obtained after re-oxidizing the crude product **(Xc)** with Jones reagent, back exchanging the deuterium atoms attached to C-2 and C-4, and reducing the 3-keto group with $NaBH_4$ in methanol. Upon derivatization the four 3ξ/5ξ isomers, **1-F** to **4-F** were obtained.

Scheme X. Preparation of 3ξ-OSiDMTB-17β-hydroxy-17β-methyl-5ξ-androstanes-6,6-d_2 **(1-F to 4-F).**



In order to place deuterium at positions C-7 or C-8, allylic oxidation of C-7 was explored as a starting point. The starting 17α-methyl-5-androstene-3β,17β-diol, **XIa**, (**Scheme XIA**) was acetylated by acetic anhydride in dry pyridine[139] or derivatized with TBDMSiCl reagent, and then oxidized by the method of Dauben[140,141] to the corresponding 5-en-7-ones, $\mathbf{XIc_1}$ or $\mathbf{XIc_2}$. To incorporate deuterium at C-8 base-cataly-

Scheme XIA. Attempted in preparation of 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-6,6,8β-d_3 **(4-H)**.



zed enolization of $\mathbf{XIc_1}$ and $\mathbf{XIc_2}$ was attempted. Although this route appeared to be very attractive, it led unfortunately, to elimination of the C-3 oxygen function, yielding $\mathbf{XId_1}$, which was identified as follows. Its strong absorption under a UV lamp suggested the presence of a conjugated double bond. Its deuterium magnetic resonance spectrum

showed the presence of five deuterium atoms, Fig. 2, and GC/MS analysis of **XId$_1$** showed a molecular ion of m/z 305, compared to m/z 300 expected for the unlabeled analogue. An analogous product was also reported for 3β-acetoxy-5-androsten-7-one, but involving two reaction steps, i.e. acid hydrolysis of the 3β-acetyl group, followed by equilibration of the product in basic deuteriomethanol[142].

Hydrogenation of **XIc$_1$** in the presence of a Pd/C catalyst, yielded only 3β-acetyloxy-17β-hydroxy-17α-methyl-5α-androstan-7-one (**XId$_2$**). Similarly, saturation of the 5-6 double bond of a 7-keto steroid with hydrogen in ethyl acetate or 1,4-dioxane as solvent, in the presence of 10% palladium-on-charcoal, gave only the 5α-H-7-keto isomer[143].

To obtain both 5α- and 5β-isomers, by analogy with reduction of 4-en-3-one steroids by sodium borohydride in refluxing pyridine, the same procedure was employed to reduce the conjugated double bond in **XIc$_2$**. This gave only a minor yield of the 5β-H,3α-OR isomer (**XId$_3$**) together with unidentified products.

We still required to have both 5ξ-isomers, so an alternative approach was tried. Hydrogenation of **XIc$_1$** (**Scheme XIB**) in the presence of 5% rhodium-on-charcoal catalyst in methanol/cyclohexane solution, led to a mixture of the two 5ξ-isomers (**XId$_4$**). Oxidation of the 7-hydroxy group by Jones reagent to the 7-ketone (**XIe**), followed by equilibration of the saturated ketone (**XIe**) in basic deuteriomethanol for 3 days, furnished the 6,6,8β-d$_3$ compounds (**XIf**), which were converted to the corresponding p-toluenesulfonylhydrazones[144] (**XIg**) (in methanol-d$_1$ to prevent previously incorporated deuterium atoms from back-exchanging). After back-exchange of deuterium in the tosylhydrazone substituent, the product **XIh** was reduced with an excess of sodium borohydride (to suppress formation of olefins[145,146]) to give, unfortunately, only the 5α-H isomer (**XIi**). In addition, this isomer was contaminated with an unlabeled 17α-methyl-5α-androstane-3ξ,17β-diol. To determine at which step the unlabeled products were formed the entire process was repeated, each step being

Figure 2. The 300 MHz deuterium nmr spectrum of compound **XId$_1$**, assigned as 17β-hydroxy-17α-methyl-3(4),5-androstadien-7-one-2,2,4,6,8β-d$_5$: the spectrum shows the presence of five types of deuterium signals.

Sample HMB4 2H spectrum in CHCl3, unlocked.
reference=CDCl3, HOGWASH enhanced

Current Data Parameters

NAME	hmb4d
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

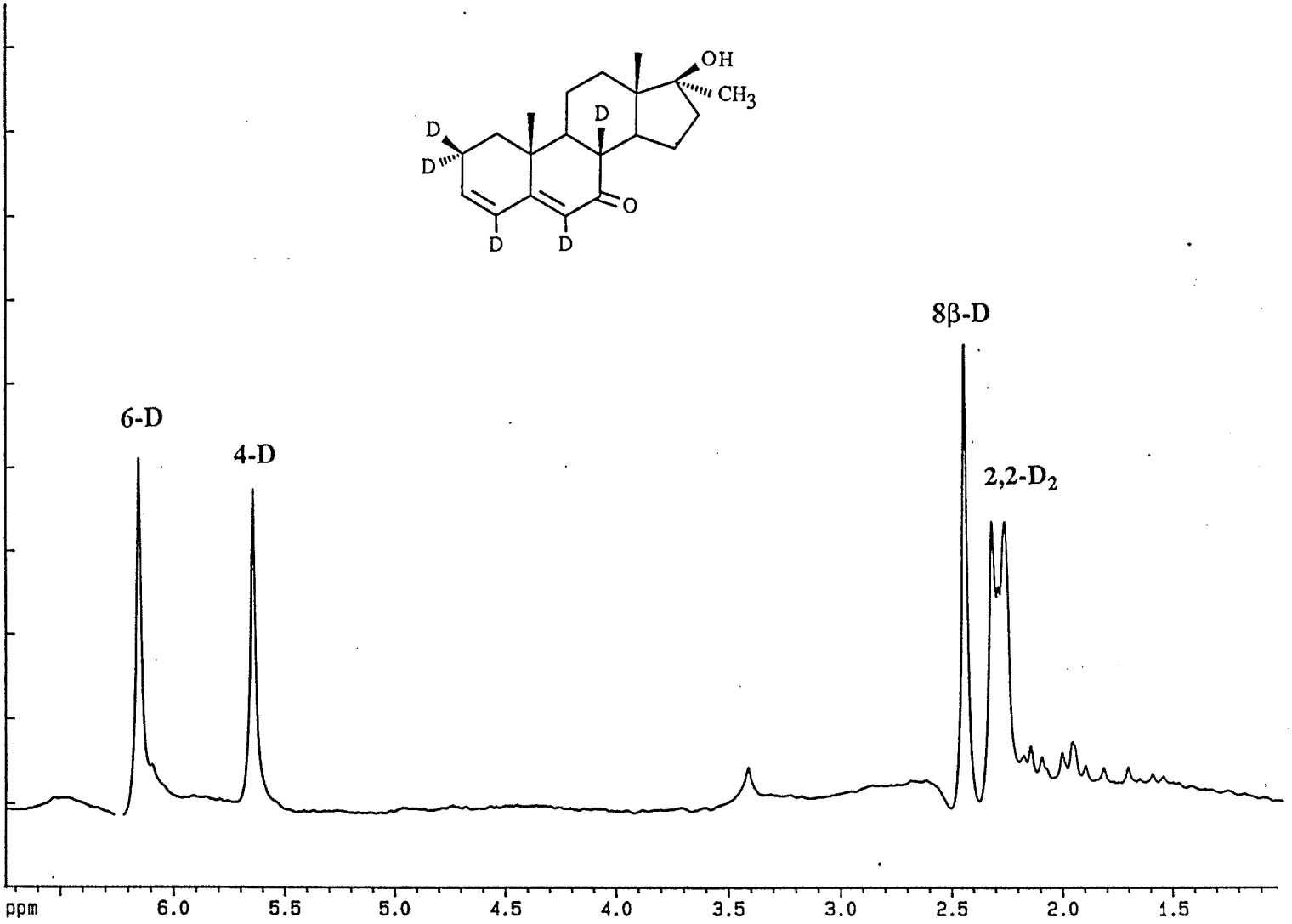
PULPROG	
TD	8192
NS	-1
DS	0
SW	47.808 ppm
SWH	2202.64 Hz
FW	2800.00 Hz
HZpPT	0.134439 Hz
AQ	1.8595840 sec
DW	227.0 usec
SOLVENT	CHCl3
NUCLEUS	deut.
BF1	46.07 MHz
O1	2283.74 Hz
DECNUC	
BF2	300.13 MHz
O2	6500.00 Hz
TE	300 K
AQ_mod	qseq
RG	24784
DR	12
DP	16 dB
DECSTAT	BB
Date	900213
Time	8.08

F2 - Processing parameters

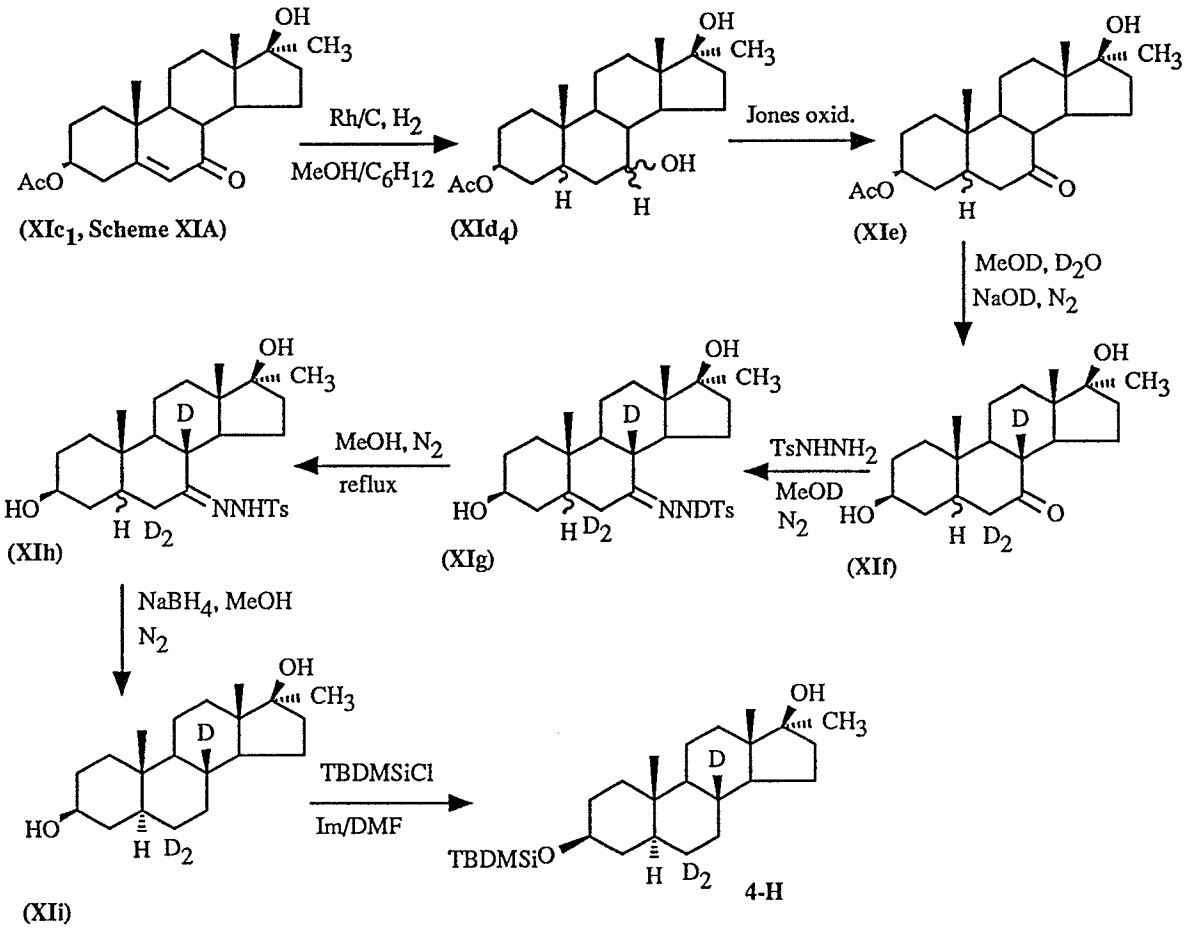
SI	16384
SF	46.0723934 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	4.00

1D NMR plot parameters

CX	32.00 cm
F1P	6.744 ppm
F1	310.73 Hz
F2P	0.736 ppm
F2	33.90 Hz
PPMCM	0.18777 ppm/cm
HZCM	8.65064 Hz/cm



Scheme XIB. Successful preparation of 3β-OSiDMTB-17α-methyl-5α-androstane-6,6,8β-d_3 **(H-4)**.

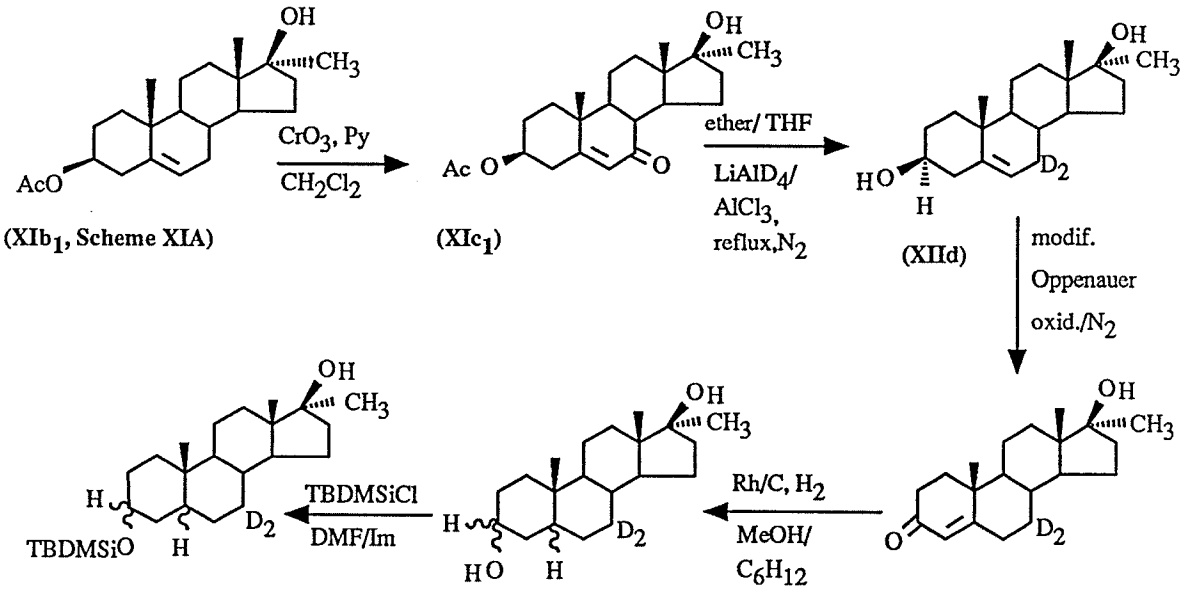


monitored by GC/MS analysis. This revealed that reduction of the allylic 7-ketone, **XIc_1**, with the Rh/C catalyst, was the source of the undesired product. After reduction of the C-7 keto group dehydroxylation occurred.

The key intermediate for the preparation of 7,7-d_2 labeled 5ξ-H compounds, **1-G** to **4-G**, was the same one used for incorporation of deuterium at the 6,6,8β positions, i.e. **XIc_1**. Elimination of the 7-ketone function of **XIIc** with $LiAlD_4/AlCl_3$[141] in ether/THF

(**Scheme XII**) gave the 7,7-d_2 compound (**XIId**). The presence of two deuterium atoms was confirmed by the ^{13}C NMR spectrum, Fig. 3. Modified Oppenauer[147] oxidation of **XIId** (where N-methyl-4-piperidone functions as the hydride acceptor) gave 17β-hydroxy-17α-methyl-4-androsten-3-one (**XIIe**), which was reduced, in the usual way, with 5% rhodium-on-charcoal in a methanol/cyclohexane solvent to 17α-methyl-5ξ--androstane-3ξ,17β-diol-7,7β-d_2 isomers (**XIIf**), together with unidentified side products.

Scheme XII. Preparation of 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstanes-7,7-d_2 (**1-G to 4-G**).



1-G : 2-G : 3-G-: 4-G = 1 : 2 : 2 : 1.6

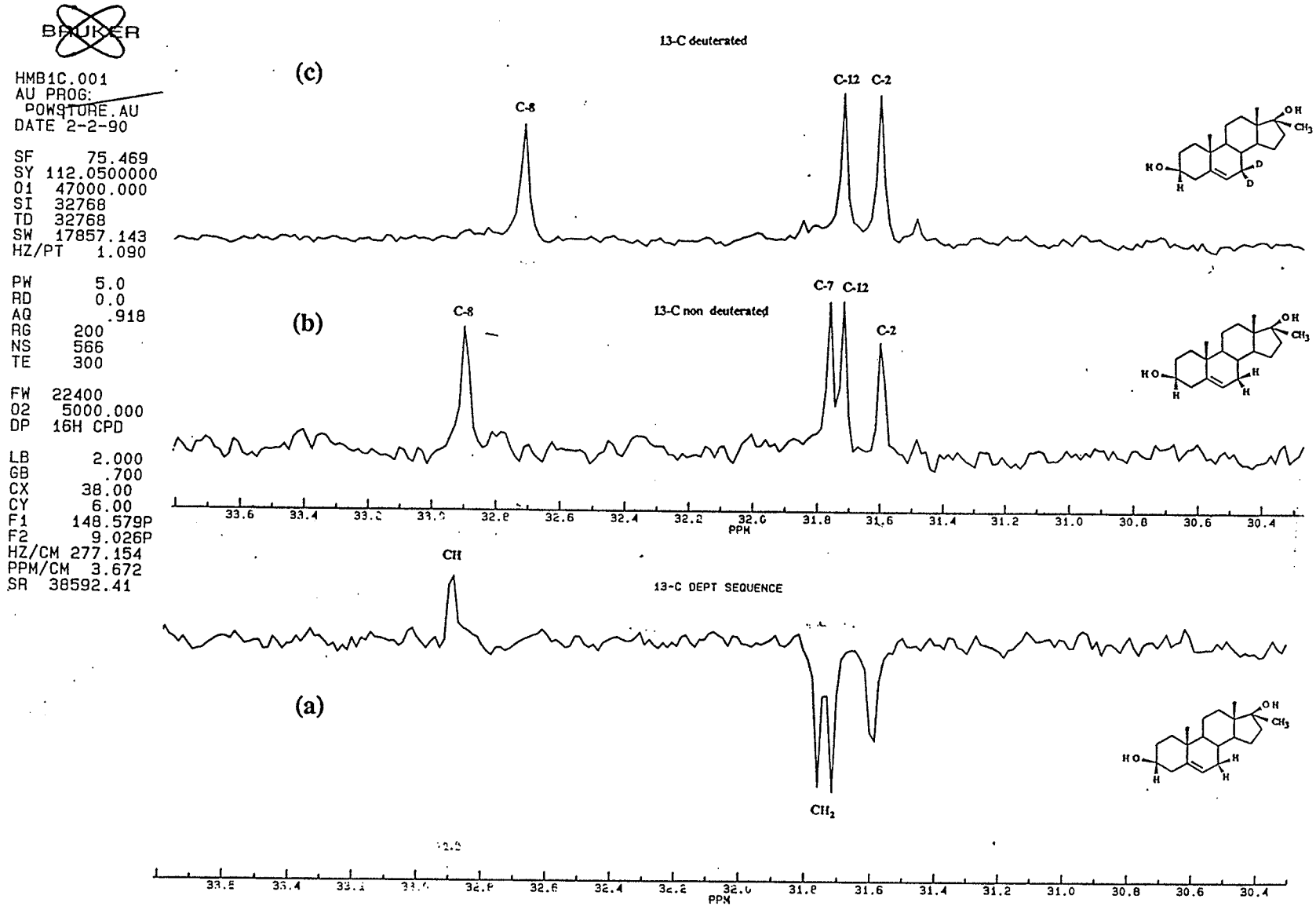
1-G (3α-OR, 5α-H)
2-G (3α-OR, 5β-H)
3-G (3β-OR, 5β-H)
4-G (3β-OR, 5α-H)

Figure 3. The 300 MHz partial ^{13}C nmr spectra of unlabeled and deuterium-labeled compound **XIId**, assigned as 17α-methyl-5-androstene-3,17β-diol.

(a) Partial proton-decoupled spectrum of unlabeled compound (DEPT B35).

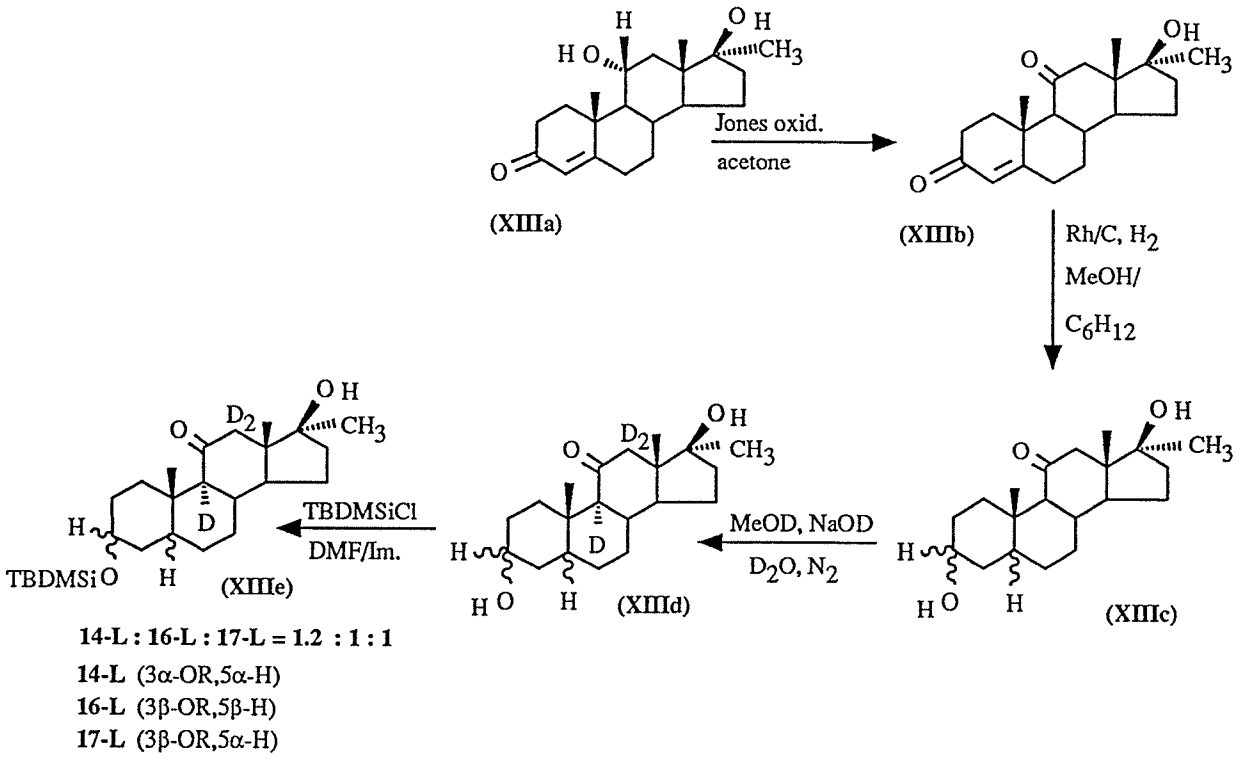
(b) Partial normal spectrum of the unlabeled compound showing C-2, C-7, C-8 and C-12 resonances[149].

(c) Normal spectrum of the labeled compound, C-7,7-d_2. Two deuterium atoms coupled to the ^{13}C-7 give five lines, i.e. $L = 2nI + 1 = 5$, where $I = 1$ for ^{2}H, n = number of deuteriums attached to ^{13}C-7. Thus, the ^{13}C-7 signal is split into five lines of 1:2:3:2:1 intensity which are buried in the background. Therefore, this peak ($\delta = 31.78$ ppm) is not observed in the spectrum, compare (b) to (c). Additionally, the ^{13}C-8 peak is shifted upfield[149], because of the isotope effect.


BRUKER
HMB1C.001
AU PROG:
POWSTURE.AU
DATE 2-2-90
SF 75.469
SY 112.0500000
O1 47000.000
SI 32768
TD 32768
SW 17857.143
HZ/PT 1.090
PW 5.0
RD 0.0
AQ .918
RG 200
NS 566
TE 300
FW 22400
O2 5000.000
DP 16H CPD
LB 2.000
GB .700
CX 38.00
CY 6.00
F1 148.579P
F2 9.026P
HZ/CM 277.154
PPM/CM 3.672
SR 38592.41
(c)
13-C deuterated
C-8
C-12
C-2
(b)
13-C non deuterated
C-8
C-7
C-12
C-2
(a)
CH
CH2
13-C DEPT SEQUENCE
PPM

To introduce a deuterium atom at C-9, commercially available 17α-methyl-4-androstene-11α,17β-diol-3-one (**XIIIa**) was selected as the starting material (**Scheme XIII**). Oxidation of **XIIIa** by Jones reagent in acetone led to the 3,11-dione (**XIIIb**). Catalytic hydrogenation of **XIIIb** in methanol/cyclohexane solution for 4 hr using Rh/C catalyst provided 17α-methyl-5ξ-androstane-3ξ,17β-diol-11-ones (**XIIIc**). According to the Djerassi *et al.*[148], hydrogenation of 4-en-3-keto steroids bearing an 11-one group, in the presence of a Pd/C catalyst, leads to the 5α-H isomer only. However, substitution of Pd/C for the Rh/C catalyst, in our experiment, gave the 5β isomer (30%) in addition to the 5α-H isomer (70%).

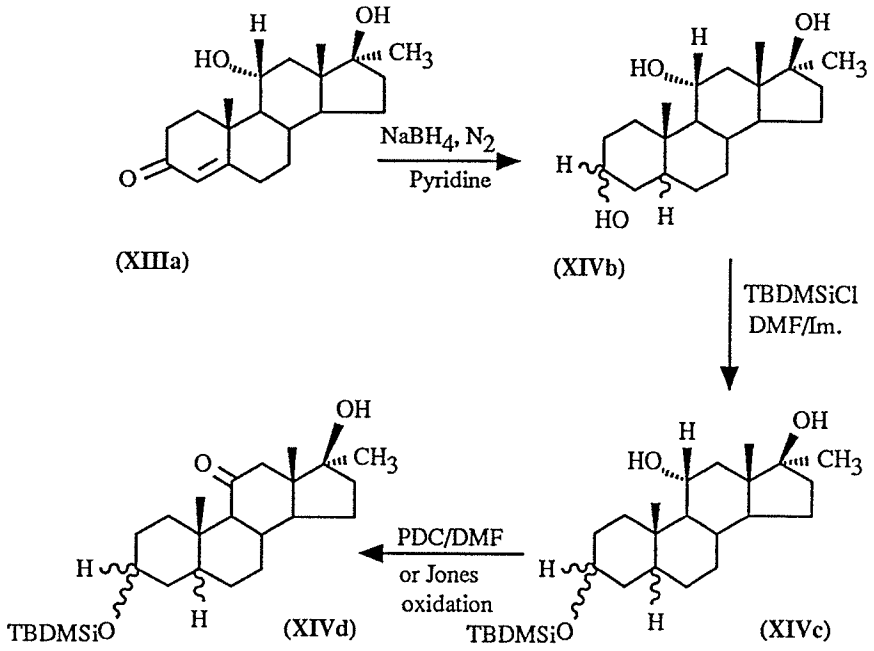
Scheme XIII. Preparation of the three isomers of 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstanes-11-one-9α,12,12-d_3.



As anticipated[150], because of steric interference, the C-11 keto function was not reduced by the heterogenous catalyst, as shown by GC/MS analysis. The product of hydrogenation (**XIIIc**) was then refluxed in the standard $MeOD/D_2O$ equilibration solution. The enolizable hydrogen atoms at C-9 and C-12 were replaced by deuterium to give **XIIId.** Analysis of the derivatized products, **XIIIe**, showed the presence of the three isomers 5α-H,3α-OH (**14-L**), 5β-H,3β-OH (**16-L**), and 5α-H,3β-OH (**17-L**) only, in the ratio 1.2 : 1 : 1, respectively.

Alternatively, reduction of **XIIIa** with $NaBH_4$ in anhydrous pyridine (**Scheme XIV**) furnished a mixture of three 5ξ-H,3ξ,11α,17β-triols (**XIVb**). The product **XIVb**

Scheme XIV. Preparation of 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstan-11-one (**15**, 5β-H,3α-OR; **16**, 5β-H,3β-OR; and **17**, 5α-H,3β-OR).



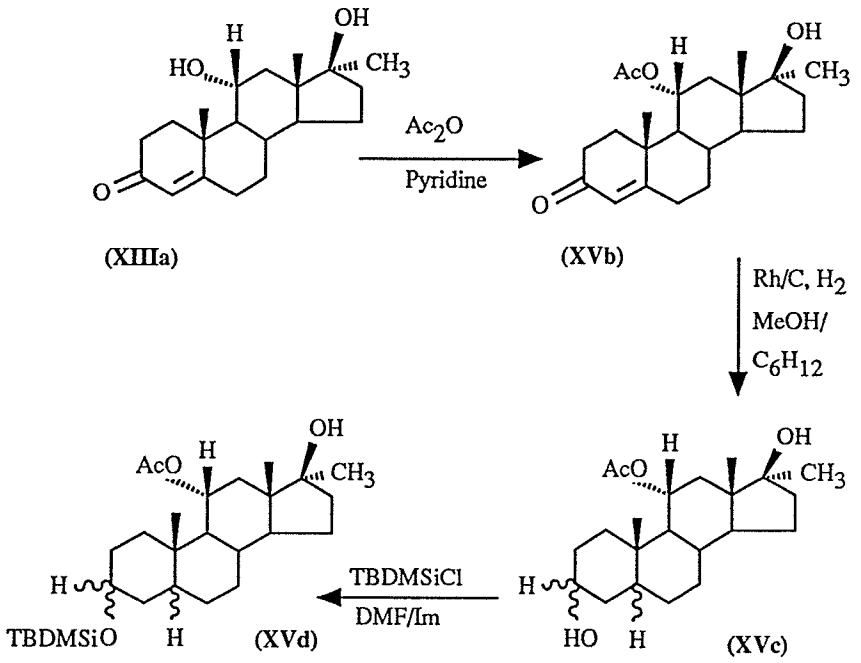
15 : 16 : 17 = 1 : 0.5 : 0.2

15 (3α-OR,5β-H)
16 (3β-OR,5β-H)
17 (3β-OR,5α-H)

was then selectively derivatized at C-3 with the TBDMSiCl reagent[153] to give **XIVc**, which was next oxidized under mild conditions, by pyridinium dichromate[152] in DMF, to **XIVd.** Similarly, oxidation of **XIVc** with Jones reagent in acetone gave the expected 11-ketone, **XIVd.** It is worth noting that acidic Jonesconditions did not remove the silyl substituent. To date, stability of the TBDMSi-ether derivatives to Jones oxidation conditions has not been reported. GC/MS analysis of the product showed the presence of the 3α- and 3β-OSiDMTB epimers of the 5β-H isomer plus the 5α-H,3β-OR **(15)** isomer, in the ratio 5β-H,3α-OR **(15)** : 5β-H,3β-OR **(16)**: 5α-H,3β-OR **(17)** = 1 : 0.5 : 0.2, respectively, (R = TBDMSi).

The following method **(Scheme XV)** was next developed to prepare all four isomers. Acetylation of 17α-methyl-4-androsten-3-one-11α,17β-diol, **XVa,**

Scheme XV. Preparation of 3ξ-OSiDMTB-11α-acetyloxy-17β-hydroxy-17α-methyl--5ξ-androstane isomers **(9-12).**



9 : 10 : 11 : 12 = 0.4 : 0.16 : 1 : 0.1

9 (3α-OR,5α-H) **11** (3β-OR,5β-H)
10 (3α-OR,5β-H) **12** (3β-OR,5α-H)

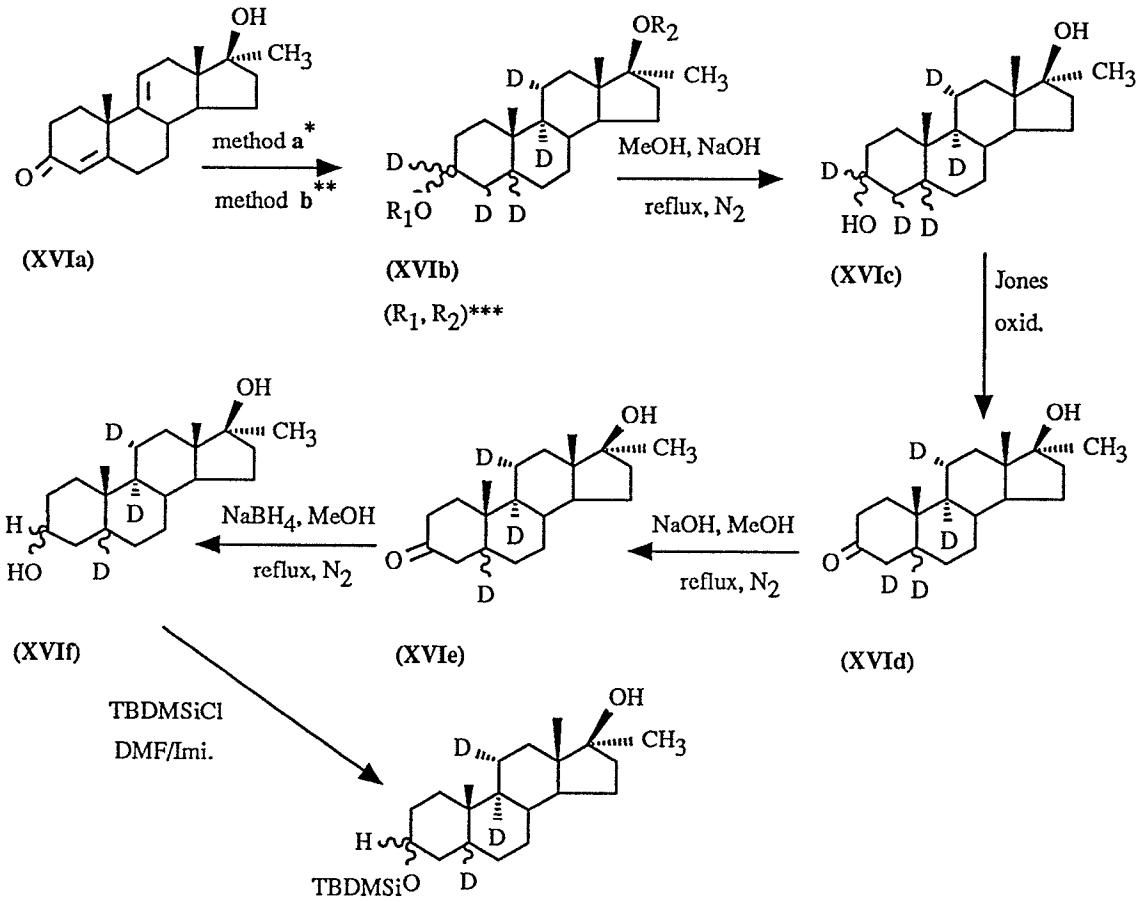
followed by reduction of **XVb** with Rh/C catalyst in MeOH/cyclohexane solution, furnished a mixture of **XVc** isomers, which were then derivatized at O-3 with the TBDMSiCl reagent to give **XVd.** GC/MS analysis showed the presence of two 5ξ-isomers each having two 3ξ-epimers in the ratio 5α-H,3α-OH (**9**) : 5β-H,3α-OH (**10**) : 5β,3β-OH (**11**) : 5α-H,3β-OH (**12**) = 0.4 : 0.16 : 1 : 0.1.

An attempt to synthesize C-9 deuterium-labeled compounds lacking a C-11 oxygen function, by conversion of **XIIId**, in acetic acid[153-155], to the p-toluenesulfonylhydrazone derivative, followed by reduction with $NaBH_4$, was not pursued, after it was established that the formation of the 11-ONNHTs derivative did not proceed.

In another attempt to introduce a deuterium atom at C-9, 17β-hydroxy-17α-methyl-4,9(11)-androstadien-3-one (**XVIa**), was reduced with a pretreated Adams' catalyst (PtO_2) either in acetic acid-d_4(method a) or in CD_3COOD/ethyl acetate solution (method b) (**Scheme XVI**). This gave a mixture of partially acetylated 17α-methyl-5ξ-androstane-3ξ,17β-diol-3,4,5ξ,9α,11α-d_5 isomers (**XVIb**) and, in addition, side products with an unreduced double bond, and some dehydrated compounds. The acetylated products were first saponified, and then oxidized with Jones reagent to the 3-ketone (**XVId**). Base-catalyzed back-exchange of deuterium at C-4 and subsequent reduction of the ketone, **XVIe**, with $NaBH_4$ in methanol, gave a mixture of the four isomers of 17α-methyl-5ξ-androstan-3ξ,17β-diol-5ξ,9α,11α-d_3 (**XVIf**), and some by-products. Deuteration of **XVIa** with non-pretreated PtO_2 catalyst in CD_3COOD/AcOEt gave a mixture of similar products but with a higher content of unsaturated compounds.

Attempts to simultaneously reduce both double bonds of the 17β-hydroxy-17α-methyl-4,9(11)-dien-3-one steroid (**XVIa**) with deuterium in the presence of 5% Rh/C in methanol/cyclohexane, 10% Pd/C in CD_3COOD, or $[(C_6H_5)_3P]RhCl$ in 1,4 dioxane, gave negative results. GC/MS analysis showed that, in any event, the 9,11 double bondwas not reduced. The results of several attempted reductions showed that PtO_2

Scheme XVI. Preparation of 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstane-5-ξ,9α,11α-d_3 (**1-I** to **4-I**)



1-I : 2-I : 3-I : 4-I = 0.006 : 0.16 : 0.0004 : 1

1-I (3α-OSiDMTB, 5α-D) **3-I** (3β-OSiDMTB, 5β-D)

2-I (3α-OSiDMTB, 5β-D) **4-I** (3β-OSiDMTB, 5α-D)

* PtO_2, D_2, CD_3COOD

** PtO_2, D_2, CD_3COOD/AcOEt

*** $R_1 = H$, $R_2 = H$

R_1= Ac-d_3, $R_2 = H$

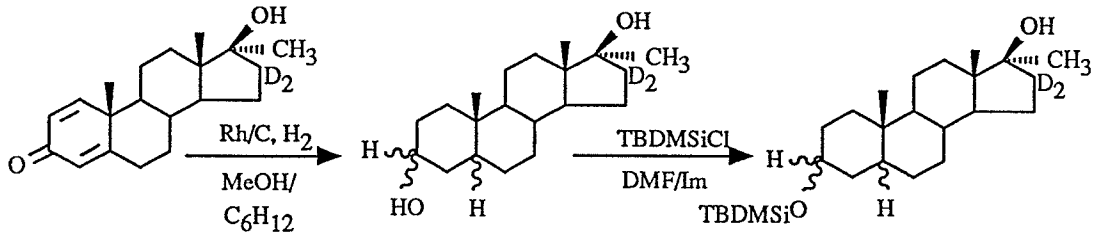
$R_1 = H$, R_2= Ac-d_3

R_1 = Ac-d_3 R_2 = Ac-d_3

catalyst provided the most convenient method for reduction of the 4,9(11) double bonds, but in low yield and gave poor isotopic purity of the desired products.

The availability of 16,16-d_2[128] and 17α-methyl-d_3[128] analogues of 17α-hydroxy-17α-methyl-1,4-androstadien-3-one offered a direct route to the labeled 17α-methyl-

Scheme XVII. Preparation of 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstanes-16,16-d_2 (**1-J** to **4-J**).

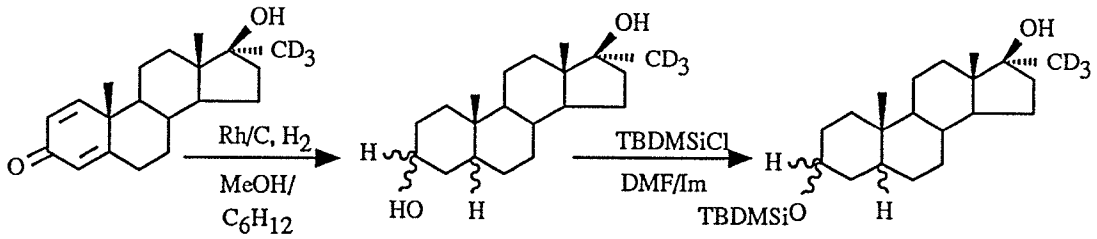


(XVII)[128] (XVIIb)

1-J : 2-J : 3-J : 4-J = 2.5 : 1 :12.5 : 0.25

1-J (3α-OR,5α-H) **3-J** (3β-OR,5β-H)
2-J (3α-OR,5β-H) **4-J** (3β-OR,5β-H)

Scheme XVIII. Preparation of 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-d_3-5ξ-androstanes (**1-K** to **4-K**).



(XVIII)[128] (XVIIIb)

1-K : 2-K : 3-K : 4-K = 2.5 : 1 :12.5 : 0.25

1-K (3α-OR,5α-H) **3-K** (3β-OR,5β-H)
2-K (3α-OR,5β-H) **4-K** (3β-OR,5α-H)

5ξ-androstane-3ξ,17β-diols by hydrogenation of the 1,4-dien-3-ones in methanol/cyclohexane solution in the presence of the Rh/C catalyst (**Schemes XVII** and **XVIII**). These compounds were synthesized to study the electron impact induced elimination of a water molecule from ring D of mono-3ξ-OSiDMTB steroids.

II.B.4. Syntheses of Specific Compounds.

Syntheses of compounds for Scheme I.

17β-hydroxy-17α-methyl-5α-androstan-3-one-2,2,4,4-d_4 (**Ib**).

The starting material, 17β-hydroxy-17α-methyl-5α-androstan-3-one, **Ia**, (100 mg, 0.328 mmol) was dissolved in 2 mL of the NaOD/MeOD/D_2O of the standard reagent (1M; section III.B.2, procedure F) and refluxed in a current of nitrogen for 1/2 hr. The solvent was evaporated *in vacuo* and the wet residue was extracted with ether, and deuterium oxide was withdrawn by pipette. The ether was evaporated *in vacuo* and the exchange process was repeated twice more. The final product was extracted with ether. The ether layer was washed with cold water, saturated sodium chloride, dried over anhydrous magnesium sulfate, and evaporated to dryness.

17β-hydroxy-17α-methyl-5α-androstan-3ξ-ol-2,2,4,4-d_4 (**Ic**).

The product, **Ib**, (22 mg, 0.07 mmol) was reduced with sodium borohydride (20 mg, 0.52 mmol) in refluxing methanol-d_1 (1.5 mL) for 1 hr and worked-up according to procedure A (section III.B.2).

3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-2,2,4,4-d_4 (**1-C** and **4-C**)

The product, **Ic**, was derivatized according to procedure B (section III.B.2). GC/MC analysis showed two epimers in the ratio 5α-H,3α-OR (**1-C**) : 5α-H,3β-OR (**4-C**) = 1 : 5, (R = TBDMSi), identified by reference spectra and the diagnostic [M -

t-Bu]$^+$ ion, m/z 367, in their mass spectra.

Syntheses of compounds for Scheme II.

17β-hydroxy-17α-methyl-5β-androstan-3-one-2,2,4,4-d_4 **(IIb)**

The starting material, 17β-hydroxy-17α-methyl-5β-androstan-3-one, **IIa**, (100 mg, 0.328 mmol), was dissolved in 2 mL of the NaOD/MeOD/H_2O standard reagent and refluxed for 20 min. Subsequent steps were performed exactly as for **Ib.**

17β-hydroxy-17α-methyl-5β-androstan-3ξ-ol-2,2,4,4-d_4 **(IIc).**

The equilibration product, **IIb**, (10 mg, 0.032 mmol) was refluxed in tetrahydrofuran (2 mL) and ethyl alcohol (0.5 mL) with sodium borohydride (15 mg, 0.39 mmol) for 1 hr, and then worked-up according to procedure A (section III.B.2).

3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-2,2,4,4-d_4 **(2-C and 3-C)**

The reduction product, **IIc**, was derivatized according to procedure B. GC/MS analysis showed two 3ξ-epimers in the ratio 5β-H,3α-OR (**2-C**) : 5β-H,3β-OR (**4-C**) = 4 : 1 (R = TBDMSi), identified by GC retention times and the diagnostic [M -*t*-Bu]$^+$ ion, m/z 367, in their mass spectra.

Syntheses of compounds for Scheme III.

17α-methyl-5α-androstan-3ξ,17α-diol-3ξ-d_1 **(IIIb)**

The starting material, 17β-hydroxy-17α-methyl-5α-androstan-3-one, **IIa**, (1 mg, 0.036 mmol) was refluxed with sodium borodeuteride (13 mg, 0.31 mmol) in dry tetrahydrofuran, in a current of nitrogen for 1 hr. Excess sodium borodeuteride was then decomposed by 10% NH_4Cl solution. After deuterium gas evolution ceased the residue was extracted with ether. *Work-up:* the organic layer was washed with water, brine, and then dried over $MgSO_4$. Evaporation of the solvent afforded a white residue of **IIIb.**

3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-3ξ-d_1 (**1-D** and **4-D**)

The reduction product, **IIIb**, was derivatized according to procedure B. GC/MS analysis showed two 3-epimers in the ratio 5α-H,3α-OR (**1-D**) : 5α-H,3β-OR (**4-D**) = 1 : 2 (R = TBDMSi), identified by GC retention times and the diagnostic $[M - t\text{-Bu}]^+$ ion, m/z 364, in their mass spectra.

Syntheses of compounds for Scheme IV.

17α-methyl-5β-androstan-3ξ,17β-diol-3ξ-d_1 (**IVb**)

The starting material, 17β-hydroxy-17α-methyl-5β-androstan-3-one, **IVa**, (40 mg, 0.132 mmol) was refluxed with sodium borodeuteride (22 mg, 0.526 mmol) in ethanol (3 mL) in a current of nitrogen for 1hr. The work-up was the same as for compound **IIIb**.

3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-3ξ-d_1 (**2-D** and **3-D**)

The product, **IVb**, was derivatized according to procedure B to give **IVc**. GC/MS analysis showed two epimers in the ratio 5β-H,3α-OR (**2-D**) : 5β-H,3β-OR (**3-D**) = 14 : 1 (R = TBDMSi), identified by GC retention times and the diagnostic $[M - t\text{-Bu}]^+$ ion, m/z 364, in their mass spectra.

Syntheses of compounds for Scheme V.

17α-methyl-5ξ-androstan-3ξ-17β-diol-3ξ,5ξ-d_2 (**Vb**)

The starting material, 17β-hydroxy-17α-methyl-4-androsten-3-one, **Va**, (200 mg, 0.662 mmol) was refluxed in anhydrous pyridine (2 mL) with sodium borodeuteride (100 mg, 2.3 mmol) for 10hr in a current of nitrogen. *Work-up:* the reaction mixture was cooled to room temperature, poured into ice-water, and allowed to stand for 30 minutes; it was then acidified with 5% hydrochloric acid and extracted with ether. The organic layer was washed with water, 5% sodium bicarbonate, water, brine, dried over Na_2SO_4, and evaporated to dryness.

17β-hydroxy-17α-methyl-5ξ-androstan-3-one-5ξ-d_1 **(Vc)**

The crude product, **Vb**, was subjected to Jones oxidation and worked-up according to procedure C.

17α-methyl-5ξ-androstan-3ξ,17β-diol-5ξ-d_1 **(Vd)**

The oxidation product, **Vc**, (10 mg, 0.032 mmol) was refluxed in ethanol (2 mL) with sodium borohydride (20 mg, 0.52 mmol) in an atmosphere of nitrogen for 2 hr. Work-up was as described for **IIb**, above.

3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstane-5ξ-d_1 **(1-E to 4-E)**

The product, **Vd**, was derivatized according to procedure B. GC/MS analysis showed four isomers 5α-H,3α-OH **(1-E)** : 5β-H,3α-OR **(2-E)** : 5β-H,3β-OR **(3-E)** : 5α-H,3β-OR **(4-E)** in the ratio 1 : 15 : 4 : 19 (R = TBDMSi), respectively, identified by GC retention times and the diagnostic $[M - t\text{-Bu}]^+$ ion, m/z 364, in their mass spectra.

Syntheses of compounds for Scheme VI.

17β-methyl-5ξ-androstan-3ξ,17α-diol-3ξ,5ξ-d_2 **(VIb)**

The starting material, 17α-hydroxy-4-androsten-3-one, **VIa**, (50 mg, 0.165 mmol) was refluxed in anhydrous pyridine (2 mL) with sodium borodeuteride (90 mg, 2.14 mmol)for 14 hr in a current of nitrogen. Work-up was performed exactly as for **Vb**.

17α-hydroxy-17β-methyl-5ξ-androstan-3-one-5ξ-d_1 **(VIc)**

The crude product, **VIb**, was subjected to Jones oxidation in acetone and worked-up according to procedure C.

17β-methyl-5ξ-androstan-3ξ,17α-diol-5ξ-d_1 **(VId)**

The oxidation product, **VIc**, (30 mg) was refluxed in methanol (2 mL) with sodium borohydride (20 mg) in an atmosphere of nitrogen for 1hr. Work-up was the same as described for **IIIb**.

3ξ-OSiDMTB-17α-hydroxy-17β-methyl-5ξ-androstane-5ξd_1 **(5-E to 8-E)**

The product, **VId**, was derivatized according to procedure B. GC/MS analysis

showed the four isomers in the ratio 5α-H,3α-OR (**1-E**) : 5β-H,3α-OR (**2-E**) : 5β-H,3β-OR (**3-E**) : 5α-H,3β-OR (**4-E**) = 7 : 57 : 2 : 35 (R = TBDMSi), identified by GC retention times and the diagnostic $[M - t\text{-Bu} - H_2O]^+$ ion, m/z 345, in their mass spectra.

Syntheses of compounds for Scheme VII.

17α-methyl-5ξ-androstan-3ξ,17β-diol-1,3,5ξ-d_5 (**VIIb**)

The starting material, 17β-hydroxy-17α-methyl-1,4-androstadien-3-one, **VIIa**, (150 mg, 0.5 mmol) was refluxed in anhydrous pyridine (5 mL) with $NaBD_4$ (150 mg, 3.59 mmol) for 14 hr in a current of nitrogen. Work-up was performed exactly as for compound **Vb**.

17β-hydroxy-17α-methyl-5ξ-androstan-3-one-1,5ξ-d_4 (**VIIc**)

The crude d_3 product, **VIIb**, was subjected to Jones oxidation and worked-up according to procedure E.

17α-methyl-5ξ-androstan-3ξ,17β-diol-1ξ,5ξ-d_2 (**VIId**)

The dideuterioketone, **VIIc**, (50 mg), was reduced with $NaBH_4$ in refluxing methanol solvent (2 mL) and worked-up according to procedure A.

3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstane-1ξ,5ξ-d_2 (**1-A'** to **4-A'**)

The reduction product, **IXd**, was derivatized with TBDMSiCl standard reagent according to procedure B. GC/MS analysis showed the presence of two 5ξ-isomers, each having 3ξ-epimers in the ratio 5α-H,3α-OR (**1-A'**) : 5β-H,3α-OR (**2-A'**) : 5β-H,-3β-OR (**3-A'**) : 5α-H,3β-OR (**4-A'**) = 0.14 : 1 : 0.4 : 0.7 (R = TBDMSi).

Syntheses of compounds for Scheme VIII

17α-hydroxy-17β-methyl-5α-androstan-3-one-1,2,4,5α-d_4 (**VIIIb**)

For this reaction the catalyst and solvent were pretreated with D_2 gas for 20 minutes prior to addition of steroid. The starting material, 17α-methyl-17β-hydroxy-1,4-androstadien-3-one, **VIIIa**, (200 mg, 0.666 mmol) and tris(triphenylphosphine)-

rhodium chloride (200 mg) in methanol-d_1 (0.5 mL) and 1,4-dioxane (5 mL) were stirred in a deuterium atmosphere at room temperature for 10 days; reaction was then complete as monitored by TLC. (The reduction products showed no conjugated double bonds when tested under a UV lamp). After evaporation of the solvent *in vacuo,* the residue was dissolved in ethyl ether and chromatographed on a column of alumina (3g), swollen with ether. Elution with ether gave a residue still contaminated with catalyst; therefore, the residue was recrystallized from methanol/methylene chloride.

17α-hydroxy-17β-methyl-5α-androstan-3-one-1α,5α-d_2 **(VIIIc)**

The recrystallized product, **VIIIb**, (50 mg, 0.162 mmol) was back-exchanged in methanol (2mL) with sodium hydroxide (180 mg) for 1 hr and worked-up according to procedure E.

17α-methyl-5α-androstan-3ξ,17β-diol-1α,5α-d_2 **(VIIId)**

The product, **VIIIc**, (10 mg, 0.26 mmol) was reduced with sodium borohydride (10 mg, 0.26 mmol) in methanol (2 mL) and worked-up according to procedure A.

3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-1α,5α-d_2 (**1-A** and **4-A**)

The product, **VIIId**, was derivatized according to general procedure B. GC/MS analysis showed the presence of two 3ξ-epimers in the ratio 5α-H,3α-OH (**1-A**) : 5α-H,3β-OH (**4-A**) = 1 : 7, identified by GC retention times and the diagnostic $[M - t\text{-Bu}]^+$ ion, m/z 365, in their mass spectra.

Syntheses of compounds for Scheme IX.

17β-hydroxy-17α-methyl-5ξ-androstane-3-one-1,2β,4,5ξ-d_4 **(IXb)**

The starting material, 17β-hydroxy-17α-methyl-1,4-androstadien-3-one, **IXa**, (50 mg, 0.166 mmol) was added to dry 1,4-dioxane (3 mL) containing 10% Pd/C (50 mg) which had been pretreated with deuterium gas for 4.5 hr. The mixture was stirred in the presence of deuterium gas for 1hr; monitoring by TLC under a UV lamp showed that the conjugated double bonds had been reduced.

17β-hydroxy-17α-methyl-5ξ-androstan-3-one-1,2β,4,5ξ-d_4 **(IXc)**

The reduction of **IXa** (see above), with deuterium in the presence of palladium as a catalyst gave small amounts of the C-3 alcohols. Therefore, reoxidation of **IXb** reaction was necessary. The **IXb** products (*ca* 50 mg) were oxidized with Jones reagent and worked-up according to procedure C to give **IXc**.

17β-hydroxy-17α-methyl-5ξ-androstan-3-one-1β,5ξ-d_2 **(IXd)**

The d_4 product, **IXc**, (*ca* 20 mg) was back-exchanged with 1M methanolic sodium hydroxide (2 mL) for 3 hr and worked-up according to procedure E.

17α-methyl-5ξ-androstan-3ξ,17β-diol-1β,5ξ-d_2 **(IXe)**

A methanol solution of the dideuterioketone, **IXd**, (10 mg in 2 mL) was reduced with sodium borohydride (10 mg) and worked-up according to procedure B (section II.B.2).

3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstane-1β,5ξ-d_2 **(1-B to 4-B)**

The product, **IXe**, was derivatized according to procedure C. GC/MS analysis showed the presence of four isomers in the ratio 5α-H,3α-OR **(1-B)** : 5β-H,3α-OR **(2-B)** : 5β-H,3β-OR **(3-B)** : 5α-H,3β-OR **(4-B)** = 0.006 : 16 : 3 : 1 (R = TBDMSi), identified by GC retention times and the diagnostic $[M - t\text{-Bu}]^+$ ion, m/z 365, in their mass spectra.

Syntheses of compounds for Scheme X.

17β-hydroxy-17α-methyl-4-androsten-3-one-2,2,4,6,6-d_5 **(Xb)**

The starting material, 17β-hydroxy-17α-methyl-4-androsten-3-one, **Xa**, (200 mg, 0.662 mmol) was treated with the NaOD/MeOD/D_2O standard reagent (3 mL) in a current of nitrogen for 18 hr (procedure F). After evaporation of the solvent *in vacuo*, the wet residue was extracted with ether and deuterium oxide was withdrawn by a pipete. The ether solvent was evaporated, *in vacuo,* and the entire process was repeated twice more. The final product was extracted with ether. The ether layer was washed with cold water, brine, and dried over anhydrous $MgSO_4$. Evaporation of ether afforded a yellow

solid, **Xb**.

17α-methyl-5ξ-androstan-3ξ,17β-diol-2,2,4,6,6ξ-d_5 (**Xc**)

The crude product, **Xb**, (30 mg, 00974 mmol) in methanol (1 mL) and cyclohexane (4 mL) was reduced over a 5% rhodium-on-charcoal catalyst (16 mg) in an atmosphere of deuterium gas for 14 hr. The catalyst was filtered off and the solution was evaporated to dryness *in vacuo* to give **Xc**.

17α-hydroxy-17β-methyl-5ξ-androstan-3-one-2,2,4,6,6ξ-d_5 (**Xd**)

The total crude product, **Xc**, was oxidized in acetone with Jones reagent and worked-up according to procedure C (section II.D.2).

17α-hydroxy-17β-methyl-5ξ-androstan-3-one-6,6-d_2 (**Xe**)

The oxidation product, **Xd**, (*ca* 20 mg, 0.064 mmol), was subjected to alkaline back-exchange equilibration by refluxing for 2 hr with 1N methanolic sodium hydroxide (2 mL) and worked-up according to procedure E (section II.B.2).

17α-methyl-5ξ-androstan-3ξ,17β-diol-6,6-d_2 (**Xf**)

The crude product, **Xe**, (10 mg, 0.032 mmol) was reduced with $NaBH_4$ (30 mg, 0.79 mmol) in refluxing methanol (1.5 mL) and worked-up according to procedure A.

3ξ-OSiDMTB-17α-hydroxy-17β-methyl-5ξ-androstane-6,6-d_2(**1-F to 4-F**)

The reduction product, **Xf**, was derivatized according to procedure B. GC/MS analysis showed the presence of four isomers in the ratio 5α-H,3α-OR (**1-F**) : 5β-H,3α-OR (**2-F**) : 5β-H,3β-OR (**3-F**) : 5α-H,3β-OR (**4-F**) = 0.2 : 4 : 1 : 2 (R = TBDMSi), identified by GC retention times and the diagnostic $[M - t\text{-Bu}]^+$ ion, m/z 365, in their mass spectra.

Syntheses of compounds for Schemes XIA and XIB.

(Scheme XIA)

3β-acetyloxy-17β-hydroxy-17α-methyl-5-androstene (**XIb_1**)

The starting material, 17α-methyl-5-androsten-3β,17β-diol, **XIa**, (500 mg, 1.644 mmol), was acetylated by acetic anhydride in pyridine and worked-up according to procedure G (section II.B.2).

3β-acetyloxy-17β-hydroxy-17α-methyl-5-androsten-7-one (**XIc$_1$**)

Chromium trioxide (0.780 g, 7.8 mmol), dried over P_2O_5, was added to pyridine (1.27 mL, 1.246 g, 15.5 mmol) in methylene chloride (20 mL). After stirring this reagent for 30 minutes at room temperature under anhydrous conditions, the acetylation product, **XIb$_1$**, (500 mg, 1.445 mmol) in methylene chloride (20 mL) was added and the mixture was stirred for over 48 hr. The supernatant solution was decanted, and the black residue was washed with ether (3 x 20 mL). The ether washings were combined with the supernatant solution and the resulting mixture was then extracted with aqueous sodium bicarbonate (5 x 5 mL). The sodium bicarbonate washings were extracted once with ether (4 mL). The combined ether extracts were then washed with 5% HCl (4 x 4 mL), 5% sodium bicarbonate, water, saturated NaCl (1 x 4 mL), dried over $MgSO_4$, and evaporated to give a yellow residue. This product had a conjugated double bond (as tested by TLC and a UV lamp) and a molecular mass of 360 (GC/MS) and is the desired product, **XIc$_1$**.

17β-hydroxy-17α-methyl-3(4),5-androstadien-7-one (**XId$_1$**)

The oxidation product, **XIc$_1$**, (50 mg) was subjected to alkaline equilibration in methanol-d_1 (5 mL) for 3 days and then worked-up according to procedure E. GC/MS analysis showed the presence of only one compound, with a molecular ion of m/z 305. The deuterium nmr spectrum showed five types of deuterium resonance (Fig. 2, section II.B.3.)

Similarly, the oxidation product, **XIc$_2$**, (50 mg) subjected to the above conditions, gave **XId$_1$**, with a molecular ion of m/z 305, as monitored by GC/MS analysis

3β-acetyloxy-17β-hydroxy-17α-methyl-5α-androstan-7-one (**XId$_2$**)

The oxidation product, **XIc$_1$**, (100 mg) was hydrogenated in the presence of 10%

Pd/C (50 mg) in 1,4 dioxane (10 mL, distilled over CaH_2) for 4hr. The product, monitored by TLC under a UV lamp, lacked a conjugated double bond. GC/MS analysis showed one chromatographic peak, indicating the presence of one isomer. The presence of the diagnostic $[M - CH_3COOH]^+$ ion, m/z 300, indicated that the 7-keto function had not been reduced to the 7-hydroxy group.

3β-OSiDMTB-17β-hydroxy-17α-methyl-5-androstene (**XIb$_2$**)

The starting material, **XIa** (0.358 mg, 1.2 mmol), dissolved in DMF (5 mL), was allowed to react with the TBDMSiCl/Im reagent (section II.B.2; 1.3 mmol in 2 mL of DMF) overnight, and then the solution was extracted with ether. The ether layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated to yield a residue of **XIb$_2$**.

3β-OSiDMTB-17β-hydroxy-17α-methyl-5-androsten-7-one (**XIc$_2$**)

Chromium trioxide (1.91g, 0.0191 mol), dried over P_2O_5, was added to pyridine (3.2 mL) in methylene chloride (50 mL). After stirring the mixture for 30 minutes at room temperature under anhydrous conditions, **XIb$_2$** (*ca* 400 mg), in methylene chloride (10 mL), was added and the mixture was stirred for over 48hr. Work-up was performed according to the procedure used for **XIb$_1$**. The product on TLC, under a UV lamp, showed the presence of a conjugated double bond. GC/MS analysis of the product, showed the diagnostic $[M - t\text{-Bu}]^+$ ion, m/z 375, anticipated for **XIc$_2$**.

(Scheme XIB)

3β-acetyloxy-7ξ,17β-dihydroxy-17α-methyl-5ξ-androstane (**XId$_4$**)

The crude product, **XIc$_1$**, (200 mg, 0.633 mmol) in methanol (3 mL) and cyclohexane (5 mL) was stirred in a hydrogen atmosphere in the presence of 5% rhodium-on-charcoal (100 mg) for 6 hr. Filtration from catalyst and evaporation of the solvent gave a glassy residue. The product on TLC, under a UV lamp, lacked a conjugated double bond. GC/MS analysis showed that the 7-one functional group had

been reduced to the 7-alcohol.

17α-methyl-5ξ-androstan-3,17β-diol-7-one 3 acetate **(XIe)**

The crude product, **Xd_3,** was oxidized with Jones reagent and worked-up according to procedure C (section II.D.2). GC/MS analysis showed the presence of two 5ξ-epimers, in the ratio 5β-H, 3β-OAc : 5α-H,3β-OAc = 1 : 7.

17α-methyl-5ξ-androstan-3β,17β-diol-7-one-6,6,8β-d_3 **(XIf)**

The crude product, **XIe**, (150 mg) was subjected to alkaline equilibration in methanol-d_1 (5 mL) and worked-up according to procedure F. The solution was acidified with 5% DCl, and extracted with ether. The ether layer was washed with cold water, dried over Na_2SO_4, and evaporated, to yield a residue of **XIf.**

17α-methyl-5ξ-androstan-3β,17β-diol-7-NNDTs-6,6,8β-d_3 **(XIg)**

The crude product, **XIf**, and p-toluenesulfonylhydrazine (100 mg) were refluxed overnight in methanol-d_1 (3 mL) in a current of nitrogen and the solvent evaporated *in vacuo* to give a residue of **XIg**.

17α-methyl-5ξ-androstan-3β,17β-diol-7-NNHTs-6,6,8β-d_3 **(XIh)**

The crude product, **XIg**, was refluxed in methanol (2 mL), in a current of nitrogen for 1hr, to back-exchange a deuterium atom of the tosylhydrazine derivative. The solvent was evaporated *in vacuo* to give a residue of **XIh.**

17α-methyl-5α-androstan-3β,17β-diol-6,6,8β-d_3 **(4-H)**

The tosylhydrazine derivative, **XIh**, was refluxed with $NaBH_4$ (60 mg) in methanol (2 mL) in a current of nitrogen for 3hr. Water was added and the residue was extracted with ether. The ether layer was washed with 5% HCl, water, 5% sodium bicarbonate, water, brine, dried over Na_2SO_4 and evaporated to give **XIf.** GC/MS analysis showed the presence of only the 3β-OH,5α-H **(4-H)** isomer.

Syntheses of compounds for Scheme XII.

17α-methyl-5-androsten-3β,17β-diol-7,7-d_2 **(XIId)**

Lithium aluminum deuteride (55 mg, 1.3 mmol) in dry ether (3.5 mL) under nitrogen, cooled in an ice-bath, was cautiously treated with aluminum chloride (348 mg, 3.41 mmol) in dry ether (3.5 mL). The mixture was stirred for 0.5 hr under reflux, cooled to room temperature, and 17β-hydroxy-17α-methyl-5-androsten-7-one, **XIc**, (50 mg, 0.151 mmol) in ether (2 mL), was added while maintaining a gentle reflux. After refluxing for 2 hr, the reaction mixture was cooled, and water (2 mL) was carefully added. After separation of the aqueous layer, the organic phase was washed with 5% HCl, water, 5% $NaHCO_3$, water, dried over anhydrous $MgSO_4$, and the solvent was evaporated to give a white residue. The ^{13}C nmr spectrum showed the presence of two deuterium atoms at C-7, Fig. 3 (c).

17β-hydroxy-17α-methyl-4-androsten-3-one-7,7-d_2 (**XIIe**)

The crude product, **XIId**, (20 mg, 0.0654 mmol) in anhydrous toluene (12 mL), was refluxed in a current of nitrogen, and 5 mL of solvent was distilled off. The solution was then treated with freshly distilled (under reduced pressure) 1-methyl-4-piperidone (3.8 mL, 3.5 g, 31 mmol), in toluene (5 mL), and then the solution was heated until 5 mL of toluene was distilled off. Then aluminum isopropoxide (0.2g, 1.0 mmol) in toluene (5 mL) was added, the mixture was refluxed for a further 2 hr, and then 5 mL of toluene was distilled off. The reaction mixture, which had turned an orange color, was cooled to room temperature, diluted with ether (25 mL) and washed with 5% sulphuric acid (2 x 25 mL), which was re-extracted with ether before being rejected. The combined ethereal extracts were washed with water, 5% sodium bicarbonate, water, brine, and then dried over anhydrous Na_2SO_4. Evaporation of the solvent furnished a white residue which, when tested by TLC under UV lamp, showed the presence of a conjugated double bond, anticipated for **XIIe.**

17α-methyl-5ξ-androstan-3ξ,17β-diol-7,7-d_2 (**XIIf**)

The crude product (15 mg) from the previous step was hydrogenated in the presence of the 5% rhodium-on-charcoal catalyst (12 mg) in methanol (0.5 mL)

/cyclohehane (2 mL) for 4 hr. Filtration from the catalyst and evaporation of the solvent gave a glassy residue assumed to be **XIIf.**

3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstane-7,7-d_2 **(1-G to 4-G))**

The reduction product from the previous step was derivatized according to procedure B. The final product consisted of two 5ξ-isomers each having two 3ξ-epimers, as shown by GC/MS, in the ratio 5α-H,3α-OR **(1-G)** : 5β-H,3α-OR **(2-G)** : 5β-H,3β-OR **(3-G)** : 5α-H,3β-OR **(4-G)** = 1 : 2 : 2 : 1.6 (R = TBDMSi). The mass spectrum showed the diagnostic $[M - t\text{-Bu}]^+$ ion, m/z 365.

Syntheses of compounds for Scheme XIII

17β-hydroxy-17α-methyl-4-androsten-3,11-one **(XIIIb)**

The starting material, 17α-methyl-4-androsten-11α,17β-diol-3-one, **XIIa,** (100 mg, 0.314 mmol) was dissolved in acetone (20 mL) and oxidized with Jones reagent; the product was worked-up according to procedure C (section II.B.2).

17α-methyl-5ξ-androstan-3ξ,17β-diol-11-one **(XIIIc)**

The oxidized product from the previous step, assumed to be **XIIIb,** (80 mg, 0.253 mmol) was hydrogenated in the presence of 5% rhodium-on-charcoal (40 mg) in methanol (2 mL) and cyclohexane (4 mL) solution according to procedure D (section II.B.2). Evaporation of the solvent gave a glassy residue assumed to be **XIIIc.**

17α-methyl-5ξ-androstan-3ξ,17β-diol-11-one-9α,12,12-d_3 **(XIIId)**

The product of hydrogenation, **XIIIc,** (30 mg, 0.094 mmol), was refluxed in the NaOD/MeOH/D_2O standard equilibration solution (2 mL) for 1 hr (procedure F). The solvents were removed *in vacuo*, ether was added to the wet residue, and deuterium oxide was withdrawn by pipette. The ether solvent was removed *in vacuo* and the entire process was repeated twice more. Work-up was performed as for **Ib.**

3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstan-11-one-9α,12,12-d_3 **(14-L, 16-L, and 17-L)**

The crude product from the previous step, assumed to be **XIIId**, was derivatized according to procedure B. GC/MS analysis showed that the mixture consisted of two epimers (3α and 3β OH) of the 5α-isomer and the 5β-H,3β-OH isomer, in the ratio 5α-H,3α-OR **(14-L)** : 5β-H,3β-OR **(16-L)** : 5α-H,3β-OR **(17-L)** = 1.2 : 1 : 1, respectively, (R = TBDMSi). Surprisingly, under the above hydrogenation conditions, the compound of 5β-H,3α-OR **(15-L)** configuration (equatorial OH) was not detected. The mass spectra showed the diagnostic fragment ion, $[M - t\text{-Bu}]^+$, m/z 378, in each case.

Syntheses of compounds for Scheme XIV.

17α-methyl-5ξ-androstane-3ξ,11α,17β-triol **(XIVb)**

The starting material, 17β-methyl-4-androsten-11α,17β-diol-3-one, **XIVa**, (500 mg, 1.57 mmol) was refluxed in anhydrous pyridine (5 mL) with $NaBH_4$ (860 mg, 22.3 mmol) for 14 hr in a current of nitrogen. Work-up was performed, as for **Vb**, to yield a white product.

3ξ-OSiDMTB-17α-methyl-5ξ-androstan-11α,17β-diol **(XIVc)**

The reduction product from the previous step, **XIVb**, (133 mg, 0.412 mmol) was selectively derivatized at C-3 with 1M TBDMSiCl/imidazole reagent (2 mL) at room temperature for 1.5 hr. The solution was extracted with ether. The ether layer was washed with distilled water (3 x 1 mL), and dried over anhydrous sodium sulphate. Evaporation of ether under a gentle stream of nitrogen furnished **XIVc**, as determined by GC/MS.

3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstan-11-one **(XIVd)** **(15, 16, 17)**

The selectively derivatized steroid, **XIVc**, (100 mg, 0.230 mmol), was oxidized with pyridinium dichromate, $[C_5H_5NH^+]_2[Cr_2O_7^{2-}]$, (200 mg, 0.531 mmol) in

dimethylformamide (2 mL) at room temperature for 12 hr. The reaction mixture was worked up by pouring it into water (15 mL) and the oxidation products were extracted with ether. The ether layer was washed with water, brine, dried over $MgSO_4$, and evaporated to give a residue of **XIVd.** The final product consisted of two epimers (3α and 3β OH) of the 5β-H isomer and only the 5α-H,3β-OH isomer, in the ratio 5β-H,3α-OR **(15)** : 5β-H,3β-OR **(16)** : 5α-H,3β-OR **(17)** = 1 : 0.5 : 0.2 (R = TBDMSi). The mass spectrum showed the diagnostic $[M - t\text{-Bu}]^+$ ion, m/z 365. Under the above reduction conditions, the 5α-H,3α-OR **(14)** isomer (axial OH) was not detected.

Syntheses of compounds for Scheme XV.

11α-acetyloxy-17β-hydroxy-17α-methyl-4-androstene **(XVb).**

The starting material, 17α-methyl-4-androsten-11α,17β-diol-3-one, **XVa,** (250 mg, 0.786 mmol) was treated with acetic anhydride (1.25 mL) and pyridine (2.5 mL) for 16 hr. Work-up was performed according to procedure G. GC/MS analysis showed a single component and no starting material.

11α-acetyloxy-17α-methyl-5ξ-androstane-3ξ,17β-diols **(XVc)**

The acetylation product, **XVb,** (50 mg, 0.139 mmoL) was treated with a hydrogen atmosphere in the presence of 5% Rh/C (30 mg) in methanol (2 mL) and cyclohexane (0.5 mL) for 18hr and worked-up according to the general procedure D (section II.B.2). The product on TLC, a under UV lamp, lacked a conjugated double bond, consistent with the formation of **XVc.**

3ξ-OSiDMTB-11α-acetyloxy-17β-hydroxy-17α-methyl-5ξ-androstane **(XVd)(9 - 12)**

The reduction product, **XVc,** was derivatized with TBDMSiCl reagent according to procedure B (section II.B.2). GC/MS analysis showed the presence of two 5ξ-H isomers each having 3ξ-OTBDMSi epimers, in the ratio 5α-H,3α-OR **(9)** : 5β-H,3α-OR **(10)** : 5β-H,3β-OR **(11)** : 5α-H,3α-OR **(12)** = 0.4 : 0.16 : 1 : 0.1 (R = TBDMSi). The mass spectra of **XIXd** isomers showed diagnostic $[M - t\text{-Bu}]^+$ fragment ions, m/z 361.

Syntheses of compounds for Scheme XVI

17α-methyl-5ξ-androstan-3ξ,17β-diol-3ξ,4ξ,5ξ,9α,11α-d_5 **(XVIb)**

Method a. The starting material, 17β-hydroxy-17α-methyl-4,9(11)-androstadien-3-one, **XVIa**, (100 mg, 0.333 mmol) was stirred with pretreated Adams' catalyst (PtO_2/100 mg) in acetic acid-d_4 (3 mL) in an atmosphere of deuterium gas for 24 hr (procedure D). The solution was worked-up by pouring into ice/water and extracting with ether. The organic layer was washed with 5% sodium bicarbonate, water, brine, and then dried over $MgSO_4$ to furnish a glassy residue. GC/MS analysis showed, in addition to the expected compounds (**XVIb**), the presence of by-products of the following types: a compound acetylated at C-3 or C-17, and compounds with unsaturated bonds as resulting from dehydration.

Method b. A solution of **XVIa**, (100 mg, 0.333 mmol), in ethyl acetate (3 mL) and acetic acid-d_4 (1 mL) was stirred in an atmosphere of deuterium at room temperature with Adams' catalyst for 12 hr. Work-up, as above, furnished a glassy residue. GC/MS analysis showed, in addition to the expected compounds (**XVIb**), the presence of by-products of the following types: a compound acetylated at C-3 or C-17, and compounds with unsaturated bonds.

17β-hydroxy-17α-methyl-5ξ-androstan-3-one-4,5ξ,9α,11α-d_4 **(XVId)**

Because of the presence of acetylated compounds, deacetylation of the product was required. Thus, the crude residue, **XVIb**, (*ca* 80 mg, 0.227 mmol), was refluxed in 1.7 M methanolic sodium hydroxide solution (1 mL) for 2 hr. Work-up was performed according to procedure A (section II.B.2). The hydrolysed products, **XVIc**, were oxidized with Jones reagent and worked-up, according to procedure E, to furnish a white residue assumed to be **XVId**.

17β-hydroxy-17α-methyl-5ξ-androstan-3-one-5ξ,9α,11α-d_3 **(XVIe)**

The oxidation product from the previous step, **XVId**, (*ca* 60 mg, 0.20 mmol), was back-exchanged in 1M methanolic sodium hydroxide solution (2 mL) under nitrogen gas

for 2.5 hr. Work-up (procedure E, section II.B.2) furnished a residue assumed to be **XVIe.**

17α-methyl-5ξ-androstan-3ξ,17β-diol-5ξ,9,11α-d_3 **(XVf)**

The product, **XVIe,** (*ca* 5 mg, 0.16 mmol) was refluxed with $NaBH_4$ (20 mg, 0.53 mmol) in methanol (2 mL) for 1 hr and worked-up according to procedure A (section II.B.2).

3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstan-5ξ,9α,11α-d_3 **(1-I to 4-I)**

The reduction product, **XVIf,** was derivatized according to procedure B (section II.B.2) to furnish **XVIg,** which consisted of two 5ξ-isomers each having two 3ξ-epimers, in the ratio 5α-H,3α-OR **(1-I)** : 5β-H,3α-OR **(2-I)** : 5β-H,3β-OR **(3-I)** : 5α-H,3β-OR **(4-I)** = 0.006 : 0.16 : 0.004 : 1 (R = TBDMSi). The mass spectra of the isomers showed diagnostic $[M - t\text{-Bu}]^+$ fragment ions, m/z 366.

Syntheses of compounds for Scheme XVII.

17α-methyl-5ξ-androstan-3ξ,17β-diol-16,16-d_2 **(XVIIb)**

The starting material, 17β-hydroxy-17α-methyl-4,9(11)-androstadien-3-one-16,16-d_2, **XVIIa,** (10 mg, 0.33 mmol), dissolved in methanol (0.6 mL) and cyclohexane (2.5 mL), with 5% rhodium-on-charcoal (15 mg) was under hydrogen at room temperature for 6 hr (procedure D). The mixture was filtered to remove catalyst and the solvent was evaporated to yield a glassy residue **XVIIb.**

3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstan-16,16-d_2 **(1-J to 4-J)**

The reduction product from the previous step was derivatized according to procedure B to afford the final compounds, **XVIIc,** which consisted of two 5ξ-isomers each having two 3ξ-epimers, in the ratio 5α-H,3α-OR **(1-J)** : 5β-H,3α-OR **(2-J)** : 5β-H,3β-OR **(3-J)** : 5α-H,3β-OR **(4-J)** = 2.5 : 1 : 12.5 : 0.25 (R = TBDMSi). The mass spectra of the **XVIIc** isomers showed diagnostic $[M - t\text{-Bu}]^+$ ions, m/z 365.

Syntheses of compounds for Scheme XVIII.

17α-methyl-d_3-5ξ-androstan-3ξ,17β-diol (**XVIIIb**)

The starting material, 17β-hydroxy-17α-methyl-d_3-1,4-androstadien-3-one (15 mg, 0.049 mmol) in methanol (0.25 mL) and cyclohexane (2 mL) and 5% rhodium-on-charcoal was stirred under hydrogen at room temperature for 10 hr (procedure D; section II.B.2). The catalyst was filtered off and the solvent was evaporated to give a glassy residue.

3ξ-OSiDMTB-17β-hydroxy-17α-methyl-d_3-5ξ-androstane (**1-K** to **4-K**)

The crude product from the previous step was derivatized according to procedure C to afford the product (**XVIIc**), which consisted of two 5ξ-isomers each having two 3ξ-epimers in the ratio 5α-H,3α-OR (**1-K**) : 5β-H,3α-OR (**2-K**): 5β-H,3β-OR (**3-K**) : 5α-H,3β-OR (**4-K**) = 8.4 : 1 : 39 : 1.3 (R = TBDMSi). The mass spectra of the **XVIIIc** isomers showed diagnostic $[M - t\text{-Bu}]^+$ ions, m/z 366.

III. RESULTS and DISCUSSION.

III.A. The chromatographic characteristics of steroid mono-TBDMSi ethers.

The eight *t*-butyldimethylsilyl (TBDMSi) ether derivatives **1-8** (Scheme i-4) of 17ξ-methyl-5ξ-androstane-3ξ,17ξ-diol were studied by GC/MS. They were prepared by reaction of the individual steroid with TBDMSiCl/Im/DMF reagent[47-49] in 100% yield as judged by gas chromatography. The derivatized compounds could be stored for several months at room temperature without noticeable decomposition[92]. The stability of these derivatives is presumably a combination of the interaction of the lone pair of electrons on the oxygen atoms with vacant d orbitals on silicon and of the steric bulk of the alkyl groups; the latter would inhibit an attack by water[73].

All 3-TBDMSi ether derivatives were studied on a DB-5 capillary column under the same GC/MS conditions. A chromatogram for each compound was individually recorded to serve as a standard and a reference mass spectrum was obtained. For each of the 17β- and 17α-hydroxy series, a mixture of all four TBDMSi-ethers was chromatographed to determine the order of elution, as well as to evaluate the retention time for a single derivative, as shown in Fig. 4 (a, b). The retention time was measured from the time of injection of the sample. Individual peaks in a chromatogram were identified by comparing their mass spectra with reference spectra. The four isomers **1-4** and the four isomers **5-8** eluted as resolved peaks as shown in Fig. 4 (a, b). The elution order, irrespective of the 17-hydroxy orientation, is: first 5α-H,3α-OSiDMTB (**1, 5**), second 5β-H,3α-OSiDMTB (**2, 6**), third 5β-H,3β-OSiDMTB (**3, 7**) and fourth 5α-H,3β-OSiDMTB (**4, 8**). The retention times for **2** and **5**, as well as for **3** and **7**, are quite similar (Table 3). In order to determine the exact order of elution of **1-8**, a mixture was chromatographed under the same GC/MS conditions. The chromatogram, presented

Figure 4. Gas chromatograms of a mixture of (a) **1-4**, (b) **5-8**; (c) and (d) **1-8** on a DB-5 capillary column with mass spectrometric detection (temperature programmed as described in section II.A.1.). Figures (a), (b), and (c) are total ion chromatograms, computer reconstructed from repeatedly scanned spectra. (d) The m/z 363 ion current chromatogram corresponds to the fragment ion $[M - 57]^+$ of all isomers.

Figure 4.

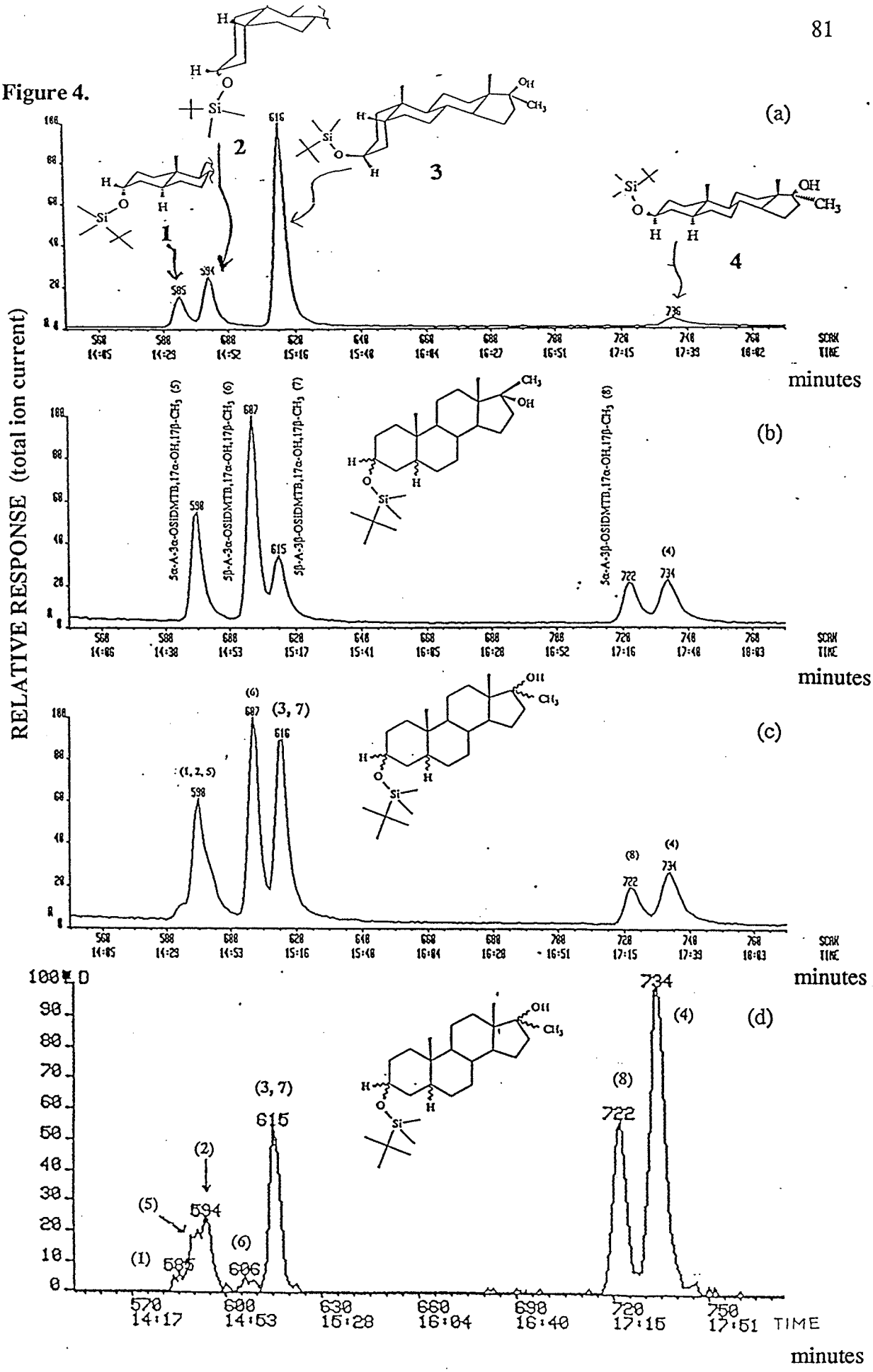

RELATIVE RESPONSE (total ion current)
(a)
(b)
(c)
(d)
1
2
3
4
minutes
TIME
(1, 2, 5)
(3, 7)
(6)
(8)
(4)
5α-A-3α-OSIDMTB,17α-OH,17β-CH3 (5)
5β-A-3α-OSIDMTB,17α-OH,17β-CH3 (6)
5β-A-3β-OSIDMTB,17α-OH,17β-CH3 (7)
5α-A-3β-OSIDMTB,17α-OH,17β-CH3 (8)

Table 3. Retention times, t_R (min), of the eight 3ξ-OSiDMTB-17ξ-hydroxy-17ξ-methyl-5ξ-androstane derivatives (**1-8**). (GC conditions as given in Section II.A.1).

	Isomer	t_R (min)
1	5α-H,3α-OSiDMTB,17β-OH,17α-CH_3	14.33
2	5β-H,3α-OSiDMTB,17β-OH,17α-CH_3	14.45
3	5β-H,3β-OSiDMTB,17β-OH,17α-CH_3	15.10
4	5α-H,3β-OSiDMTB,17β-OH,17α-CH_3	17.31
5	5α-H,3α-OSiDMTB,17α-OH,17β-CH_3	14.40
6	5β-H,3α-OSiDMTB,17α-OH,17β-CH_3	14.59
7	5β-H,3β-OSiDMTB,17α-OH,17β-CH_3	15.11
8	5α-H,3β-OSiDMTB,17α-OH,17β-CH_3	17.17

in Fig. 4 (c), shows only five resolved peaks.

Isomers **1**, **2**, and **5** and isomers **3** and **7**, were not resolved, and gave the chromatographic peaks centered on mass spectral scans #590 and #616, respectively. In an attempt to distinguish between the **1**, **2** and **5** isomers, the m/z 363 fragment ion profile Fig. 4 (d), was constructed by the computer. The profile leads to unambigous elution order of the three isomers as **1**, **5**, and **2**, in spite of their quite similar retention times.

The configuration at C-5, the orientation of the C-3 OSiDMTB group, and the orientation of the C-17 hydroxy group are presumably responsible for differences in the retention times. The 3-equatorial substituents of **4** and **8** are more accessible than the 3-axial substituents of **1** and **5** to interact with the non-polar stationary phase; **4** and **8** therefore have longer retention times. We observe that the pseudoaxial 17α-hydroxy group influences elution of **5-8** differently from the influence of the pseudoequatorial 17β-hydroxy group on **1-4**. For example, Fig. 4 (d), the two 17-epimers **7** and **3** were not resolved (Δt_R = 0.01 min). Steroid **5** had a slightly longer retention time than its

17-epimer **1** (Δt_R= 0.07 min). For the 17-epimers, **2** and **6**, with a 5β-H structure (cis A/B ring junction) and an equatorial 3α-OSiDMTB substituent, **6** has a much longer retention time than **2**. For the 17-epimers, **4** and **8**, with a 5α-H,3β-OSiDMTB structure, an inversion of elution is observed, i.e. **8** elutes before **4**. It is noteworthy that the retention time of epimers **2** and **6**, with the 5β-H,3α-OSiDMTB structure, are shifted towards that of **1** when the 17-hydroxy group is pseudoequatorially oriented, and towards that of **7** when the 17-hydroxy group is pseudoaxially oriented. Presumably, different populations of conformers for **2** and **6** increases or reduces their average polarities[156-159], respectively.

III.B. Mass Spectrometric Properties of mono-TBDMSi Ethers.

In section III.B.1, we describe mass spectrometric properties of 3ξ-OSiDMTB-17ξ-hydroxy-17ξ-methyl-5ξ-androstanes (**1-8**) at 70 eV ionizing energy, as well as daughter (B/E scan) and parent (B^2/E scan) ion mass spectra of selected fragment ions.

In sections III.B.2. and III.B.2.1, the single hydrogen rearrangement involved in formation of the $[(CH_3)_2SiOH]^+$ ion is discussed in the light of results for appropriately labeled steroids and dependence of its relative abundance on electron beam energy. In section III.B.2.2, an alternative structure, $[HCH_3SiOCH_3]^+$, is suggested for the m/z 75 ion.

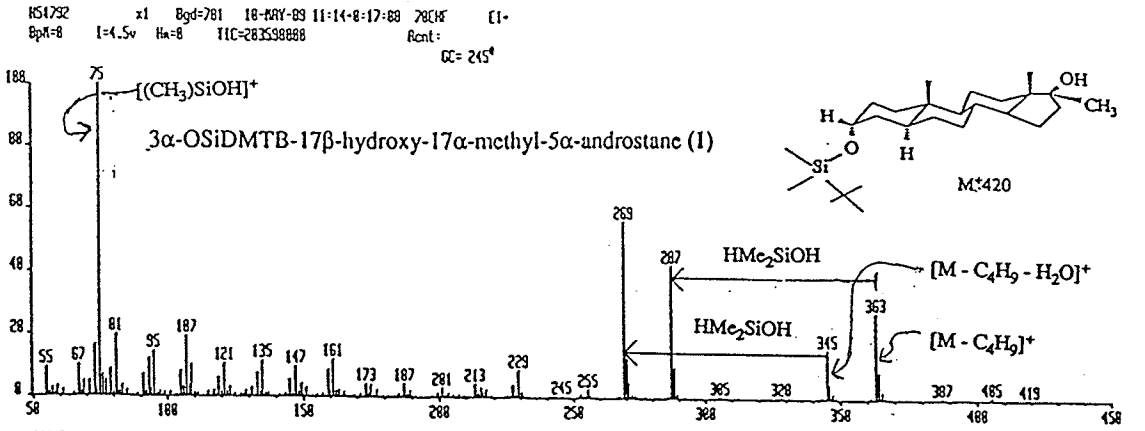
In section III.B.3, the mass spectra of four 3ξ-TBDMSi ether 11α-acetyloxy derivatives of 17α-methyl-5ξ-androstan-3ξ,11α,17β-triols (**9-12**) are discussed. A fragmentation mechanism leading to formation of the prominent m/z 117 ion is proposed.

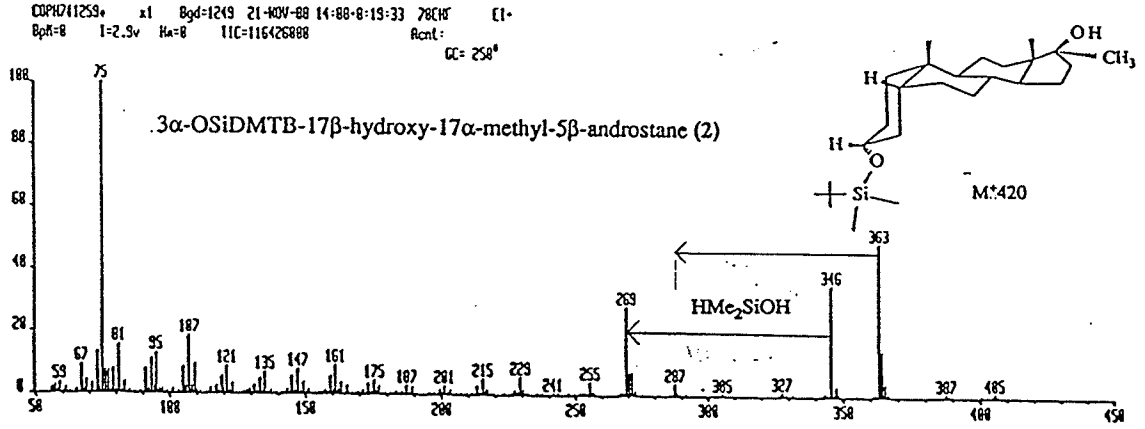
III.B.1. 3ξ-OSiDMTB-17ξ-hydroxy-17ξ-methyl-5ξ-androstanes.

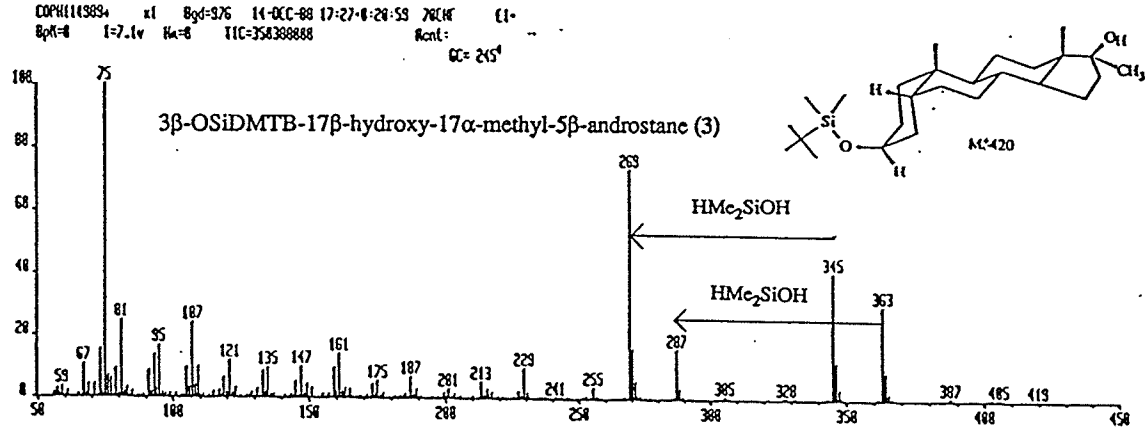
The 70 eV EI mass spectra of the eight 3ξ-OSiDMTB-17ξ-hydroxy-17ξ-methyl-5ξ-androstanes (**1-8**) are presented in Fig. 5, with a partial listing of ions and abundances given in Table 4. In general, fragmentation patterns observed in all the spectra are very similar and in agreement with earlier observations[62,67].

The spectra of **1-8** are much simpler than their underivatized analogs[160,161], the ions $[M - CH_3]^+$ (m/z 405), $[M - H_2O]^+$ (m/z 402), and $[M - (CH_3 + H_2O)]^+$ (m/z 387) are absent, or of extremely low abundance (%RA < 0.5). The molecular ion peak is not observed and the base peak corresponds to the ion, m/z 75. For all four 17β-OH,17α-CH_3 isomers (compounds **1-4**) the prominent ion of highest mass is m/z 363, formed by elimination of the *t*-butyl group from the molecular ion. As proposed

Figure 5. Normalized EI mass spectra of 3ξ-OSiDMTB-17ξ-hydroxy-17ξ-methyl-5ξ-androstanes, **1-8** (electron beam energy = 70 eV).


[(CH3)SiOH]+
3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane (1)
M+ 420
HMe2SiOH
[M - C4H9 - H2O]+
HMe2SiOH
[M - C4H9]+


3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (2)
M+ 420
HMe2SiOH
HMe2SiOH


3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (3)
M+ 420
HMe2SiOH
HMe2SiOH

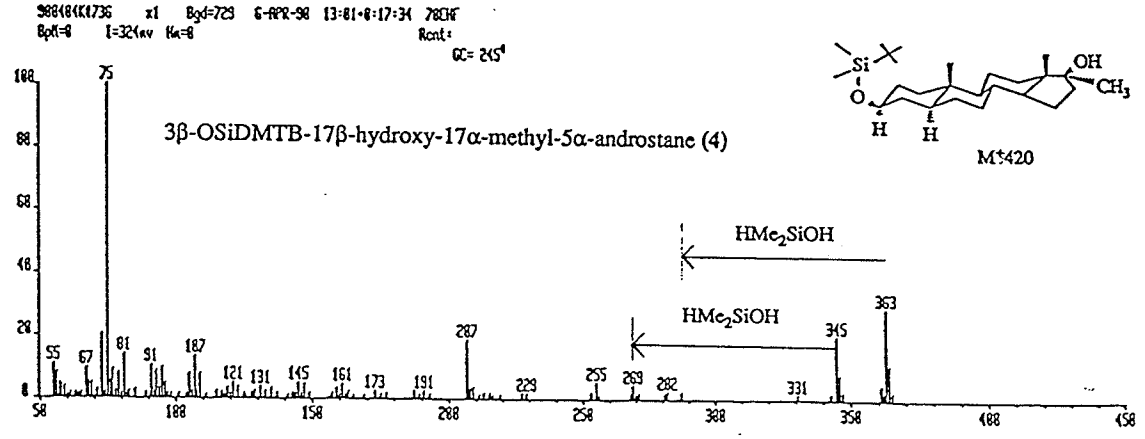

3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane (4)
M+ 420
HMe2SiOH
HMe2SiOH

Figure 5 (continued)

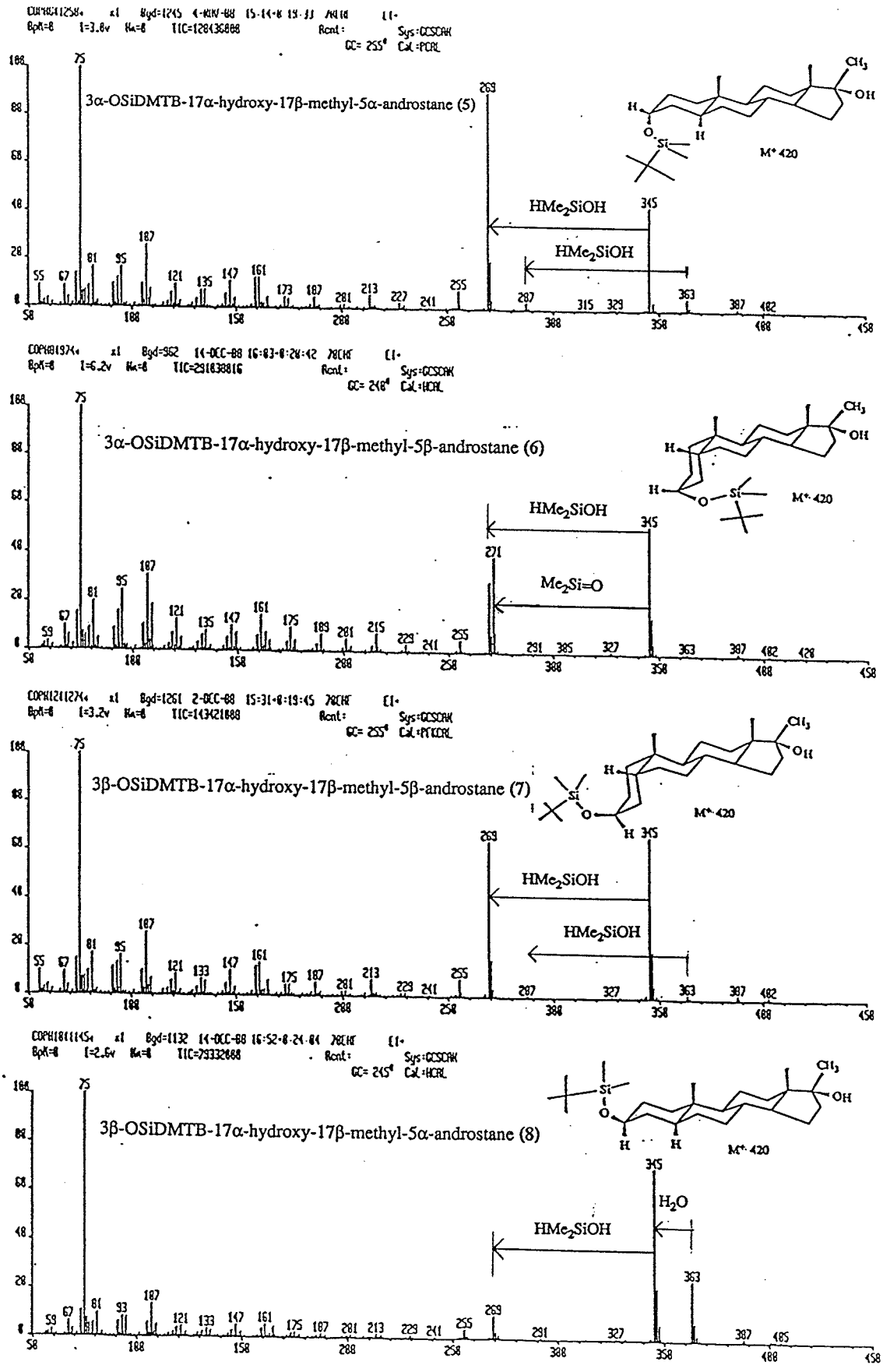


Table 4. Relative abundances (%) of significant ions in the high mass region of the EI mass spectra of compounds **1-8.**

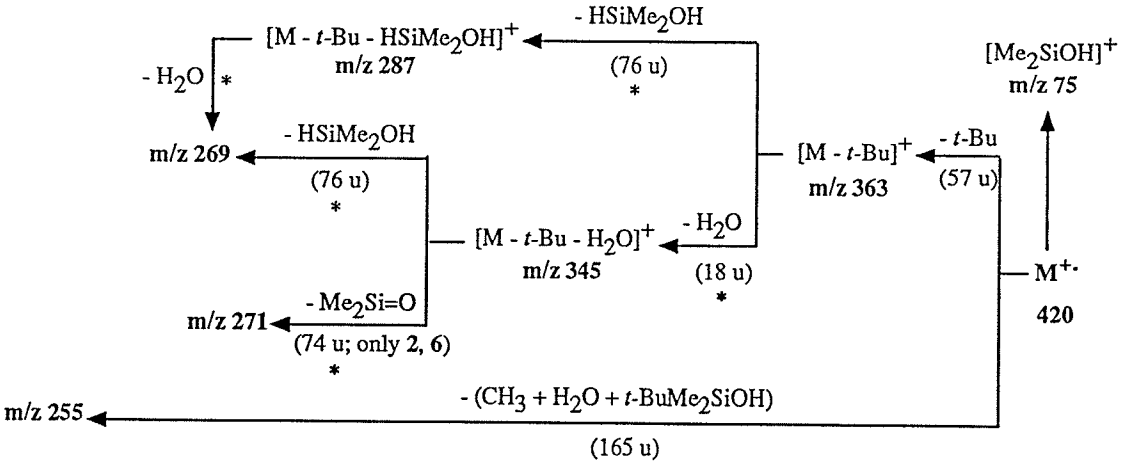
compound		structure			$[M-C_4H_9\cdot-CH_3-C_{19}H_{29}O]^+$	$[M-C_4H_9\cdot-C_{20}H_{32}O]^+$	$[M-CH_3\cdot-H_2O-C_4H_9SiMe_2OH]^+$	$M-C_4H_9\cdot-H_2O-HSiMe_2OH]^+$	$[M-C_4H_9\cdot-H_2O-HSiMe_2O\cdot]^{+\cdot}$	$[M-C_4H_9\cdot-H_2O\ Si(CH_3)_2{=}O]^+$
					$[C_3H_9Si]^+$	$[C_2H_7OSi]^+$	$[C_{19}H_{27}]^+$	$[C_{20}H_{29}]^+$	$[C_{20}H_{30}]^{+\cdot}$	$[C_{20}H_{31}]^+$
					M - 347	M - 345	M - 165	M - 151	M - 150	M - 149
		A/B	C-5/ C-3	3-OR		m/z			m/z	
					73	75	255	269	270	271
(1)	5α-A-3α-OR,17β-OH	trans	cis	a	15.49	100	4.8	63.36	---	----
(2)	5β-A-3α-OR,17β-OH	cis	trans	e	13.29	100	3.73	27.96	---	6.53
(3)	5β-A-3β-OR,17β-OH	cis	cis	a	15.35	100	3.47	73.65	---	3.71
(4)	5α-A-3β-OR,17β-OH	trans	trans	e	10.64	100	3.23	7.11	---	1.56
(5)	5α-A-3α-OR,17α-OH	trans	cis	a	13.91	100	7.29	90.01	---	1.33
(6)	5β-A-3α-OR,17α-OH	cis	trans	e	15.39	100	4.82	28.77	---	38.23
(7)	5β-A-3β-OR,17α-OH	cis	cis	a	14.82	100	7.08	64.16	---	1.84
(8)	5α-A-3β-OR,17α-OH	trans	trans	e	10.63	100	3.64	9.26	---	1.20

compound		$[M-C_4H_9\cdot-HSiMe_2OH]^+$	$[M-C_4H_9\cdot-H_2O]^+$	$[M-C_4H_9\cdot]^+$	$[M-CH_3\cdot-H_2O]^+$	$[M-H_2O]^{+\cdot}$	$[M-CH_3\cdot]^+$	$[M]^{+\cdot}$
		$[C_{20}H_{31}O]^+$	$[C_{22}H_{37}OSi]^+$	$[C_{22}H_{39}O_2Si]^+$	$[C_{25}H_{43}O_2Si]^+$	$[C_{26}H_{46}OSi]^+$	$[C_{25}H_{45}O_2Si]^+$	$[C_{26}H_{48}O_2Si]^{+\cdot}$
		M - 133	M - 75	M - 57	M - 33	M - 18	M - 15	---
					m/z			
		287	345	363	387	402	405	420
(1)	5α-A-3α-OR,17β-OH	30.63	20.95	19.34	>0.5	>0.5	>0.5	>0.5
(2)	5β-A-3α-OR,17β-OH	3.52	34.99	48.55	0.63	>0.5	0.88	>0.5
(3)	5β-A-3β-OR,17β-OH	15.88	40.53	29.76	>0.5	>0.5	>0.5	>0.5
(4)	5α-A-3β-OR,17β-OH	0.96	34.54	45.23	>0.5	>0.5	>0.5	>0.5
(5)	5α-A-3α-OR,17α-OH	2.65	42.55	4.80	0.89	>0.5	>0.5	>0.5
(6)	5β-A-3α-OR,17α-OH	>0.5	52.17	0.45	1.04	>0.5	>0.5	>0.5
(7)	5β-A-3β-OR,17α-OH	0.25	66.54	1.76	1.49	>0.5	>0.5	>0.5
(8)	5α-A-3β-OR,17α-OH	>0.5	70.48	24.38	1.05	>0.5	>0.5	>0.5

R = TBDMSi; a = axial ; e = equatorial

before[67,92] these $[M - 57]^+$ ions can serve as a molecular weight indicator and as a diagnostic ion for single ion monitoring studies. The major ions in the high mass region of **1-8** are: m/z 363, 345, 287, 271, 269, and 255. The m/z 271 ion is abundant only for the two 17-epimers (**2, 6**) with the 5β-H,3α-OSiDMTB structure.

Scheme 10 presents selected fragmentation pathways of compounds **1-4**, supported by B/E linked scanning mass spectra for a mixture of the compounds, as noted. (These spectra were obtained for a solid sample admitted on the probe, so that resolved components could not be studied). Three competitive fragmentations of the molecular ions are observed: (a) elimination of a *t*-butyl radical, to form m/z 363 ions; (b) elimination of a methyl radical, water, and *t*-butyldimethylsilanol to form m/z 255; and (c) elimination of a *t*-butyl radical and olefinic steroid, to form m/z 75. The $[M - t\text{-Bu}]^+$ ions subsequently decompose by elimination of H_2O and $HSiMe_2OH$, in either order, to form m/z 269 ions by competitive pathways.



*supported by metastable decomposition (B/E scan)

Scheme 10. Selected fragmentation pathways of 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstanes (**1-4**).

A representative daughter ion spectrum of m/z 363 (Fig. 6(a)), for the mixture of compounds (**1-4**), confirms the fragmentation of $[M - C_4H_9]^+$ ions to m/z 345, 287, and 269 ions by loss of H_2O, $HSiMe_2OH$, and (H_2O + $HSiMe_2OH$), respectively. A spectrum for m/z 345 decomposition (compounds **1-4**) is shown in Fig. 6 (b). The most abundant ion, m/z 75, in the normal spectrum was a minor species in these spectra. The B^2/E scan data also did not reveal any parent ion of m/z 75, as shown in Fig. 7. Similarly, daughter ion spectra were obtained for m/z 287 and 269. The precursors of the m/z 255 ions have not been confirmed by B/E or by B^2/E linked scan data. Metastable, or collisionally activated, ion fragmentations observed for the compounds **1-4** are summarized in Table 5.

In the important high mass region, compounds **2** and **4** (3-equatorial TBDMSiO groups) have more abundant $[M - 57]^+$ ions than $[M - 75]^+$ ions (Table 4). In contrast, compounds **1** and **3** (3-axial TBDMSiO groups) have more abundant $[M - 75]^+$ ions than $[M - 57]^+$ and $[M - 133]^+$ ions. The $[M - 133]^+$ and $[M - 151]^+$ ions are strongly represented in the spectra of compounds **1** and **3** (5-H *cis* to 3-OSiDMTB) but are of lower abundance in the spectra of compounds **2** and **4** (5-H *trans* to 3-OSiDMTB). Thus, the 3-OSiDMTB epimers can be easily distinguished, not only in the 17β-OH,17α-CH_3 (**1-4**) series but also in the 17α-OH,17β-CH_3 (**5-8**) series, by the relative abundances of the $[M - t\text{-Bu} - H_2O - HSiMe_2OH]^+$ ions or by the $[M - t\text{-Bu} - H_2O - HSiMe_2OH]^+ / [M - t\text{-Bu}]^+$ (m/z 269 / m/z 363) ratio. Table 6 shows that these values for compounds **1** and **5** (axial 3-OSiDMTB substituent) are markedly higher than for their 3-equatorial epimers, compounds **4** and **8**. A similar relationship applies to compound **3** (axial substituent) compared to its 3-equatorial epimer (compound **2**). However, the relatively high abundance of the m/z 269 ions of compounds **2** and **6** (equatorial 3α-OSiDMTB substituents) compared to the 5α-H epimers (**4** and **8**), which also have equatorial 3β-OSiDMTB substituents is related later (section **II.C.2**) to the ability of ring A to adopt a chair or a boat conformation[29]. The observed differences are a function of the relative orientations and distances between the 3-OSiDMTB and 5-H

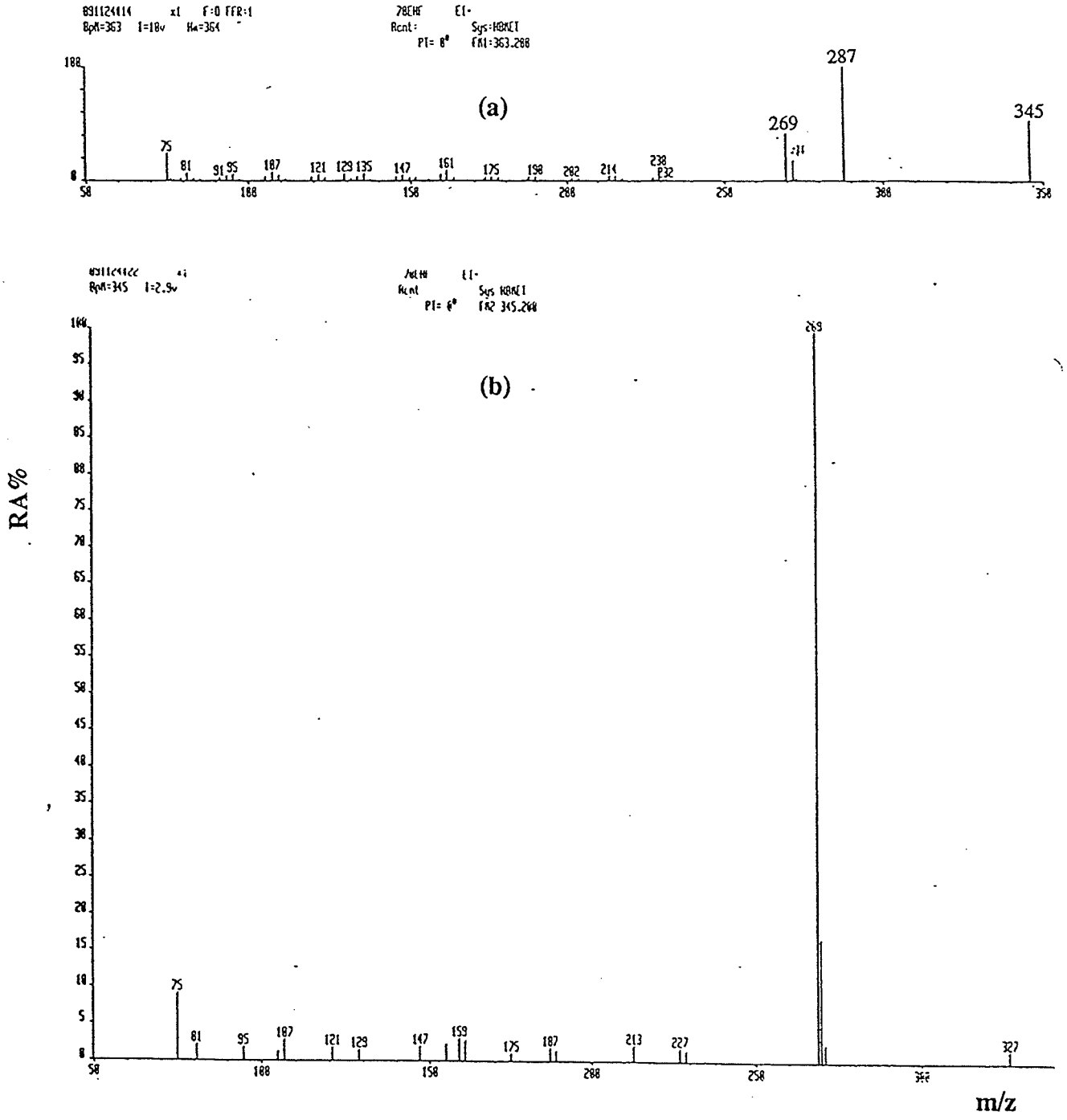


Figure 6. The daughter ion (B/E scan) spectra of (a) m/z 363, $[M - t\text{-Bu}]^+$ and (b) m/z 345 $[M - t\text{-Bu} - H_2O]^+$ from a mixture of 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstanes (**1-4**) introduced on the solid probe.

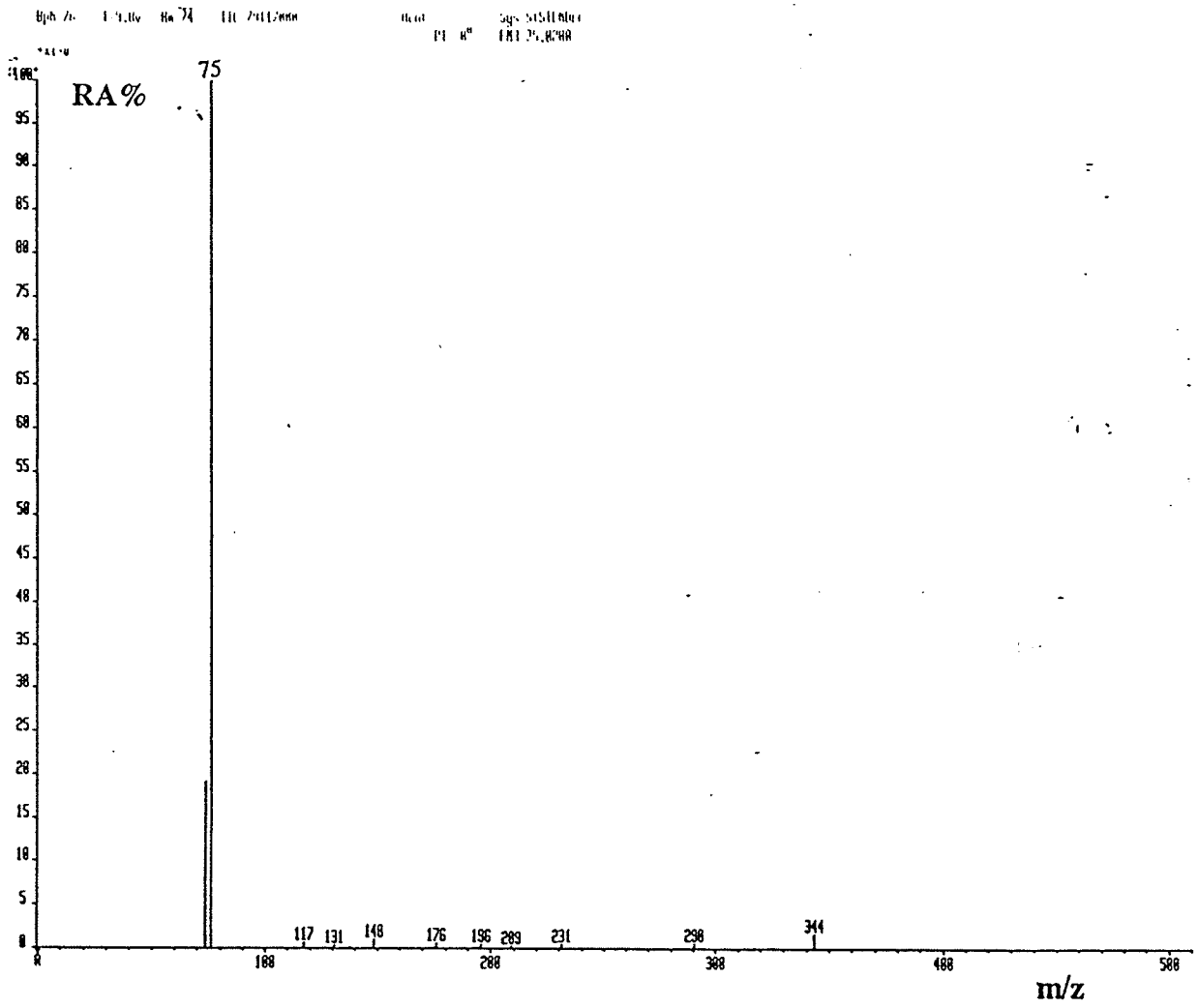
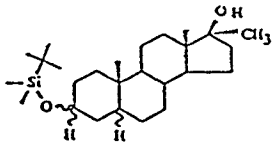


Figure 7. The parent ion (B^2/E scan) spectrum of the m/z 75 ion from a mixture of 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstanes (**1-4**) admitted to the ion source on the solid probe.

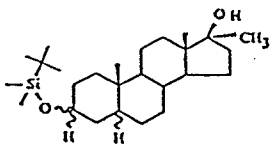
Table 5. Observed metastable ion decompositions (B/E scan) for 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstanes (**1-4**).



m/z	ion	ion
420	$C_{26}H_{48}SiO_2$	$M^{+\cdot}$
363	$C_{22}H_{39}SiO_2$	$[M-57]^+$
345	$C_{22}H_{37}SiO$	$[M-75]^+$
287	$C_{20}H_{31}O$	$[M-133]^+$
271	$C_{20}H_{31}$	$[M-149]^+$
270	$C_{20}H_{30}$	$[M-150]^{+\cdot}$
269	$C_{20}H_{29}$	$[M-151]^+$
255	$C_{17}H_{27}$	$[M-165]^+$
229	$C_{17}H_{25}$	$[M-191]^+$
227	$C_{17}H_{23}$	$[M-193]^+$
215	$C_{16}H_{23}$	$[M-205]^+$
213	$C_{16}H_{21}$	$[M-201]^+$
201	$C_{14}H_{23}$	$[M-219]^+$
189	$C_{14}H_{21}$	$[M-231]^+$
187	$C_{14}H_{19}$	$[M-233]^+$
175	$C_{13}H_{19}$	$[M-245]^+$
173	$C_{13}H_{17}$	$[M-247]^+$
161	$C_{12}H_{17}$	$[M-259]^+$
159	$C_{12}H_{19}$	$[M-261]^+$
149	$C_{11}H_{17}$	$[M-271]^+$
147	$C_{11}H_{15}$	$[M-273]^+$
135	$C_{10}H_{15}$	$[M-285]^+$
133	$C_{10}H_{13}$	$[M-287]^+$
129	$C_{10}H_{11}$	$[M-291]^+$
121	C_9H_{13}	$[M-299]^+$
119	C_9H_{11}	$[M-301]^+$
107	C_9H_{11}	$[M-313]^+$
105	C_9H_9	$[M-315]^+$
95	C_7H_{11}	$[M-325]^+$
93	C_7H_9	$[M-327]^+$
81	C_6H_9	$[M-339]^+$
75	C_2H_7SiO	$[M-345]^+$

(cont'd)

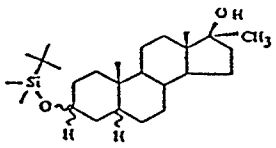
Table 5 (continued)



m/z	ion	ion
420	$C_{26}H_{48}SiO_2$	$M^{+\cdot}$
363	$C_{22}H_{39}SiO_2$	$[M-57]^+$
345	$C_{22}H_{37}SiO$	$[M-75]^+$
287	$C_{20}H_{31}O$	$[M-133]^+$
271	$C_{20}H_{31}$	$[M-149]^+$
270	$C_{20}H_{30}$	$[M-150]^{+\cdot}$
269	$C_{20}H_{29}$	$[M-151]^+$
255	$C_{17}H_{27}$	$[M-165]^+$
229	$C_{17}H_{25}$	$[M-191]^+$
227	$C_{17}H_{23}$	$[M-193]^+$
215	$C_{16}H_{23}$	$[M-205]^+$
213	$C_{16}H_{21}$	$[M-201]^+$
201	$C_{14}H_{23}$	$[M-219]^+$
189	$C_{14}H_{21}$	$[M-231]^+$
187	$C_{14}H_{19}$	$[M-233]^+$
175	$C_{13}H_{19}$	$[M-245]^+$
173	$C_{13}H_{17}$	$[M-247]^+$
161	$C_{12}H_{17}$	$[M-259]^+$
159	$C_{12}H_{19}$	$[M-261]^+$
149	$C_{11}H_{17}$	$[M-271]^+$
147	$C_{11}H_{15}$	$[M-273]^+$
135	$C_{10}H_{15}$	$[M-285]^+$
133	$C_{10}H_{13}$	$[M-287]^+$
129	$C_{10}H_{11}$	$[M-291]^+$
121	C_9H_{13}	$[M-299]^+$
119	C_9H_{11}	$[M-301]^+$
107	C_9H_{11}	$[M-313]^+$
105	C_9H_9	$[M-315]^+$
95	C_7H_{11}	$[M-325]^+$
93	C_7H_9	$[M-327]^+$
81	C_6H_9	$[M-339]^+$
75	C_2H_7SiO	$[M-345]^+$

(cont'd)

Table 5 (continued)



m/z	ion	ion
420	$C_{26}H_{48}SiO_2$	$M^{+\cdot}$
363	$C_{22}H_{39}SiO_2$	$[M-57]^+$
345	$C_{22}H_{37}SiO$	$[M-75]^+$
287	$C_{20}H_{31}O$	$[M-133]^+$
271	$C_{20}H_{31}$	$[M-149]^+$
270	$C_{20}H_{30}$	$[M-150]^{+\cdot}$
269	$C_{20}H_{29}$	$[M-151]^+$
255	$C_{17}H_{27}$	$[M-165]^+$
229	$C_{17}H_{25}$	$[M-191]^+$
227	$C_{17}H_{23}$	$[M-193]^+$
215	$C_{16}H_{23}$	$[M-205]^+$
213	$C_{16}H_{21}$	$[M-201]^+$
201	$C_{14}H_{23}$	$[M-219]^+$
189	$C_{14}H_{21}$	$[M-231]^+$
187	$C_{14}H_{19}$	$[M-233]^+$
175	$C_{13}H_{19}$	$[M-245]^+$
173	$C_{13}H_{17}$	$[M-247]^+$
161	$C_{12}H_{17}$	$[M-259]^+$
159	$C_{12}H_{19}$	$[M-261]^+$
149	$C_{11}H_{17}$	$[M-271]^+$
147	$C_{11}H_{15}$	$[M-273]^+$
135	$C_{10}H_{15}$	$[M-285]^+$
133	$C_{10}H_{13}$	$[M-287]^+$
129	$C_{10}H_{11}$	$[M-291]^+$
121	C_9H_{13}	$[M-299]^+$
119	C_9H_{11}	$[M-301]^+$
107	C_9H_{11}	$[M-313]^+$
105	C_9H_9	$[M-315]^+$
95	C_7H_{11}	$[M-325]^+$
93	C_7H_9	$[M-327]^+$
81	C_6H_9	$[M-339]^+$
75	C_2H_7SiO	$[M-345]^+$

Table 6. %RA of the $[M - t\text{-Bu}]^+$, $[M - t\text{-Bu} - H_2O]^+$, $[M - t\text{-Bu} - HSiMe_2OH]^+$, and $[M - t\text{-Bu} - H_2O - HSiMe_2OH]^+$ ions for 3ξ-OSiDMTB-17ξ-hydroxy-17ξ-methyl-5ξ-androstanes (**1-8**, R = TBDMSi). (Ratios of the %RAs of the latter ions to that of $[M - t\text{-Bu}]^+$ are shown in parentheses)

Compound	$[M - 57]^+$ m/z 363	$[M - 75]^+$ m/z 345	$[M - 133]^+$ m/z 287	$[M - 151]^+$ m/z 269
(1) 5α-H,3α-OR,17β-OH	19.4	21 (1.1)	30 (1.5)	63 (3.3)
(5) 5α-H,3α-OR,17α-OH	4.8	45 (9)	2.7 (0.6)	90 (18.8)
(2) 5β-H,3α-OR,17β-OH	49	35 (0.7)	3.5 (0.07)	28 (0.58)
(6) 5β-H,3α-OR,17α-OH	0.45	52 (116)	>0.5 (0)	29 (64)
(3) 5β-H,3β-OR,17β-OH	30	41 (1.4)	16 (0.5)	74 (2.5)
(7) 5β-H,3β-OR,17α-OH	1.8	67(38)	0.3 (0.14)	64 (36)
(4) 5α-H,3β-OR,17β-OH	45	35 (0.8)	1 (0.02)	9.3 (0.2)
(8) 5α-H,3β-OR,17α-OH	24	70 (2.9)	>0.5 (0)	7.1 (0.3)

$[M - 57]^+$ = $[M - t\text{-Bu}]^+$; **$[M - 75]^+$** = $[M - t\text{-Bu} - H_2O]^+$; **$[M - 133]^+$** = $[M - t\text{-Bu} - HSiMe_2OH]^+$; **$[M - 151]^+$** = $[M - t\text{-Bu} - H_2O - HSiMe_2OH]^+$

substituents. When the separation between them is small enough then hydrogen transfer is more probable (compounds **2** and **4**, and also **6** and **8**). The possibility of rings adopting other conformations can favour approach of the 3-OMe_2Si^+ substituent to abstractable hydrogens (see section **III.C.**, Schemes 36, 38, and 46); this leads to a higher abundance of the ions formed by this process.

These results imply that, only for the 5β-H,3α-OSiDMTB (**2, 6**) isomers, and not for 5α-H,3β-OSiDMTB (**4, 8**), does the silyloxy function (3α-OMe_2Si^+) have access to hydrogen atoms after the chair conformation of ring A flips to the inverted boat conformation. If interconversion of ring A did not assist hydrogen transfer, the abundance of the $[M - t\text{-Bu} - H_2O - HSiMe_2OH]^+$ ions (compounds **2** and **6**) should be comparable to that for the 5α-H,3β-OSiDMTB,17ξ-OH isomers (compounds **4** and **8**).

In the 17α-OH,17β-CH_3 (**5-8**) series, the $[M - t\text{-Bu}]^+$ ions of compounds **5-7** were

of very low abundance (Fig. 5) making them of little use for single ion monitoring purposes. However, abundant $[M - t\text{-Bu} - H_2O]^+$ (m/z 345) or $[M - t\text{-Bu} - H_2O - HSiMe_2OH]^+$ (m/z 269) ions can be used instead. This implies that eliminations of *t*-Bu, and of H_2O (i.e. the 17α-OH group) are both rapid (more rapid than loss of H_2O from 17β-hydroxy steroids, compounds **1-4**) suggesting that a pseudoaxial 17α-hydroxy group initiates formation of the abundant $[M - t\text{-Bu} - H_2O]^+$ ions by a different mechanism. The source of the hydrogen atom abstracted by the 17α-OH group has not been determined but the most likely origins are the sterically accessible tertiary 14α-H or the secondary 12α-H.

One of the most important implications of the mass spectra of compounds **1-8** is that the C-17 epimers, for the first time, can be very easily distinguished, with confidence, by the relative abundances of their $[M - t\text{-Bu}]^+$ ions or, more reliably, by the $[M - t\text{-Bu} - H_2O]^+/[M - t\text{-Bu}]^+$ ratio (Table 6). Thus, this ratio can be employed as a diagnostic test for distinguishing between 17(α, β) hydroxy epimers as their 3-TBDMSi ether derivatives, even if only one epimer is present.

Previously, the problem of elucidation of the stereochemistry at C-17 for 17ξ-methyl-5ξ-androstane-3ξ,17ξ-diols was not solved because of the essentially similar spectra[160,161]. Reimer[161] concluded that interpretation of the $[M - 18]^{+\cdot}/M^{+\cdot}$, $[M - 36]^{+\cdot}/M^{+\cdot}$, and $[M - 51]^+/M^{+\cdot}$ ratios was complicated by competitive losses of water from rings A and D of $[M]^{+\cdot}$. This is not surprising because ionization can occur at either hydroxy group. (When 3ξ,17ξ-diols are converted into 3ξ-TBDMSi ethers, the mass spectrum is simplified, because ionization at the 17ξ-OH group is greatly suppressed. Our preliminary studies on 17α-methyl-d_3-5ξ-androstane-3ξ,17β-diol showed that the elimination of a water molecule competes with losses of different methyl groups. The mass spectra of 17α-CH_3 and 17α-CD_3 analogues of 17α-methyl-5ξ-androstane-3ξ,-17β-diol presented in Fig. 8 show $[M - CH_3]^+$ (m/z 291) and $[M - H_2O]^{+\cdot}$ (m/z 288) ions for the unlabeled compound, and $[M - CH_3]^+$ (m/z 294; negligible abundance) as well as $([M - CD_3]^+ + [M - H_2O]^{+\cdot})$ (m/z 291) for the labeled compound.

Besides losses of an angular group(s)[162,163], the 17α-CD_3 substituent could be eliminated from the molecular ion. The $[M - CD_3]^+/[M - CH_{3(C\text{-}19+C\text{-}18)}]^+$ ratio is about 6 : 1. Proposed mechanisms for formation of the $[M - CD_3 - H_2O]^+$ (m/z 291) and $[M - H_2O - D_2O]^+$ (m/z 271) ions, observed in the **13-K** spectrum (Fig.8), are shown in Schemes 11 and 12. Loss of D_2O from ring D, and then formation of the m/z 271 ion, is accompanied by intramolecular hydrogen exchange between the 17β-OH and 17α-CD_3 groups[184], followed by transfer of the additional deuterium from the newly formed 17α-CD_2H group. Such H-D exchanges are important when competing with some other rearrangement process in an ion.

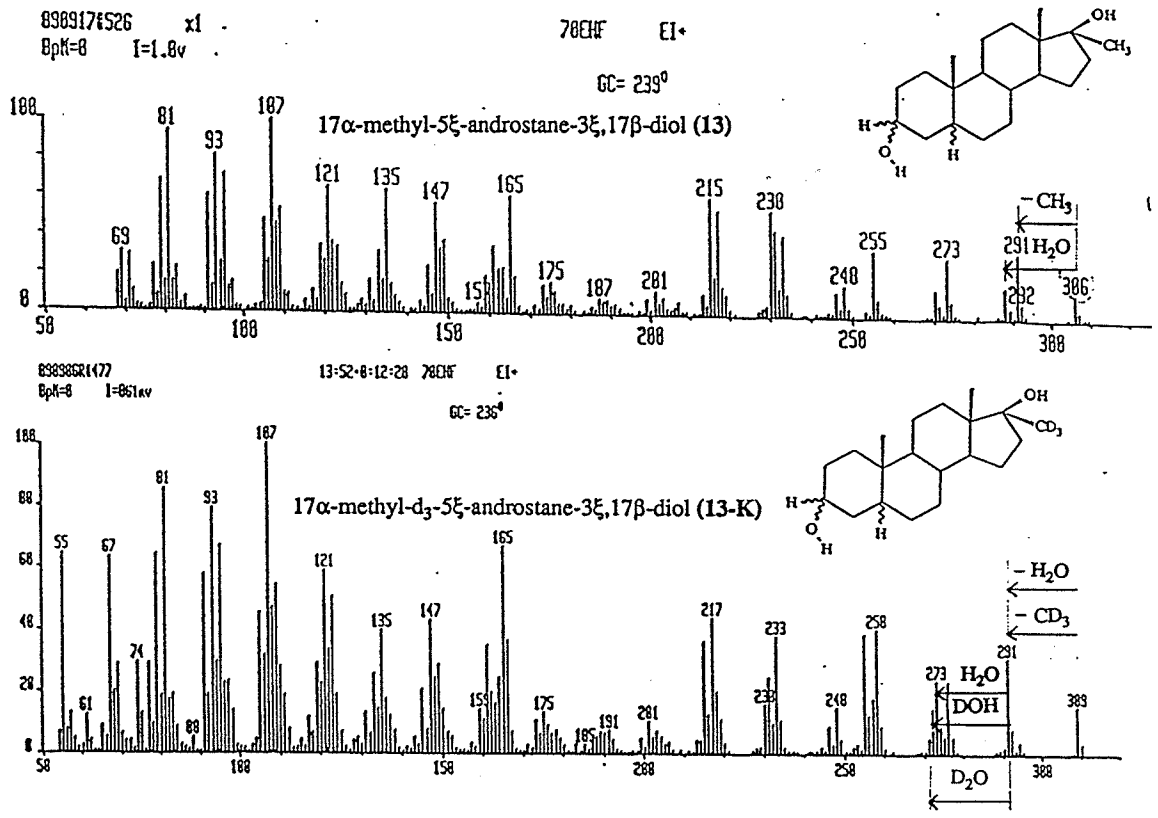
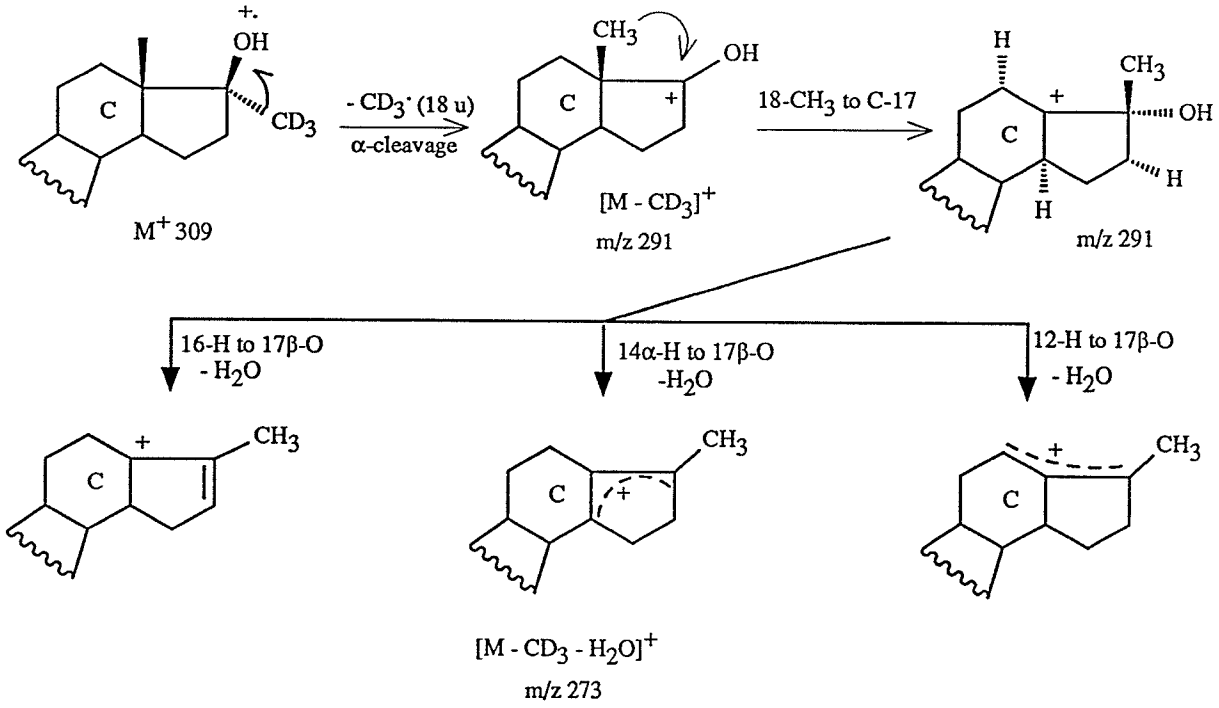
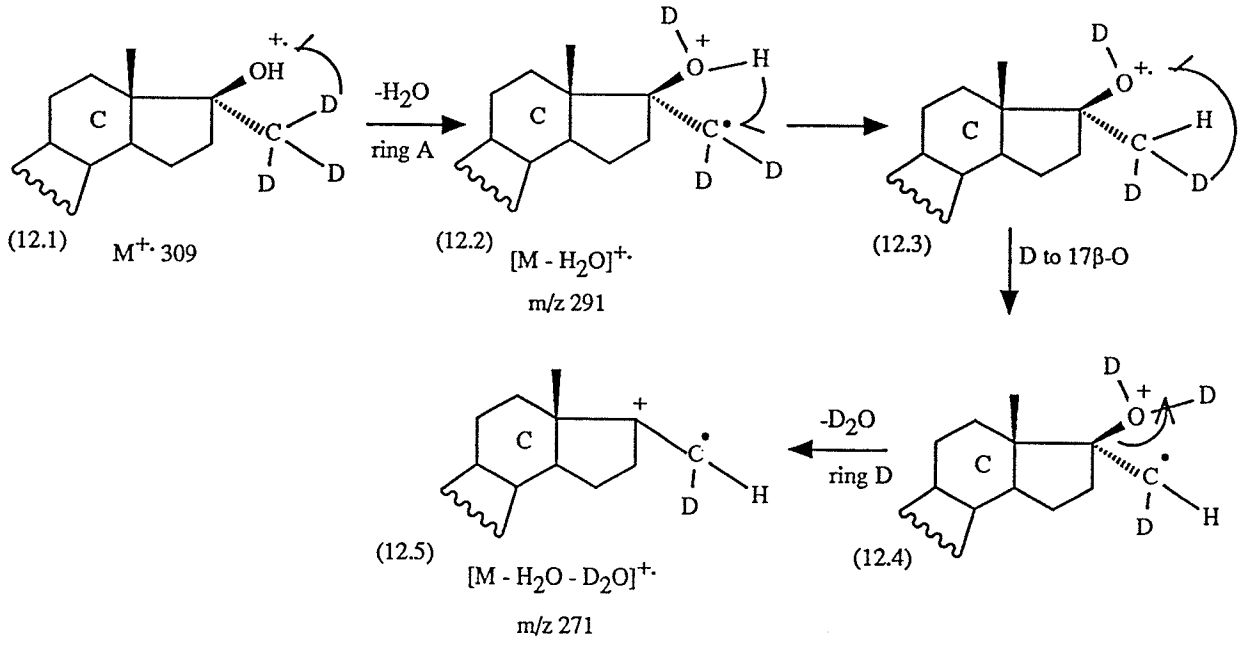


Figure 8. Mass spectra of 17α-methyl- **(13)** and 17α-methyl-d_3- **(13-K)** 5ξ-androstane-3ξ,17β-diol.



Scheme 11. Proposed mechanism of formation of the $[M - CD_3 - H_2O]^+$ ion.

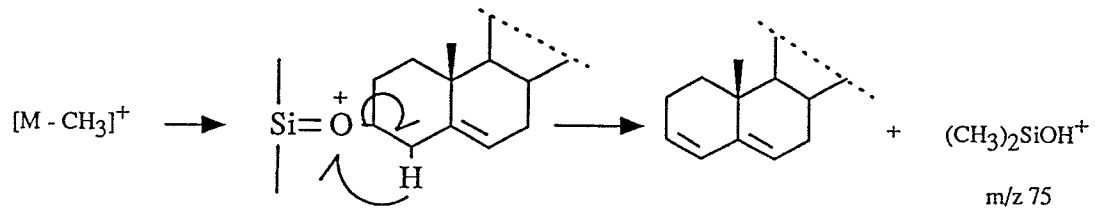


Scheme 12. Proposed elimination mechanism of D_2O from ring D of 17α-methyl-d_3-5ξ-androstane-3ξ,17β-diol (**13-K**).

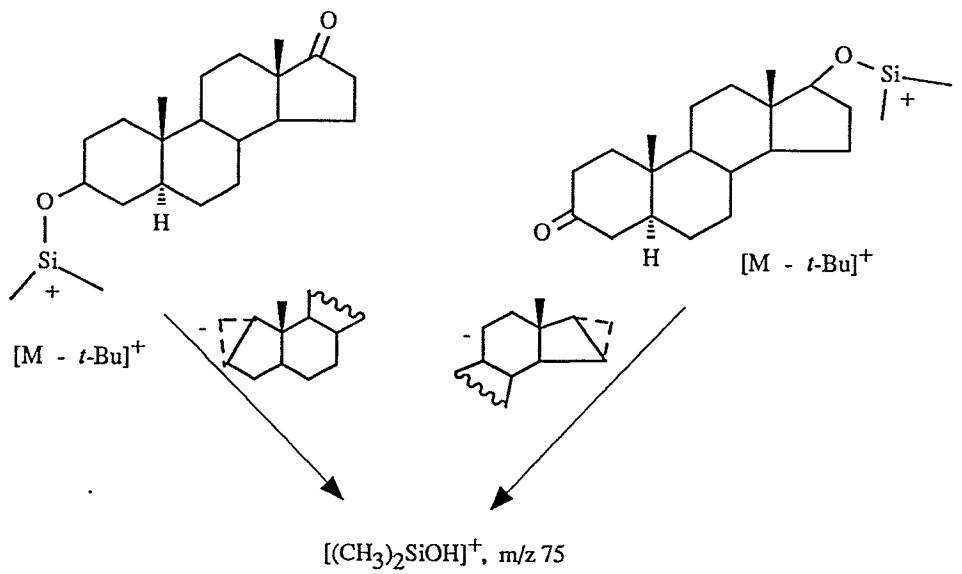
III.B.2. Single hydrogen rearrangement in formation of $[(CH_3)_2SiOH]^+$ for TBDMSi-ether derivatives 1-4.

The $[(CH_3)_3Si]^+$ (m/z 73) and $[(CH_3)_2Si{=}OH]^+$ or $[(CH_3)_2Si^+OH]$[179] (m/z 75) ions are dominant in the EI mass spectra of TMS[164], TBDMSi[64], DMIPSi[51], and ADMSi[58] ether derivatives of steroid alcohols. A survey of available spectra, as well as of our experimental results to be discussed, showed that their abundance depends upon the type of silyl derivative[62,71,101,164], the presence of an additional polar group (Fig. 14, p.125), the structure of the steroid (Fig. 14), and the ionization energy (Fig. 11, p.106). In general, the proposed mechanism[1,2,67,101, 164] for the formation of $[(CH_3)_2SiOH]^+$ has been considered to be 1,2 elimination. Diekman and Djerassi[164], investigating specifically deuterium-labeled trimethylsilyl ethers of 5-ene-3-hydroxy steroids, showed that 40% of the transferred hydrogen originated from the C-4 position (probably the 4β-hydrogen atom) and proposed the $[M - CH_3]^+$ ion as a progenitor of $[(CH_3)_2SiOH]^+$ (Scheme 13).

Quilliam and Westmore[67] and Quillaiam[101] contributed further insight concerning decomposition of 3α-TBDMSi and 17β-TBDMSi androstanolones. With support from a metastable decomposition in the mass spectrum of 17β-OSiDMTB-5α-androstan-3-one they proposed $[M - t\text{-Bu}]^+$ as a parent of m/z 75 (Scheme 14).



Scheme 13. Mechanism of formation of $[Me_2SiOH]^+$ for TMSi-cholesterol[164].



Scheme 14. Mechanism of formation of $[Me_2SiOH]^+$ for TBDMSi androstanolones[67,101].

The $[Me_2SiOH]^+$ ion, m/z 75, forms the base peak in the spectra of all eight TBDMSi-ether steroid derivatives **1-8.** This was ascribed to localization of the ionization site mainly on the ether oxygen, followed by migration of a hydrogen atom to that oxygen, and formation of $[Me_2SiOH]^+$, a *t*-butyl radical and an olefin[101].

In our work, two different linked-scanned modes, B/E and B^2/E, were employed to determine the origin of the m/z 75 ion. The B/E linked scan mass spectra of the [M - *t*-Bu]$^+$ and [M - *t*-Bu - H_2O]$^+$ parent ions produced from a mixture of compounds **1-4** (Fig. 9 a,b) showed only a minor formation of m/z 75. A search for parent ions of m/z 75 (B^2/E linked scan) was unsuccessful (Fig. 10).

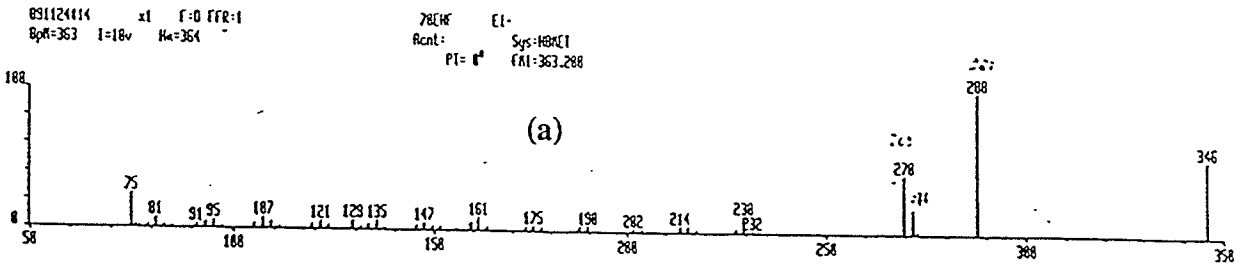
In addition to spectra recorded at the usual 70 eV electron beam energy, mass spectra at a lower beam energy were also acquired, in order to evaluate the influence of electron energy. The spectra of compounds **1-4**, recorded at 60, 30, 20, and 10 eV electron beam energies, are given in Fig. 11 and Table 7. The m/z 75 ions, which form

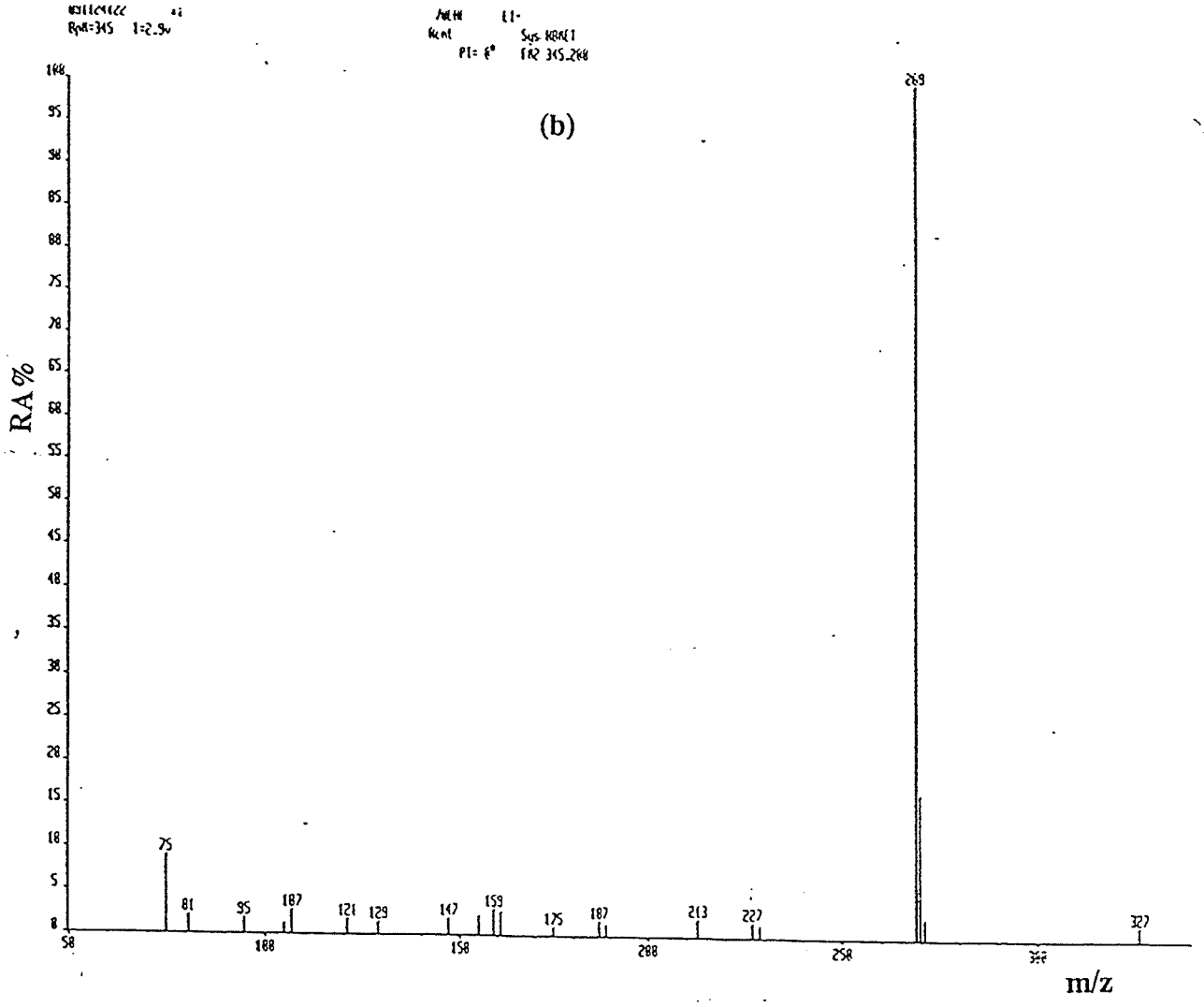
the base peak in all spectra at 70 eV, gradually decrease in relative abundance as the ionizing energy is lowered and completely disappeared at 10 eV. Morover, decreasing the ionization energy from 60 eV to 10 eV caused all peaks, except m/z 363, 345, 287, and 269, to disappear. The m/z 363 ion, rather than m/z 75, forms the base peak below 60 eV electron beam energy. The ions least affected by reduction of the electron beam energy appear to be those arising from double hydrogen rearrangement (i.e. m/z 287 and m/z 269 formed by loss of $HSiMe_2OH$ from m/z 363 and 345, respectively) and single hydrogen migration to the 17-OH group (i.e. m/z 345 and 269 formed by loss of H_2O from m/z 363 and 287, respectively).

The lowered abundance of m/z 75, at decreased ionization energy, indicates that cleavage of a C-O bond, accompanied by a single hydrogen transfer, in the formation of $[(CH_3)_2SiOH]^+$, has a higher energy barrier than does the double hydrogen rearrangement involved in the expulsion of HMe_2SiOH. The latter process apparently has a much lower energy barrier since, at lower electron beam energy, the m/z 287 peak, $[M - t\text{-Bu} - HSiMe_2OH]^+$, and the m/z 269 peak, $[M - t\text{-Bu} - H_2O - HSiMe_2OH]^+$, are still abundant. At 10 eV energy the m/z 75 ions are not formed, even though the m/z 363 and 345 ions are still abundant, suggesting that the $[M - t\text{-Bu}]^+$ and $[M - t\text{-Bu} - H_2O]^+$ ions are not the precursors of the m/z 75 ions. If an ionization site is located on the oxygen of the silyl group, it could implicate the type of fragmentation observed for mixed aliphatic ethers having a *tert*-butyl group[165]. Thus, the formation of $[Me_2SiOH]^+$ can be explained by the rearrangement of a β-hydrogen to oxygen, accompanied by C_4H_9 radical and olefin eliminations (Scheme 15). This hypothesis was tested by the deuterium-labeling studies to be described in the next sub-section.

Figure 9. Daughter ion (B/E scan) mass spectra of (a) m/z 363, $[M - C_4H_9]^+$, and (b) m/z 345, $[M - C_4H_9\cdot - H_2O]^+$, derived from a mixture of 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstanes (**1**-**4**).

Figure 9.





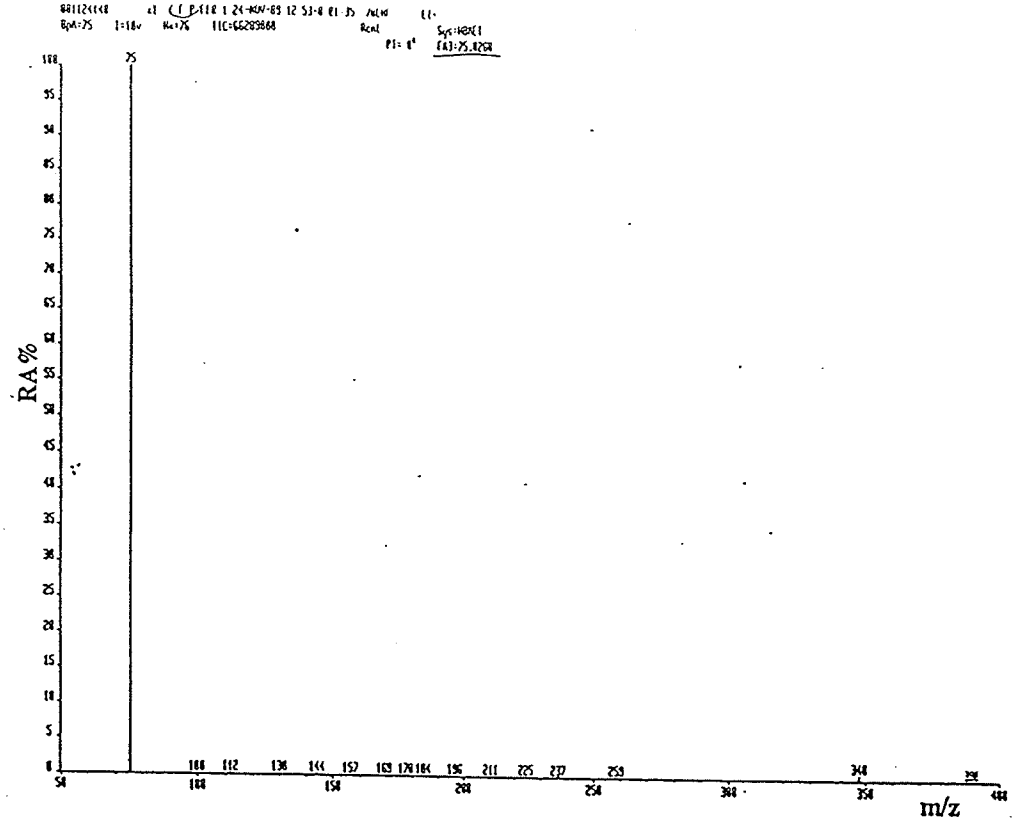


Figure 10. Search for parent ions of m/z 75 derived from a mixture of 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstanes **(1-4).** (Spectrum recorded by linked field scanning with B^2/E constant).

Table 7. Relative abundance (%) of significant ions as a function of electron beam energy for TBDMSi-ether derivatives **1-4.**

Ion	m/z	Electron beam energy (eV)			
		60	30	20	10
$[M - t\text{-Bu}]^+$	363	84	100	100	100
$[M - t\text{-Bu} - H_2O]^+$	345	11	16	17	16
$[M - t\text{-Bu} - HSiMe_2OH]^+$	287	33	46	44	30
$[M - t\text{-Bu} - H_2O - HSiMe_2OH]^+$	269	30	45	36	16
$[Me_2SiOH]^+$	75	100	63	17	0
$[(CH_3)_3Si]^+$	73	16	5	0	0

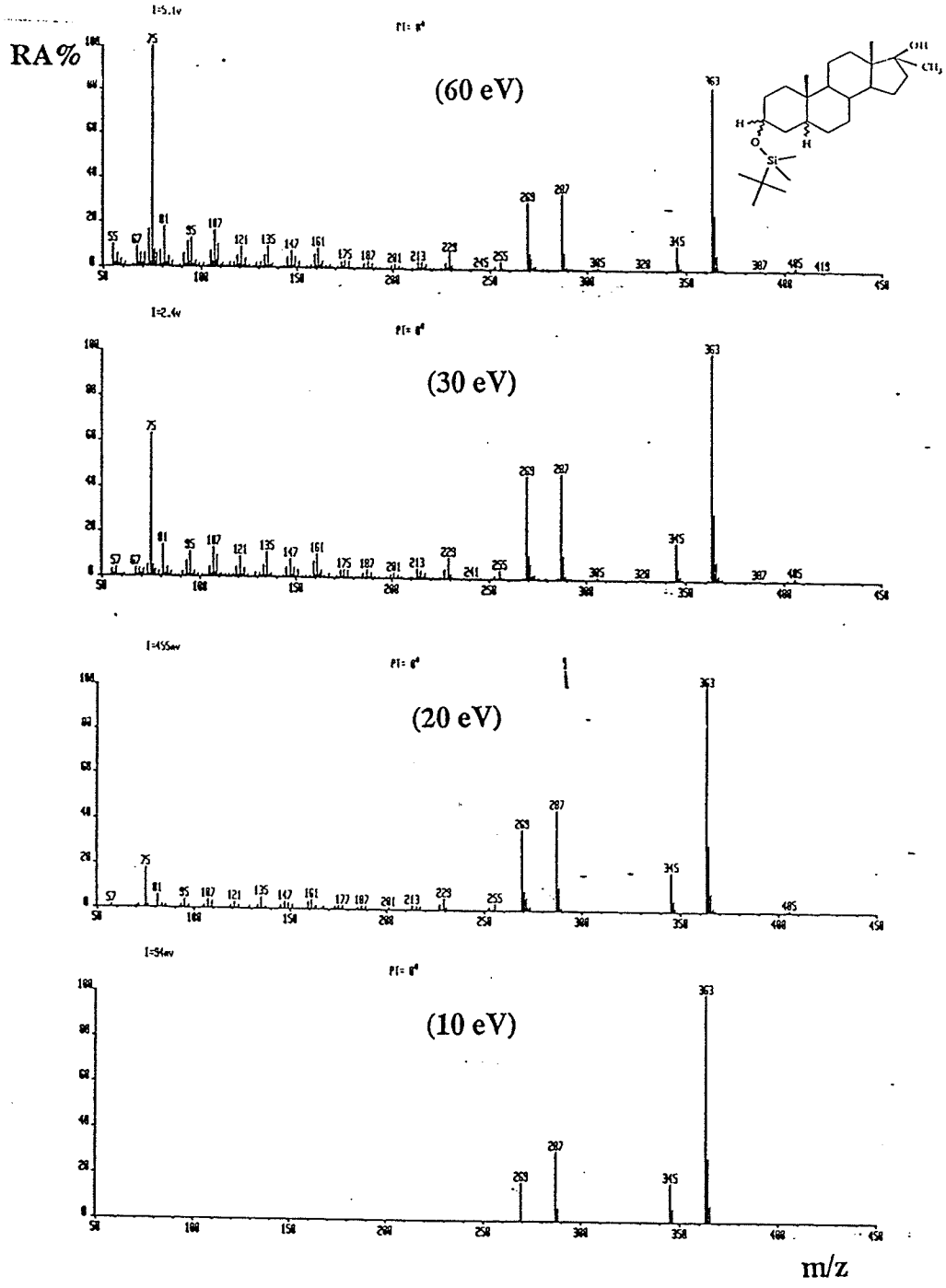
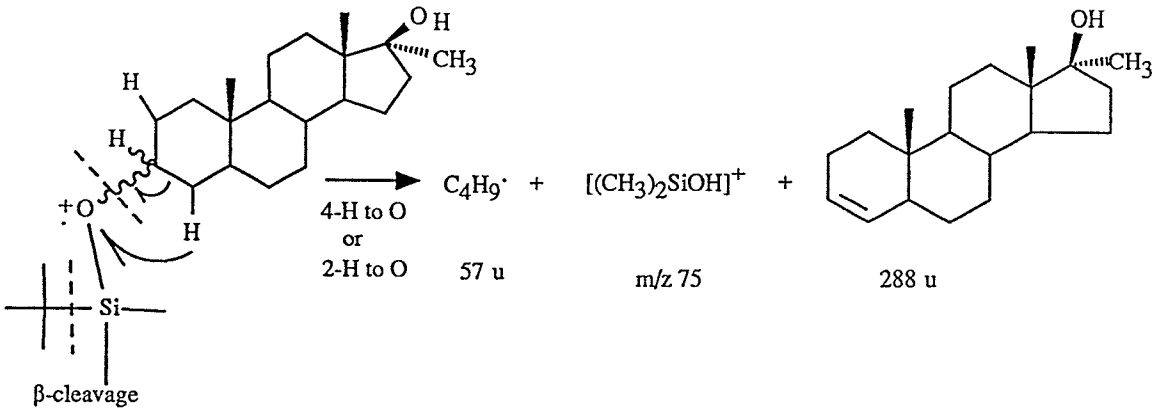


Figure 11. The mass spectra of a mixture of 3ξ-OSiDMTB-17β-hydroxy--17α-methyl-5ξ-androstanes **1-4** recorded at 60, 30, 20, and 10 eV electron beam energies.



Scheme 15. Proposed mechanism of formation of the $[(CH_3)_2SiOH]^+$ ions from the molecular ion, with migration of a β-hydrogen atom.

III.B.2.1. Formation of the Me_2SiOH^+ ion: deuterium-labeled studies

Although the main purpose for the deuterium-labeling experiments was to elucidate the mechanism of the double hydrogen rearrangement in $HSiMe_2OH$ elimination from [M - *t*-Bu]$^+$ ions, insight into rearrangement of the single hydrogen in the formation of the $[Me_2SiOH]^+$ ion is also possible. The mass spectra of the four 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstane-2,2,4,4-d_4 (**1-C** to **4-C**) isomers showed that *ca* 50% of the $[Me_2SiOH]^+$ ion arose by rearrangement of hydrogen from the C-2 or C-4 positions (Fig. 12 and Table 8). (An observed 1 m/z shift from 75 to 76 would indicate migration of a hydrogen atom from a labeled site). Data in Tables 8 (a-d) also show that small amounts of hydrogen rearrange from other sites. Scheme 16 shows the degree of positional specificity of hydrogen migration for each isomer. (The method of calculation of deuterium content of ions and of deuterium transfer in ion decomposition are described in Appendix I). In general, the contribution of the hydrogen atom from the C-3 site is almost constant (*ca* 3 - 4%), from the C-5 site it is 3-9%, from the C-6 site it is 3-5%, and from the C-7 site it is 0-2%. (There is a suggestion that a hydrogen atom from the C-16 position of compound **2** (2%), or from the 17α-CD_3 group of compound **4** (4%), are abstracted). These sources account for about 63-70% of the transferred hydrogen (Scheme 16). The remaining 30%-37% of migrating hydrogen probably comes from the *t*-butyl group (Scheme 17) or, possibly, from the 19-CH_3 group (Scheme 18).

For these, and following mechanisms, migration of a hydrogen atom prior to cleavage of the *t*-Bu-Si bond in the molecular ion is proposed. Such a transfer has been indentified in many compounds[166,167]. A typical example is the McLafferty rearrangement[168], in which a hydrogen is transferred from carbon to oxygen through a six-membered ring transition state.

Figure 12. Normalized EI mass spectra for the four 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstane-2,2,4,4-d_4 isomers (**1-C to 4-C**). (Electron beam energy = 70 eV.)

Figure 12

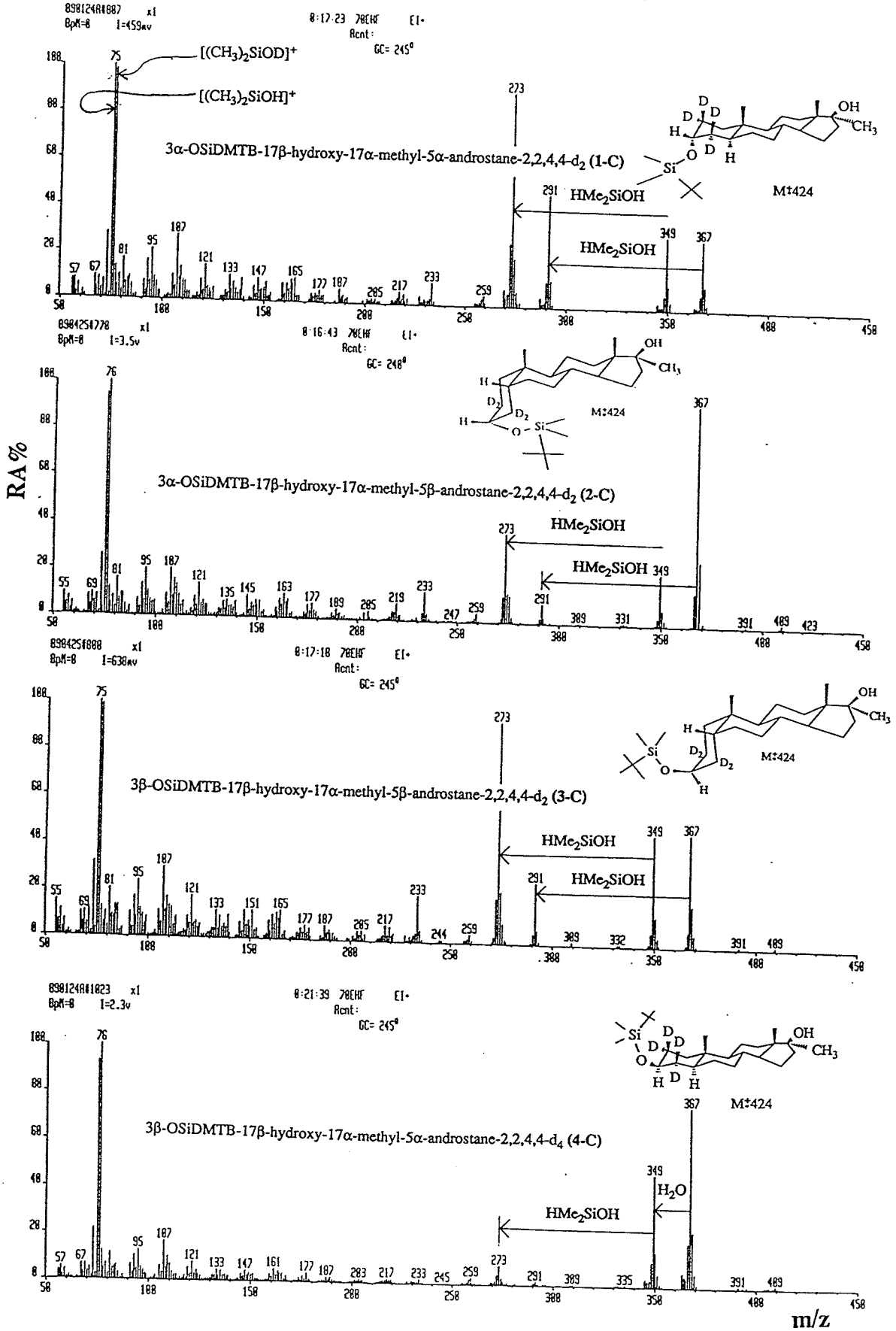

[(CH3)2SiOD]+
[(CH3)2SiOH]+
3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-2,2,4,4-d2 (1-C)
HMe2SiOH
M+424
3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-2,2,4,4-d2 (2-C)
3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane-2,2,4,4-d2 (3-C)
3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-2,2,4,4-d4 (4-C)
H2O
RA %
m/z

Table 8. % RA and deuterium content of dimethylhydroxysilicon cation. ("Migr" %, denotes percentage of single deuterium rearrangement to the Me_2SiO^+ residue calculated from the measured deuterium content of the steroid (Tables 12, 14, 16, 18, and 30) and corrected for ^{13}C, ^{17}O, ^{2}H, and ^{30}Si, ^{31}Si content.

(a) 3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane (**1**)

	m/z, RA%				isotopic composition, %			
label(s)	**75**	**76**	**77**	**78**	**d_0**	**d_1**	**d_2**	Migr, %
d_0	100	7.0	5.1	0.6	100	-	-	-
1α,5α-d_2	100	19.0	6.7	1.2	88	10	2	10
1β,5α-d_2	100	16.9	10.4	-	87	8	5	8
2,2,4,4,-d_4	100	98.4	13.9	5.8	51	47	2	47
3β-d_1	100	10.7	5.8	1.1	97	3	-	3
5α-d_1	100	14.9	5.7	1.6	93	7	-	7
6,6-d_2	100	12.3	6.7	1.5	95	5	-	5
7,7-d_2	100	9.7	6.1	1.5	98	2	-	2
5,9α,11α-d_3	100	13.0	7.2	2.3	92	5	3	5
16,16-d_2	100	7.3	6.8	1.9	100	-	-	0
17α-CD_3	100	7.8	7.3	2.1	96	-	4	0

(b) 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (**2**)

	m/z, RA%				isotopic composition, %			
label(s)	**75**	**76**	**77**	**78**	**d_0**	**d_1**	**d_2**	"Migr, %
d_0	100	7.4	7.2	0.9	100	-	-	-
1α,5β-d_2	100	12.4	7.4	1.8	92	5	3	5
1β,5β-d_2	100	12.3	6.9	2.5	93	4	3	4
2,2,4,4,-d_4	95	100	12.0	5.1	50	50	-	50
3β-d_1	100	11.0	5.4	0.9	96	4	-	4
5β-d_1	100	17.5	7.8	1.4	91	9	-	9
6,6-d_2	100	11.2	5.8	1.3	96	4	-	4
7,7-d_2	100	8.0	5.0	1.1	99	-	-	0
5β,9α,11α-d_3	100	12.6	7.4	2.6	95	5	-	5
16,16-d_2	100	10.0	6.8	1.5	95	2	3	2
17α-CD_3	100	8.0	7.7	1.8	96	-	4	-

(c) 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (3)

	m/z, RA%				isotopic composition, %			
label(s)	**75**	**76**	**77**	**78**	**d_0**	**d_1**	**d_2**	migr, %
d_0	100	7	6	0.8	100	-	-	0
1α,5β-d_2	100	17	6	1	100	8	2	8
1β,5β-d_2	100	17	7.7	3	88	7	5	7
2,2,4,4,-d_4	100	99	12.5	6	52	48	-	48
3α-d_1	100	11	6.3	-	97	3	-	3
5β-d_1	100	16.7	6	1.4	90	8	2	8
6,6-d_2	100	10.9	6	1.7	95	3	2	3
7,7-d_2	100	7.9	5.5	1.2	98	0	2	1
5β,9α,11α-d_3	100	14.1	11.3	4.2	88	6	6	6
16,16-d_2	100	6.9	6.3	1.8	98	-	2	0
17α-CH_3-d_3	100	7.7	7.18	1.97	97	-	3	0

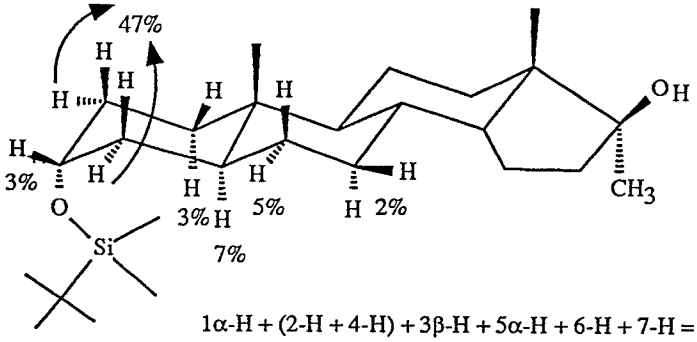
(d) 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane (4)

	m/z, RA%				isotopic composition, %			
label(s)	**75**	**76**	**77**	**78**	**d_0**	**d_1**	**d_2**	migr, %
d_0	100	7	5	0.5	100	-	-	-
1α,5α-d_2	100	13	4.4	0.8	94	5	-	5
1β,5α-d_2	100	13.4	7.8	2.0	92	5	3	5
2,2,4,4,-d_4	93	100	12.3	4.9	50	50	-	50
3α-d_1	100	11	6	2	96	4	-	4
5α-d_1	100	14.5	5.2	0.8	98	7	-	7
6,6-d_2	100	10.4	5.3	1.10	97	3	-	3
7,7-d_2	100	8	4.3	0.8	100	-	-	0
6,6,8β-d_3	100	7	6	1	100	-	-	0
5β,9α,11α-d_3	100	11	6.4	1.7	97	3	-	3
16,16-d_2	100	8	7.3	1.8	100	-	-	0
17α-CD_3	100	11.2	11	3.65	90	4	7	4

(e) 3ξ-OSiDMTB-17α-hydroxy-17β-methyl-5ξ-androstane-5ξ-d_1 (5-8)

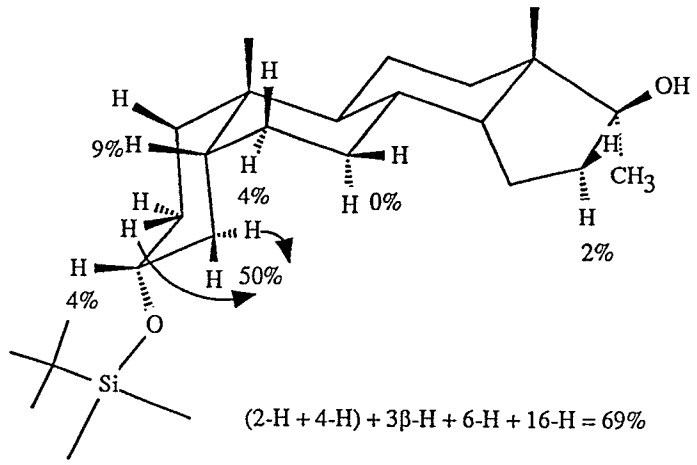
	m/z, RA%				isotopic composition, %			
isomer	**75**	**76**	**77**	**78**	**d_0**	**d_1**	**d_2**	migr, %
5α-H,3α-(5)	100	12.7	6	1.3	95	5	-	5
5β-H,3α-(6)	100	10.3	6	1.2	97	3	-	3
5β-H,3β-(7)	100	13.8	6.3	1	94	6	-	6
5α-H,3β-(8)	100	9	4.5	-	98	2	-	2

1



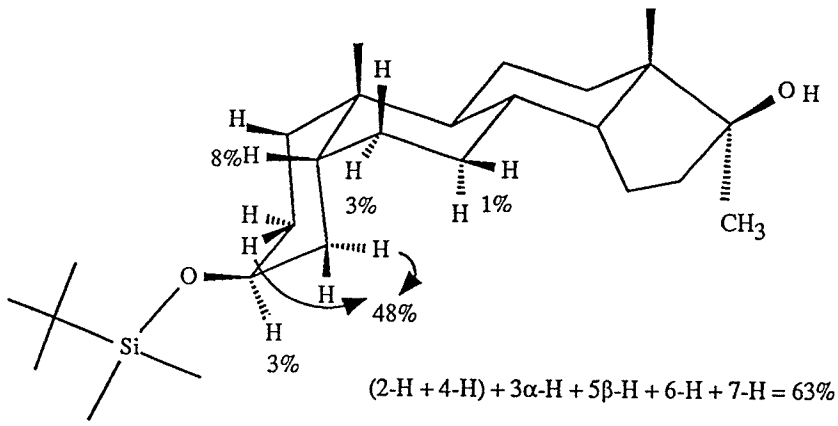
1α-H + (2-H + 4-H) + 3β-H + 5α-H + 6-H + 7-H = 67%

2



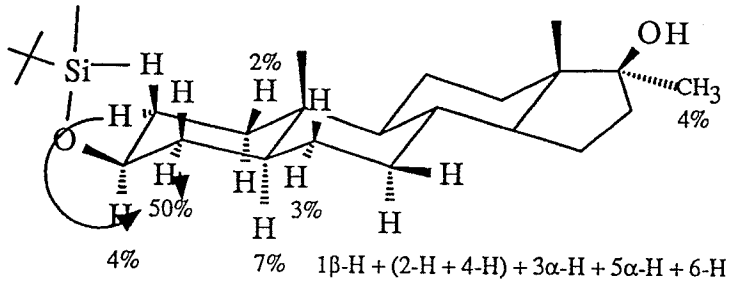
(2-H + 4-H) + 3β-H + 6-H + 16-H = 69%

3



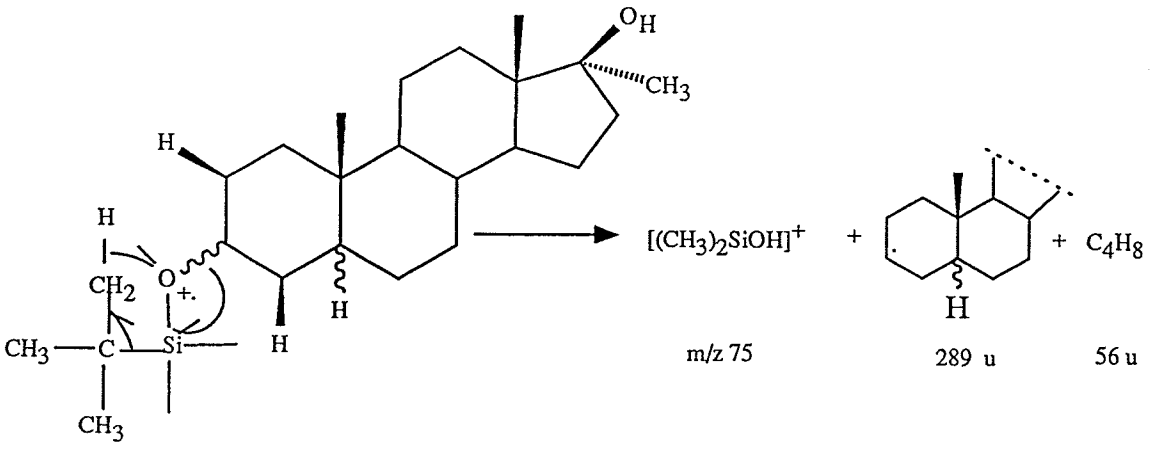
(2-H + 4-H) + 3α-H + 5β-H + 6-H + 7-H = 63%

4

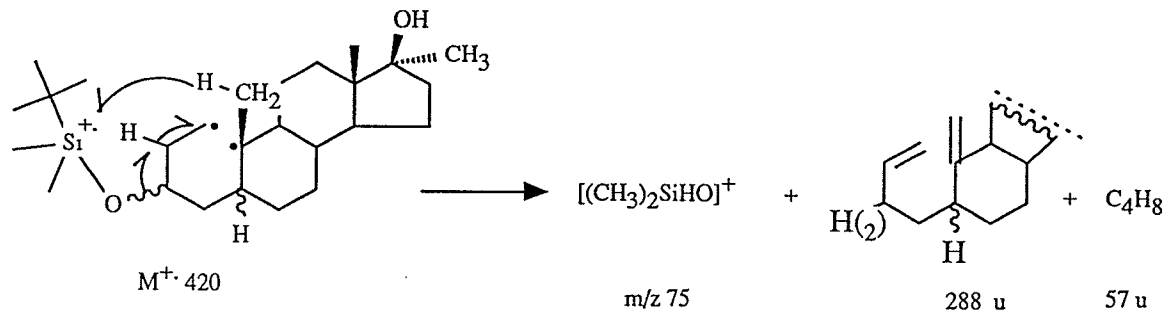


1β-H + (2-H + 4-H) + 3α-H + 5α-H + 6-H + 17α-CH_3 = 70%

Scheme 16. Origin of hydrogen incorporated into the Me_2SiOH^+ ion.

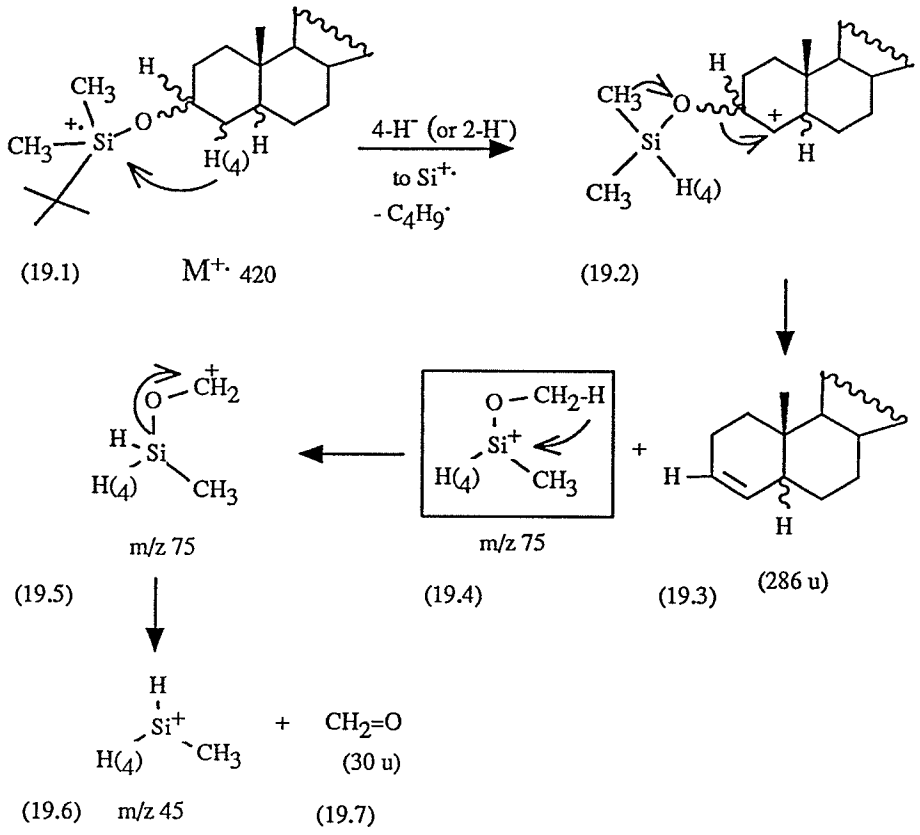


Scheme 17. Proposed mechanism formation of Me_2SiOH^+ involving transfer of a hydrogen from the *t*-butyl group.



Scheme 18. Proposed mechanism of formation of Me_2SiOH^+ involving transfer of a hydrogen from the angular 19-CH_3 group.

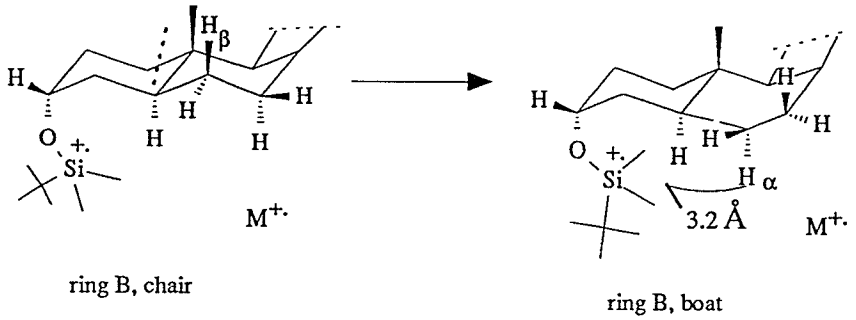
We recall that B/E and B^2/E linked scanning were not able to identify any parent ion of m/z 75. Therefore, it must be formed rapidly, in the ion source. The formation mechanisms proposed in Scheme 19 account for the *ca* 50% of the hydrogen originating from C-2 and C-4.



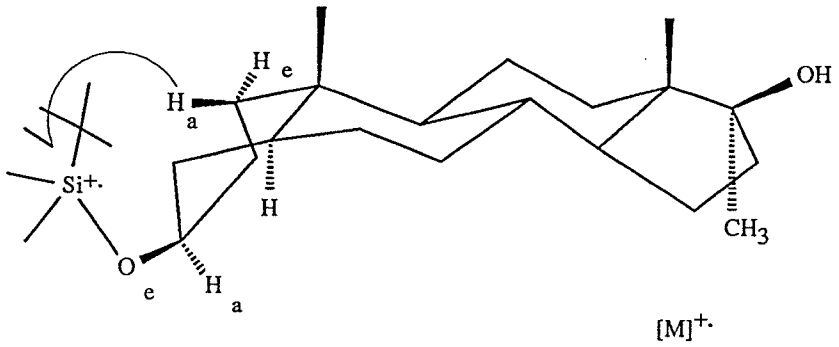
Scheme 19. Mechanism proposed for formation and decomposition of the $[HMeSiOMe]^+$ ion (19.4) for saturated TBDMSi-androstanes (**1-4**).

For the 5α-H,3α-OSiDMTB structure (**1**), abstraction of the C-6 hydrogen (3%) in the most spacially favorable boat conformation of ring B would involve a separation of *ca* 3.2 Å between 6α-H and silicon of the axial 3α-OSiDMTB group (Scheme 20). However, this distance is too large for 6-H transfer, therefore, abstraction may proceeded by fission of the C-1–C-10 bond.

For the 5α-H,3β-OSiDMTB epimer (**4**) (Scheme 21), the abstraction of 1β-H, 5α-H or 6β-H suggests more than two conformations of ring A. For example, abstra-



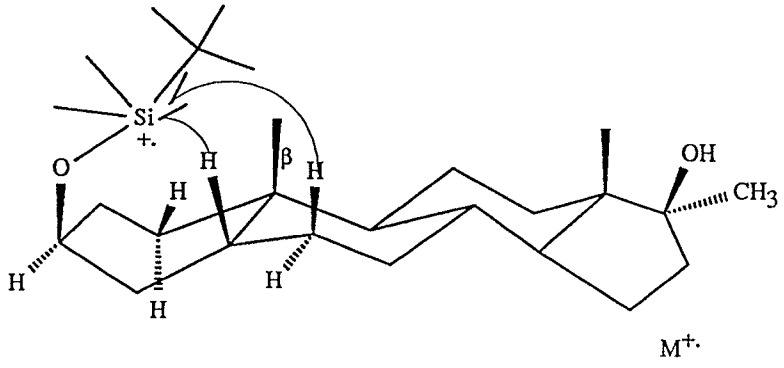
Scheme 20. Rearrangement of 6β-H to the silyloxy group in formation of $[(CH_3)_2SiOH]^+$ for the 5α-H,3α-OSiDMTB (**1**) isomer. (Distances determined by Dreiding model)



Scheme 21. Migration of 1β-H (6%) to the silyloxy group in formation of Me_2SiOH^+ ions from the 5α-H,3β-OSiDMTB isomer (**4**).

ction of axial 5α-H could occur in the chair form of ring A. Abstraction of 1β-H (6%) implies a twist-boat form of ring A in which axial 1β-H is conveniently oriented for migration. The 6β-H atom could rearrange, when ring A adopts the boat form, in which the 3β-OSiDMTB substituent and hydrogen at C-6 are both axial.

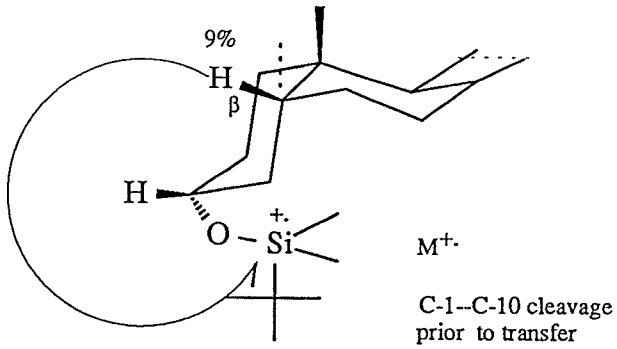
The participation of 5β-H and 6ξ-H but not 1β-H, for the 5β-H,3β-OSiDMTB isomer (**3**) implicates the boat conformation of ring A in the decomposition (Scheme 22). In this conformation of ring A, the 3β-OSiDMTB substituent and 1β-H are remote from each other. In addition, 1β-H is in an unfavourable equatorial orientation for migration. However, the axial 5β-H and 6β-H hydrogen atoms are favourably placed for rearrangement.



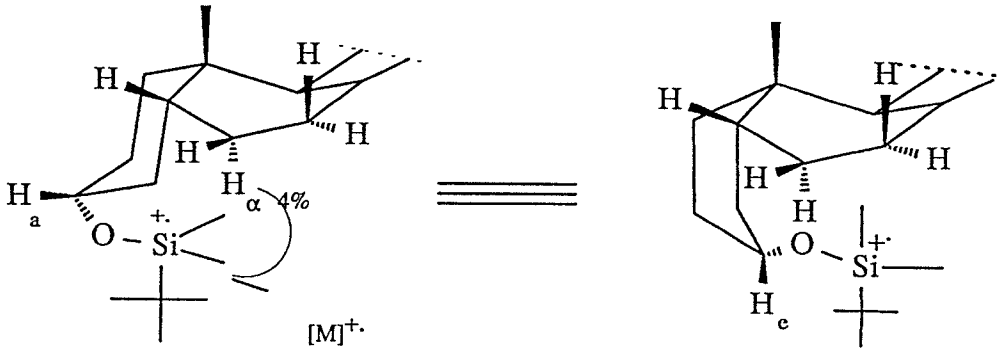
Scheme 22. Migration of 5β-H or 6β-H to the silyloxy residue in formation of the $[Me_2SiOH]^+$ ion from the 5β-H,3β-OSiDMTB isomer (**3**).

For the 5β-H,3α-OSiDMTB isomer (**2**), abstraction of hydrogen from C-6 or C-16 sites implies two different conformations of ring A and B. The chair form of ring A could allow the tertiary 5β-H to migrate to the 3α-OSiDMTB group (Scheme 23(a)). On the other hand, the observed abstraction of a hydrogen atom from C-6, but not from C-7, implies a boat structure of ring B, while at the same time ring A can also flip to an inverted boat form (Scheme 23(b)). The $[Me_2SiOH]^+$ ion incorporates about 2% of hydrogen from the C-16 site. Rearrangement of the hydrogen atom from C-16 is only possible in an inverted boat structure of ring A (Scheme 23(c)).

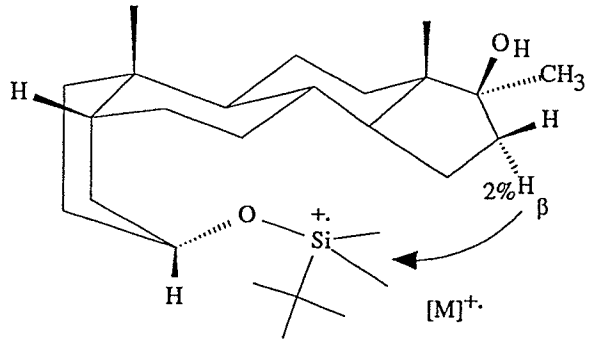
(a)



(b)



(c)



Scheme 23. The 5β-H (a), 6α-H (b), and 16α-H (c) migrations to the silyloxy residue in formation of the $[Me_2SiOH]^+$ ion from the 5β-H,3α-OSiDMTB isomer (2).

III.B.2.2. Fragmentation of $[Me_2SiOH]^+$ ions.

It is interesting to determine whether $[(CH_3)_2SiOH]^+$, an analogue of protonated acetone[169] $[(CH_3)_2COH]^+$, decomposes further. The mass-analysed ion kinetic energy (MIKE) spectrum of the m/z 75 ion from diethoxydimethylsilane showed it to fragment by losses of $H^\cdot$, H_2, and C_2H_4[170]. Our B/E linked scan spectra (Fig. 13) of the m/z 75 ion from 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane revealed six daughter ions formed by loss of $H^\cdot$, $CH_3^\cdot$, CH_4, $CH_2{=}CH_2$, HCHO, or CH_3OH (see below).

$[(CH_3)_2SiOH]^+$, m/z 75	--->	$[CH_3(CH_2)SiOH]^{+\cdot}$, m/z 74	+ $H^\cdot$, (1 u) (1)
	--->	$[CH_3Si{=}OH]^{+\cdot}$, m/z 60	+ $CH_3^\cdot$, (15 u) (2)
	--->	$[CH_2{=}Si{=}OH]^+$, m/z 59	+ CH_4, (16 u) (3)
	--->	$[H_2Si\text{-}OH]^+$, m/z 47	+ C_2H_4, (28 u) (4)
	--->	$[H_2Si\text{-}CH_3]^+$, m/z 45	+ $CH_2{=}O$, (30 u) (5)
	--->	$[CH_2{=}Si\text{-}H]^+$, m/z 43	+ CH_3OH, (31 u) (6)

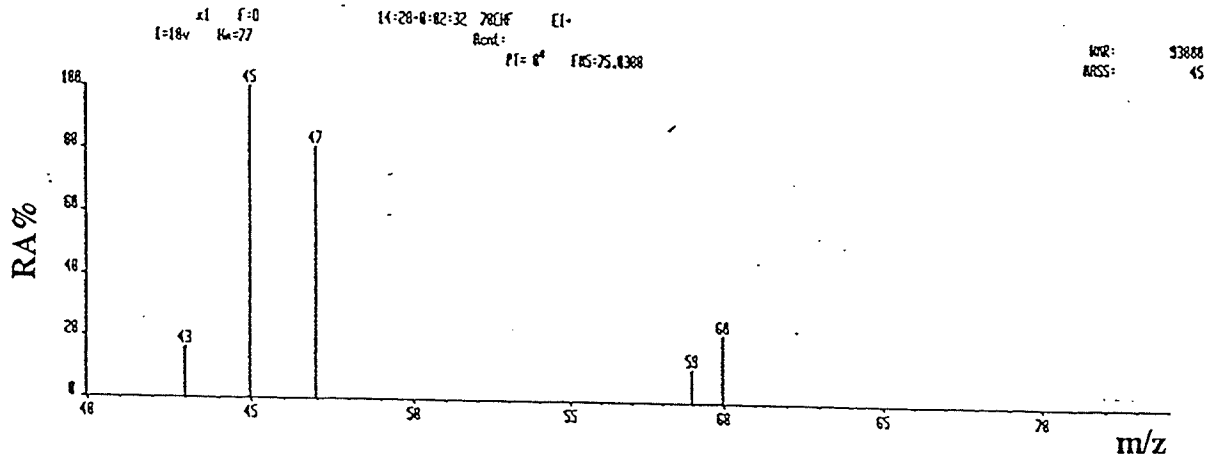
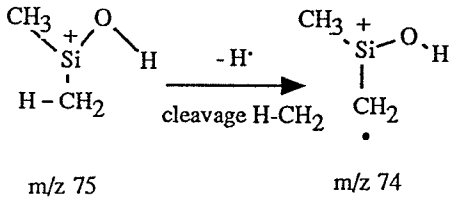
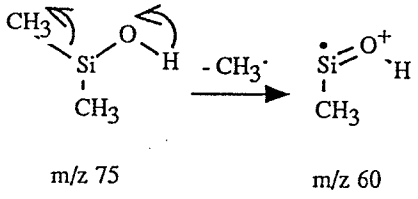


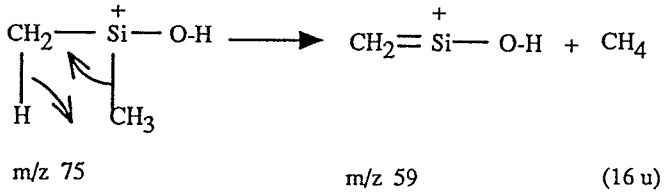
Figure 13. The daughter ion mass spectrum (B/E linked scan) of the m/z 75 ion from 3α-OSiDMTB-17α-hydroxy-17β-methyl-5β-androstane (**6**).

These fragmentations are rationalized in Schemes 24-28. Losses of hydrogen and methyl radicals are shown in Scheme 24. Loss of the hydrogen radical yields an ionized Si^+-CH_2· bond by analogy to an ionized C^+-C· bond. Loss of methane, through a four-membered transition state is shown in Scheme 25.





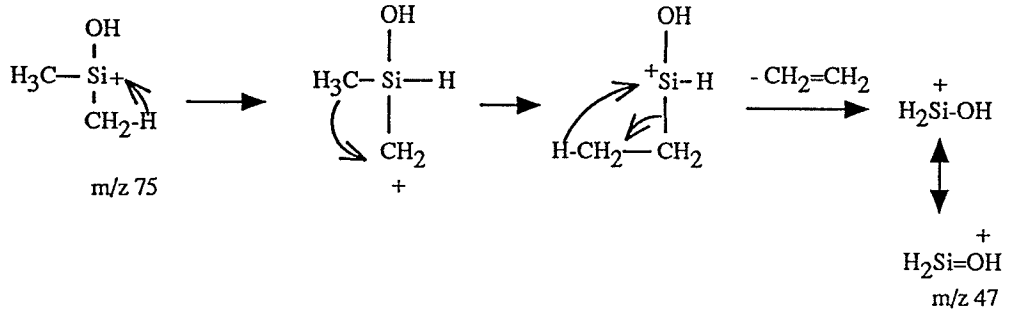
Scheme 24. Losses of hydrogen and methyl radicals from m/z 75.



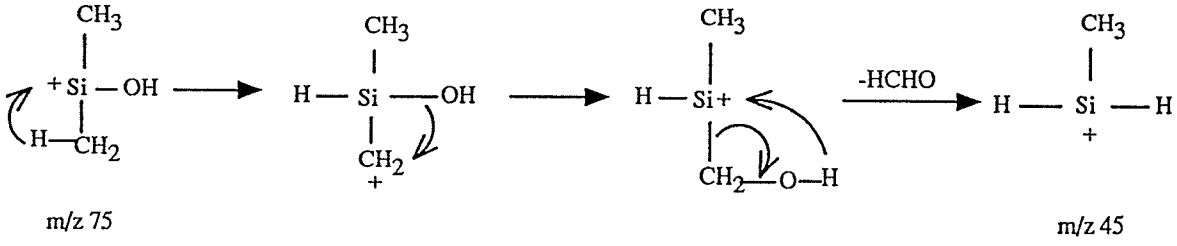
Scheme 25. The loss of methane from m/z 75.

The loss of ethylene, illustrated in Scheme 26, suggests a 1,2 hydride shift, followed by a 1,2 methyl shift and subsequent elimination of C_2H_4. A similar mechanism has been proposed for ethylene losses from protonated acetone[171], the

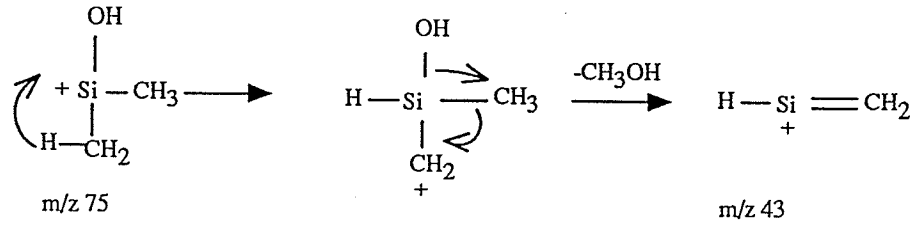
2-propylmethoxonium ion[172], and $[Si(CH_3)_3]^+$, $[(CH_3)_2SiOH]^+$, and $[HSi(CH_3)_2]^+$ ions[173]. Elimination of formaldehyde is explained in Scheme 27; after an initial 1,2 hydride shift, followed by a 1,2 hydroxide shift, 1,2 elimination of CH_2=O occurs. Loss of methanol can be explained as shown in Scheme 28.



Scheme 26. The loss of ethylene from m/z 75.



Scheme 27. The loss of formaldehyde from m/z 75.



Scheme 28. The loss of methanol from m/z 75.

All of these mechanisms assume the Me_2Si^+OH structure for m/z 75. However, an alternative structure, $H(CH_3)Si^+OCH_3$, can be proposed. The latter could be formed by intraionic rearrangement of hydride, from C-4 (or C-2), to a high positive charge density site, i.e. to silicon, as shown in Scheme 19 (subsection III.B.2.1.) followed by further rearrangement to give structure 19.4. After rearrangement 19.4 → 19.5, expulsion of CH_2=O (30 u) yields $CH_3SiH_2^+$ (19.6). Possibly, this route to formation of m/z 75 affords a better interpretation of the electron beam energy dependence on the spectra shown in Fig. 11 (subsection III.B.2).

III.B.3. Rearrangement of the 11α-acetyl group to Si^+ in formation of the $[CH_3COOSi(CH_3)_2]^+$ ion, m/z 117.

In attempts to introduce deuterium at C-9, the four 3ξ-OSiDMTB-11α-acetyloxy-17β-hydroxy-17α-methyl-5ξ-androstane isomers, **9-12**, (**XVd**, Scheme XV) were synthesized. Their mass spectra (Fig. 14 and Table 19) showed an interesting fragmentation pattern. The high mass regions show:

(a) non-detection of $M^{+\cdot}$, m/z 478,

(b) absence or low abundance of $[M - C_4H_9]^+$, m/z 421,

(c) low abundance of $[M - C_4H_9 - CH_3COOH]^+$, m/z 361, and $[M - C_4H_9 - CH_3COOH - H_2O]^+$, m/z 343, and

(d) abundant m/z 287 ions ($[M - C_4H_9 - CH_3COOH - Me_2Si{=}O]^+$ or $[M - CH_3COOH - C_4H_9SiMe_2O]^+$), and m/z 269 ions ($[M - C_4H_9 - CH_3COOH - H_2O - Me_2Si{=}O]^+$ or $[M - CH_3COOH - C_4H_9SiMe_2O - H_2O]^+$).

The base peak corresponds to the m/z 269 ion for compounds **9** (5α-H,3α-OSiDMTB) and **10** (5β-H,3α-OSiDMTB), to the m/z 117 ion for compound **11** (5β-H,3β-OSiDMTB), and to the m/z 75 ion for compound **12** (5α-H,3β-OSiDMTB) (Fig. 14).

The low abundance of the $[M - C_4H_9]^+$ ions implies their fast decomposition in the ion source, presumably by loss of the 11α-acetyloxy[147] group as CH_3COOH formed by 1,2 abstraction of the tertiary 9α-H or the secondary 12α-H. This occurs more rapidly than HMe_2SiOH elimination, because the m/z 361 and m/z 343 ions formed by elimination of CH_3COOH and ($CH_3COOH + H_2O$) are much more abundant than m/z 345 (which is absent) formed by elimination of HMe_2SiOH. The MM2 calculated geometry (Appendix II), for the $[M - t\text{-Bu}]^+$ ion of **11**, is shown in Scheme 29a. The distances between an oxygen of the CH_3COO group and the neighbouring hydrogens are dipicted. The m/z 285 and m/z 267 ions formed by loss of HMe_2SiOH from $[M - C_4H_9 - CH_3COOH]^+$ or $[M - C_4H_9 - CH_3COOH - H_2O]^+$, respectively, are insignificant for all

Figure 14. Normalized EI mass spectra (70 eV; RA%) for the four 3ξ-TBDMSi ether 11α-acetyloxy derivatives of 17α-methyl-5ξ-androstane-3ξ,11α,-17β-triols (**9**-**12**).

Figure 14.

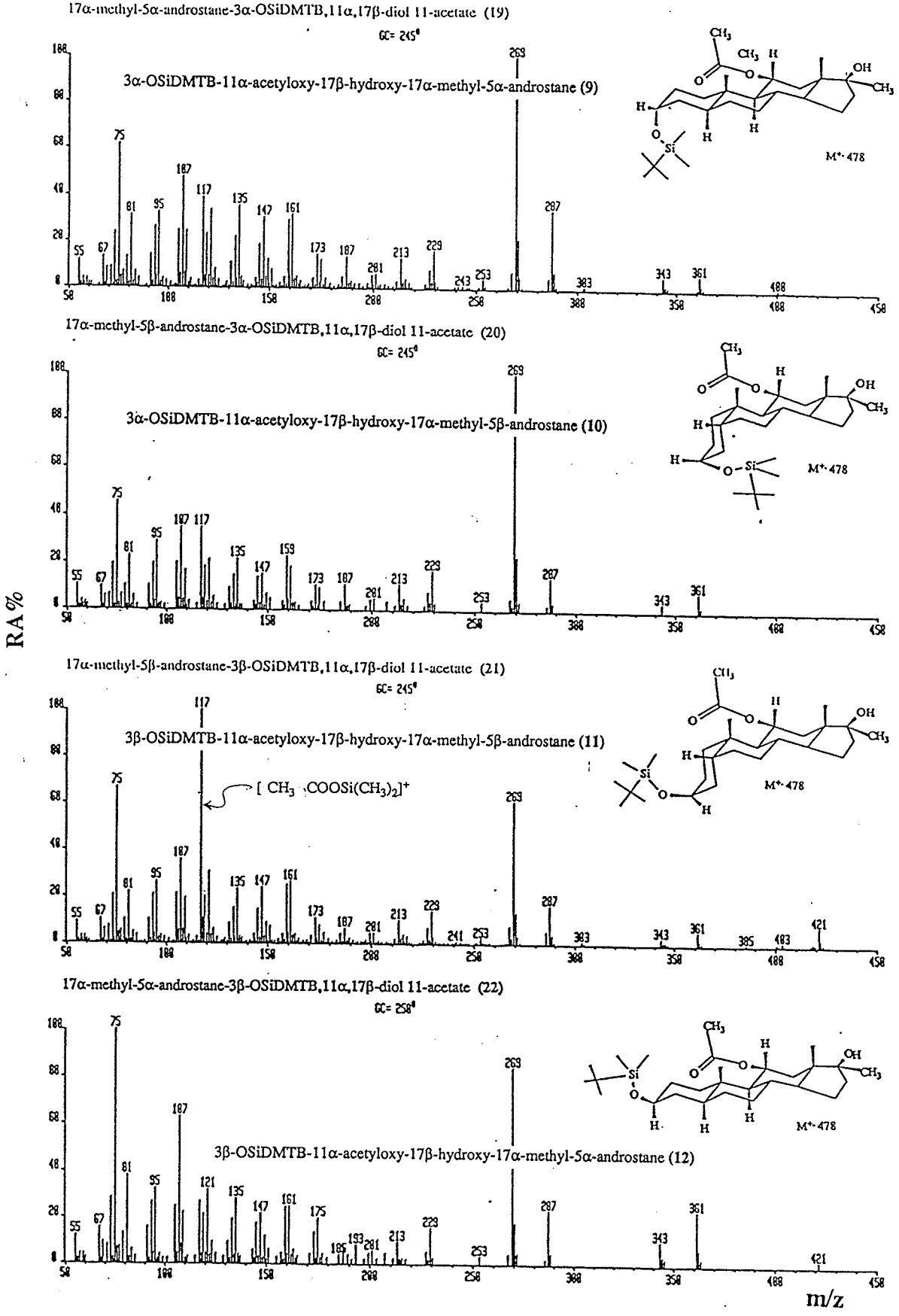
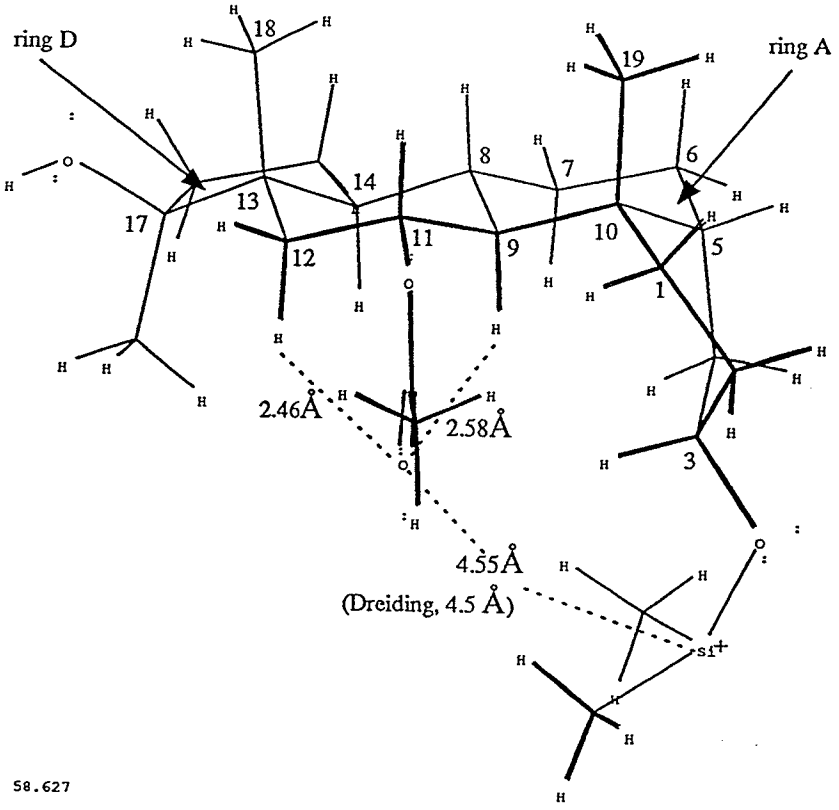

17α-methyl-5α-androstane-3α-OSiDMTB,11α,17β-diol 11-acetate (19)
3α-OSiDMTB-11α-acetyloxy-17β-hydroxy-17α-methyl-5α-androstane (9)
M+· 478
17α-methyl-5β-androstane-3α-OSiDMTB,11α,17β-diol 11-acetate (20)
3α-OSiDMTB-11α-acetyloxy-17β-hydroxy-17α-methyl-5β-androstane (10)
M+· 478
17α-methyl-5β-androstane-3β-OSiDMTB,11α,17β-diol 11-acetate (21)
3β-OSiDMTB-11α-acetyloxy-17β-hydroxy-17α-methyl-5β-androstane (11)
[CH3 ,COOSi(CH3)2]+
M+· 478
17α-methyl-5α-androstane-3β-OSiDMTB,11α,17β-diol 11-acetate (22)
3β-OSiDMTB-11α-acetyloxy-17β-hydroxy-17α-methyl-5α-androstane (12)
M+· 478
RA %
m/z

Table 9. Partial mass spectra (% RA) of 3ξ-TBDMSi ether 11α-acetyloxy derivatives of 17α-methyl-5ξ-androstane-3ξ,11α,17β-triols (**9-12**), (R = TBDMSi, electron energy = 70 eV).

Ion	m/z	5α-H, 3α-OR (**9**)	5β-H, 3α-OR (**10**)	5β-H, 3β-OR (**11**)	5α-H, 3β-OR (**12**)
$[M]^{+\cdot}$	478	---	---	---	---
$[M - C_4H_9]^+$	421	---	---	8	2
$[M - CH_3COOH - CH_3]^+$	403	---	---	2	---
$[M - C_4H_9 - CH_3COOH]^+$	361	6	8	5	23
$[M - C_4H_9 - CH_3COOH - H_2O]^+$	343	2	4	3	10
$[M - C_4H_9 - CH_3COOSi(CH_3)_2OH]^+$	303	1	---	1	---
$[M - C_4H_9 - CH_3COOH - Me_2Si{=}O]^+$ or $[M - CH_3COOH - C_4H_9SiMe_2O]^+$	287	34	14	16	23
$[M - C_4H_9 - CH_3COOH - HSiMe_2OH]^+$	285	4	2	5	2
$[M-C_4H_9 - CH_3COOH- Me_2Si{=}O - H_2O]^+$ or $[M - CH_3COOH - C_4H_9SiMe_2O - H_2O]^+$	269	100	100	61	84
$[M-C_4H_9- CH_3COOH-H_2O - HSiMe_2OH]^+$	267	7	5	8	4
$[M-CH_3-C_4H_9SiMe_2OH-CH_3COOH-H_2O]^+$	253	4	3	2	3
$[M - 351]^+ = [CH_3COOSi(CH_3)_2]^+$	117	39	35	100	27
$[M - 403]^+ = [(CH_3)_2SiOH]^+$	75	62	45	67	100
$[M - 405]^+ = [(CH_3)_3Si]^+$	73	24	19	20	28

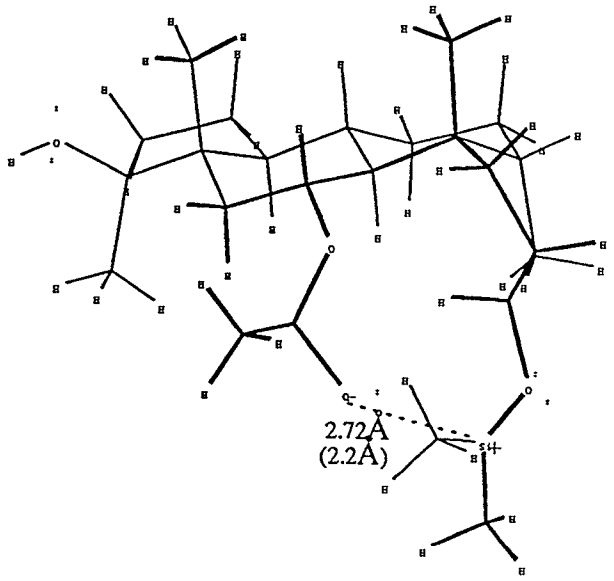
(a)



(29.1)

MMX ENERGY 58.627

(b)

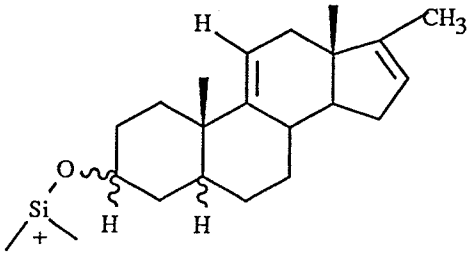


(29.2)

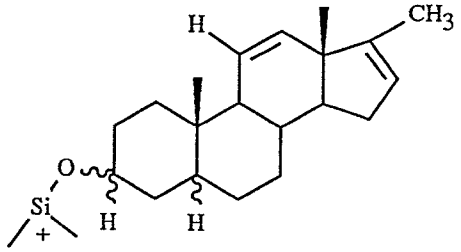
(for calculation purposes, O^- in the CH_3COO group and C^+, instead of Si^+ were used)

Scheme 29. MM2 calculated geometry for the inverted boat conformer of ring A of $[M - t\text{-Bu}]^+$ ion of compound **11** (viewed from the back). (Distances according to Dreiding models in parentheses)

four isomers. On the other hand, the prominent m/z 287 and m/z 269 ions, could be assigned as $[M - C_4H_9 - CH_3COOH - Me_2Si{=}O]^+$ and $[M - C_4H_9 - CH_3COOH - H_2O - Me_2Si{=}O]^+$, respectively. The latter could be formed by elimination of $Me_2Si{=}O$ from structures A_1 or A_2. Some proposed fragmentation pathways are presented in Scheme 30.



A_1

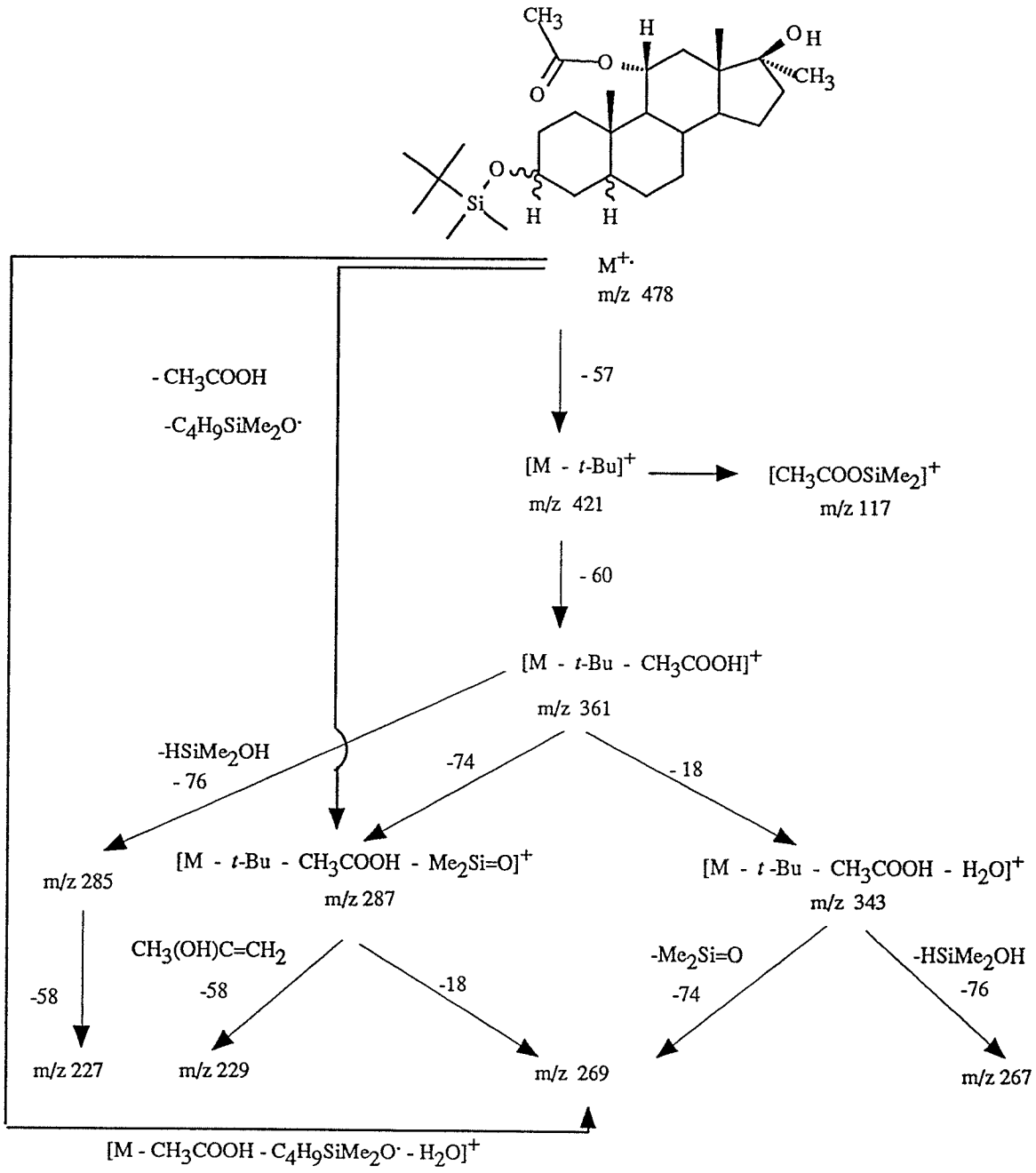


A_2

$[M - C_4H_9^{\cdot} - CH_3COOH - H_2O]^+$

m/z 343

An ion of m/z 117 is prominent in all spectra, especially that of compound **11**. This intriguing ion, formed by rearrangement of the 11α-acetyloxy group to silicon, I have assigned as $[CH_3COOSi(CH_3)_2]^+$. Exact mass measurement supported the composition $C_4H_9O_2Si^+$ (Table 10). Exact mass measurement seldom gives a single possible formula match, but other information, for example, the units of unsaturation (rings and double bonds) helps to decide between two possible formulae. The only reasonable choice of elemental composition for the m/z 117 ion is $C_4H_9O_2Si$. The mechanism proposed for its formation, for compound **11**, is presented in Scheme 31. The $[M - C_4H_9^{\cdot}]^+$ ion (31.2) is assumed to be the precursor in the 11α-acetyloxy group rearrangement to Si^+, which serves as a "supercation"[193]. The rearrangement 31.2 → 31.3 can be viewed as an example of nucleophilic attack of the carbonyl oxygen of the 11α-acetyloxy group on the electron deficient silicon centre, followed by loss of the steroid skeleton via an "unzip" sequence in which migration of the positive charge from C-11 to 3-oxygen generates the resonance-stabilized $C_4H_9Si^+$ ion (31.5). Similar modes



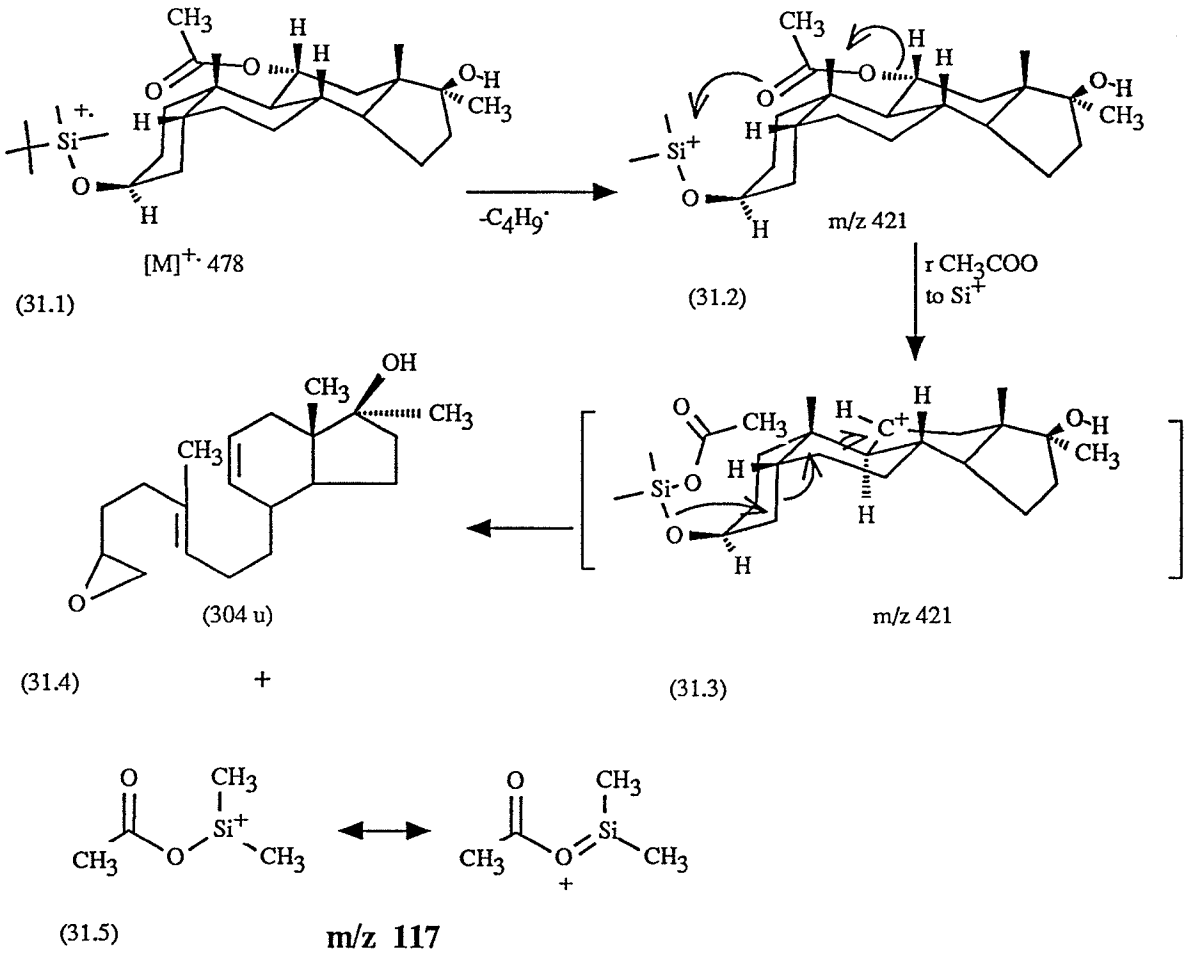
Scheme 30. Fragmentation pathways proposed for 3ξ-TBDMSi ether 11α-acetyloxy derivatives of 17α-methyl-5ξ-androstane-3ξ,11α,17β-triol (**9-12**).

Table 10. Comparison of the observed mass of the m/z 117 ion with calculated masses of ions containing C, H, O, and Si within 40 mmu.

observed m/z	C	H	O	Si	Deviation mmu	DBE
117.0341	9	9	0	0	36.3	5.5
	7	5	0	1	-18.1	6.5
	8	5	1	0	-0.1	6.5
	5	13	1	1	39.4	0.5
	7	1	2	0	-36.5	7.5
	4	9	2	1	3.0	1.5
	5	9	3	0	21.0	1.5
	3	5	3	1	-33.4	2.5
	4	5	4	0	-15.4	2.5

of formation of the $[CH_3COOSi(CH_3)_2]^+$ ion, can be proposed for **9**, **10**, and **12**. To explain the particularly high abundance of the m/z 117 ion in the spectrum of compound **11** (Fig. 14), we note that the oxygen of the 11α-CH_3COO group and the silicon of the 3β-OMe_2Si^+ residue can approach within bonding distance. This can occur when ring A adopts an inverted boat conformation, as shown in Scheme 29b. This phenomenon is reflected in a much higher abundance of the m/z 117 ion (100% RA) than for the other isomers, in which the interacting groups are separated by larger distances. For calculation purposes, the minimisation of energy of the 29.2 conformer was performed on a structure with O^- on the acetyl group and C^+ instead of silicon (no Si^+ in the PCMODEL software).

To the best of our knowledge, this is the first demonstration of EI induced intramolecular migration of the acetyloxy group (CH_3COO) to Si^+ from a distance larger than two bonds length observed in $Ph_2(CH_3COO)CSi(CH_3)_3$[174]. This novel migration



Scheme 31. Mechanism proposed for formation of the m/z 117 ion for compound **11**.

indicates a positive charge on silicon after a *t*-butyl radical elimination. Moreover, this positive charge can attract groups bearing a lone electron pair[174], i.e. ether oxygen (RO^-), methide group (CH_3^-), phenylide (Ph^-), chloride (Cl^-), bromide (Br^-), acetate (CH_3COO^-; as discussed here) or hydride (H^-; see section III.C.).

It is also interesting to note that the abundance of the m/z 75 ion is not only a function of the isomer's structure, but also of the presence of other substituents. For compound **12** it forms the base peak (Fig. 14).

III.C. Double and single hydrogen rearrangements in the elimination of $H(CH_3)_2SiOH$ and H_2O in the mass spectra of 3ξ-OSiDMTB-17ξ-hydroxy-17ξ-methyl-5ξ-androstanes (1-8).

In this section, the formation of the [M - *t*-Bu]$^+$, [M - *t*-Bu - H_2O]$^+$, [M - *t*-Bu - $HSiMe_2OH$]$^+$, [M - *t*-Bu - H_2O - $HSiMe_2OH$]$^+$ and [M - *t*-Bu - H_2O - $Me_2Si{=}O$]$^+$ ions, observed in the mass spectra of the 3ξ-OSiDMTB-17ξ-hydroxy-17ξ-methyl-5ξ-androstanes, **1-8**, are discussed. Eliminations of HMe_2SiOH, H_2O, and $Me_2Si{=}O$ involve two, one, and zero hydrogen rearrangements, respectively. For isomers **1-4**, several deuterium-labeled analogues were synthesized. Similar results are anticipated for the 17α-hydroxy-17β-methyl epimers **5-8**, for which only the C-5 position was deuterium labeled.

Immediately following are shown the mass spectra of compounds **1-4** and their deuterium-labeled analogues (Figs. 15-18). The RA% values of peak clusters for important ion species, for each labeled and unlabeled compound, are summarized in Tables 11, 13, 15, and 17. These values are used to calculate the deuterium isotopic compositions (%) of these ion species (after correcting for isotopes of carbon, hydrogen, oxygen, and silicon) shown Tables 12, 14, 16, 18.

The origins of the itinerant hydrogen atoms, in $HSiMe_2OH$ elimination from either [M - *t*-Bu]$^+$ or [M - *t*-Bu - H_2O]$^+$, determined for each isomer, are shown in Tables 19, 21, 23, 24, and 27. The results, together with fragmentation mechanisms for elimination of $HSiMe_2OH$ are discussed in sections III.C.1 - 4 for compounds **1-4**, respectively. For compound **2**, elimination of $Me_2Si{=}O$ is also discussed in Section III.C.2.

Similarly, the results for compounds **5-8** are discussed in Section III.C.5. Their mass spectra, and those of the 5ξ-d_1 analogues are shown in Fig. 19, the %RA values for

peak clusters in Table 29, deuterium contents in Table 30, and origins in Scheme 68.

The origins of transferred hydrogen atoms, in expulsion of H_2O from ring D, were also determined, and the results are presented in Table 31 in Section III.C.6. Fragmentation mechanisms are proposed.

Figure 15. Positive ion mass spectra of unlabeled (**1**) and deuterium-labeled (**1-A** to **1-K**) 3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane.

Figure 15.

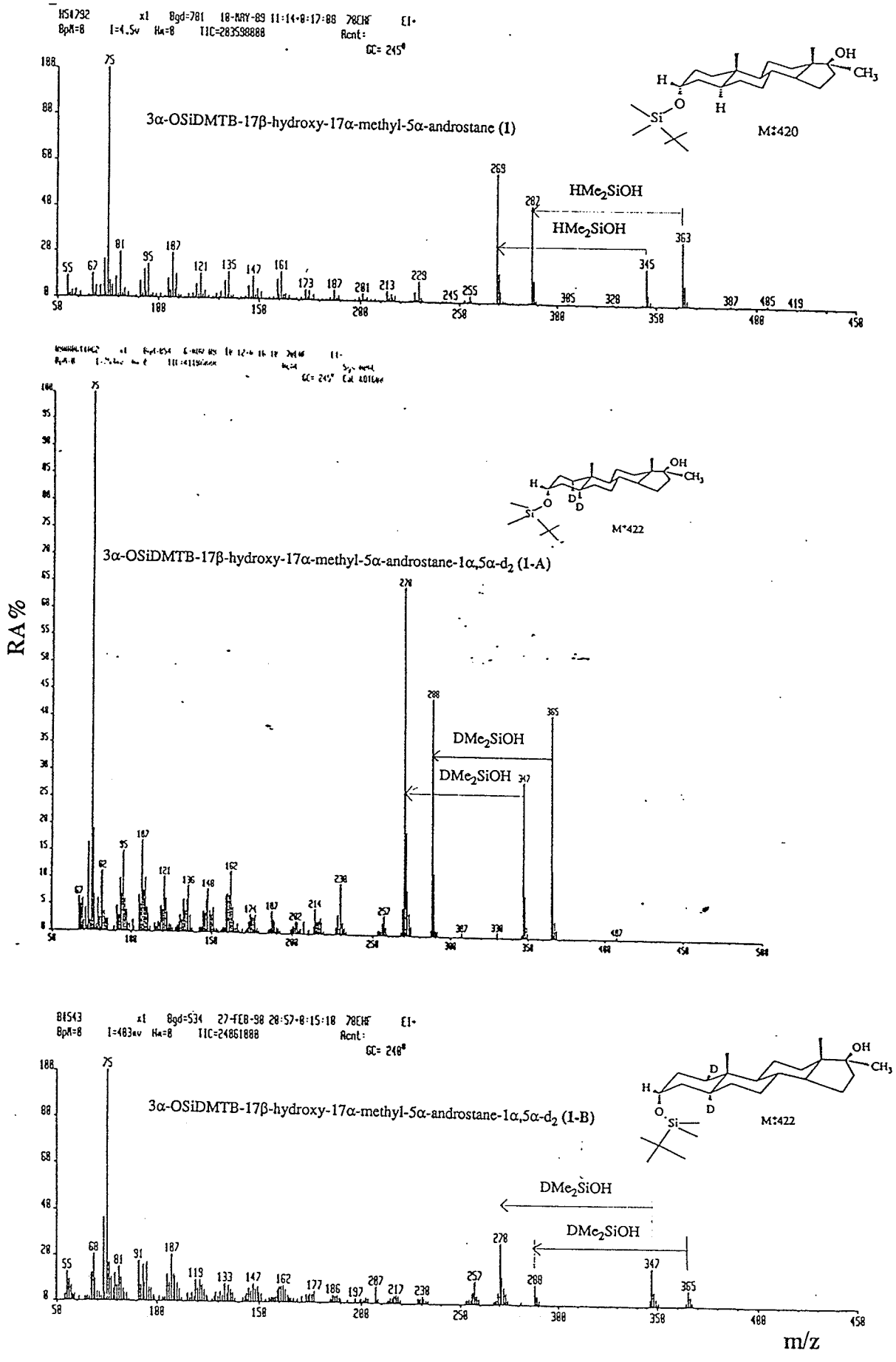

3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane (I)
M+420
HMe2SiOH
HMe2SiOH
3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-1α,5α-d2 (1-A)
M+422
DMe2SiOH
DMe2SiOH
RA %
3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-1α,5α-d2 (1-B)
M+422
DMe2SiOH
DMe2SiOH
m/z

Figure 15 (continued)

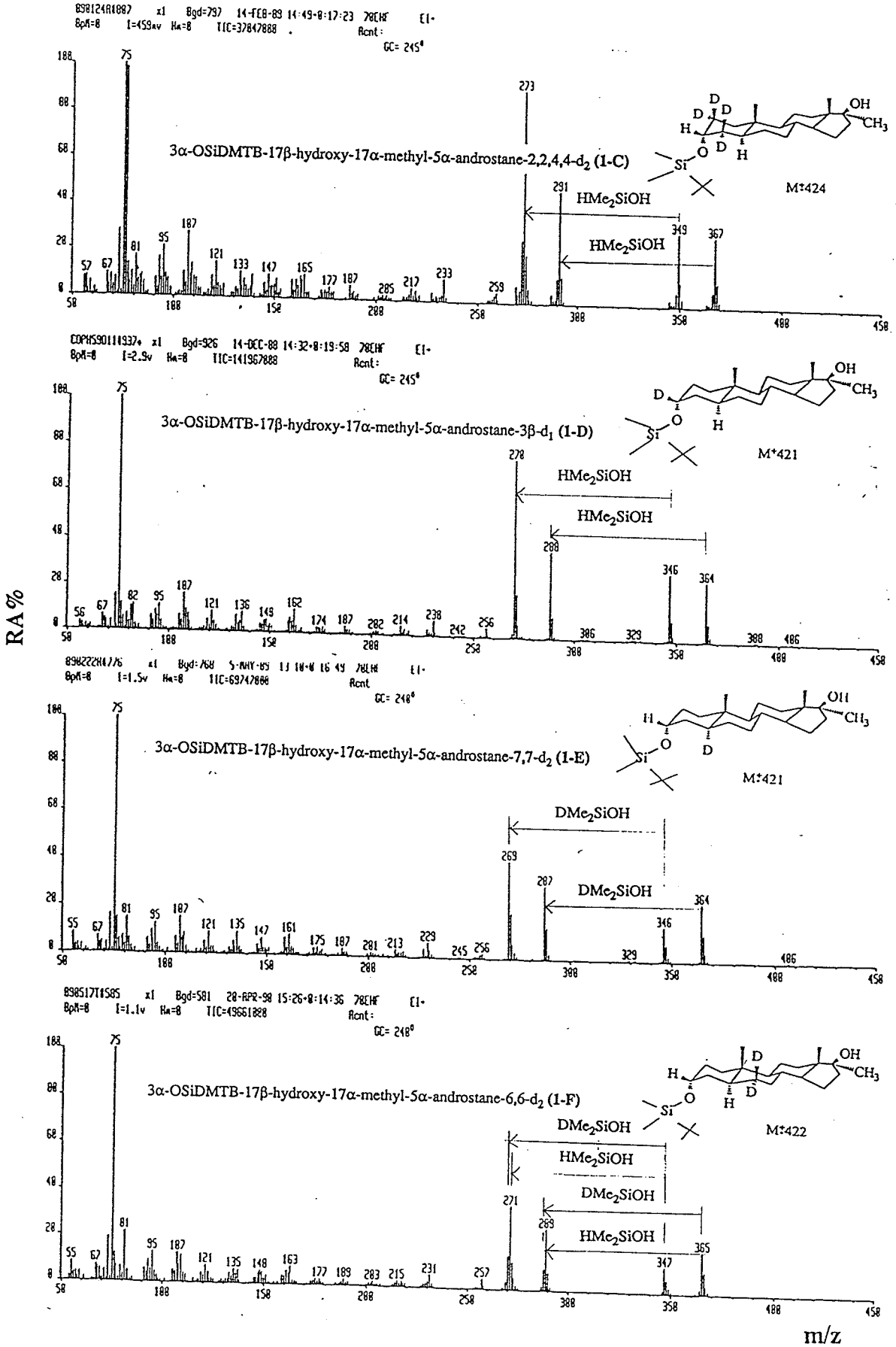
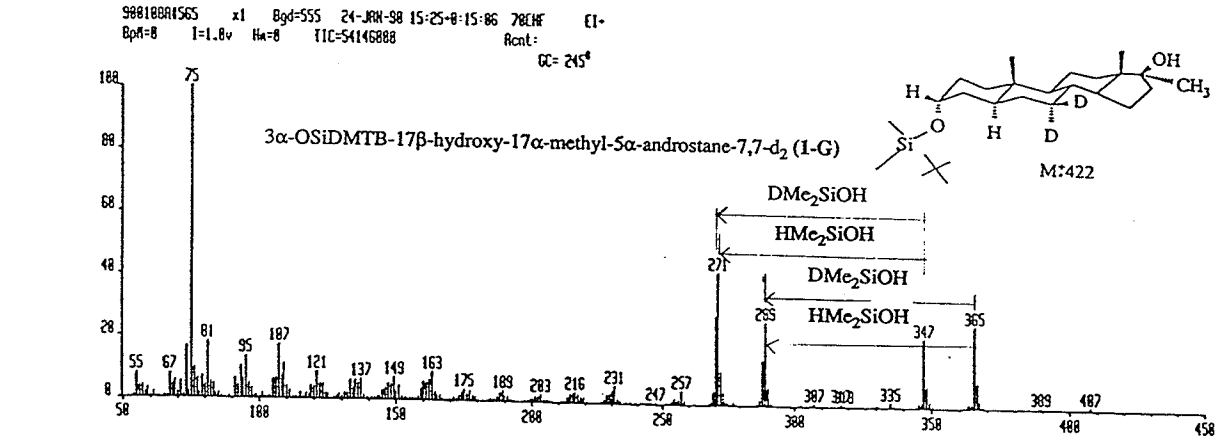
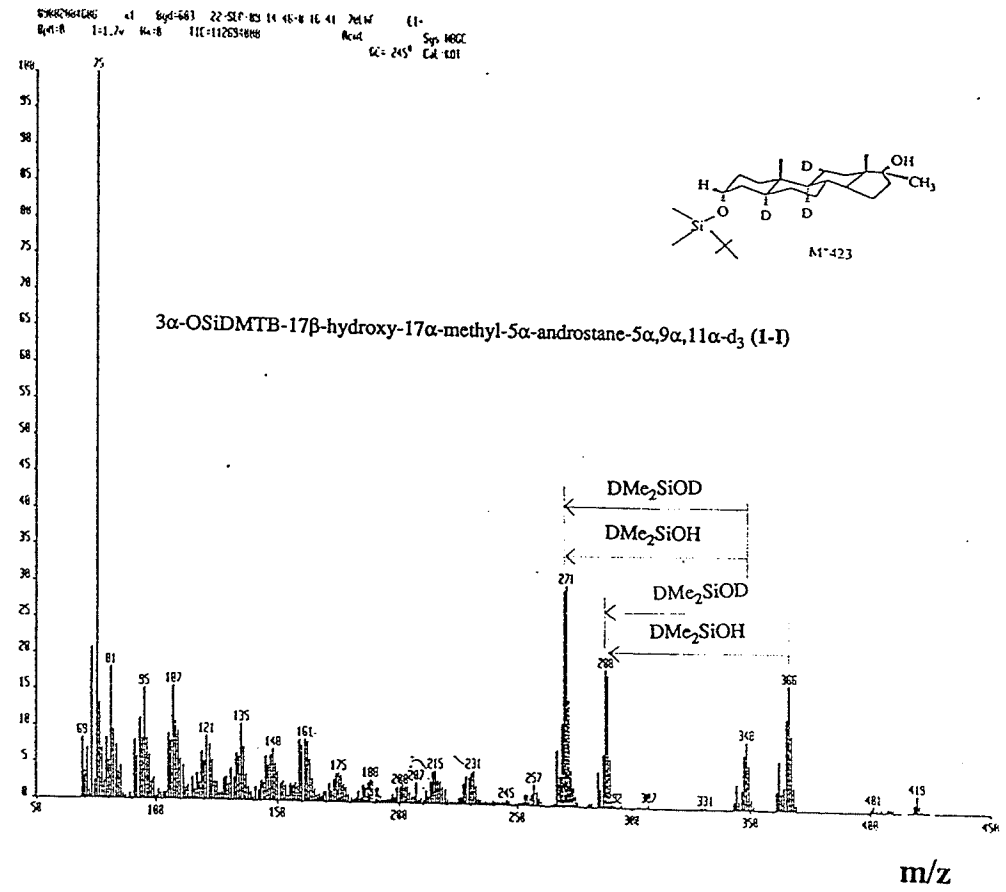


Figure 15 (continued)



RA %



m/z

Figure 15 (continued)

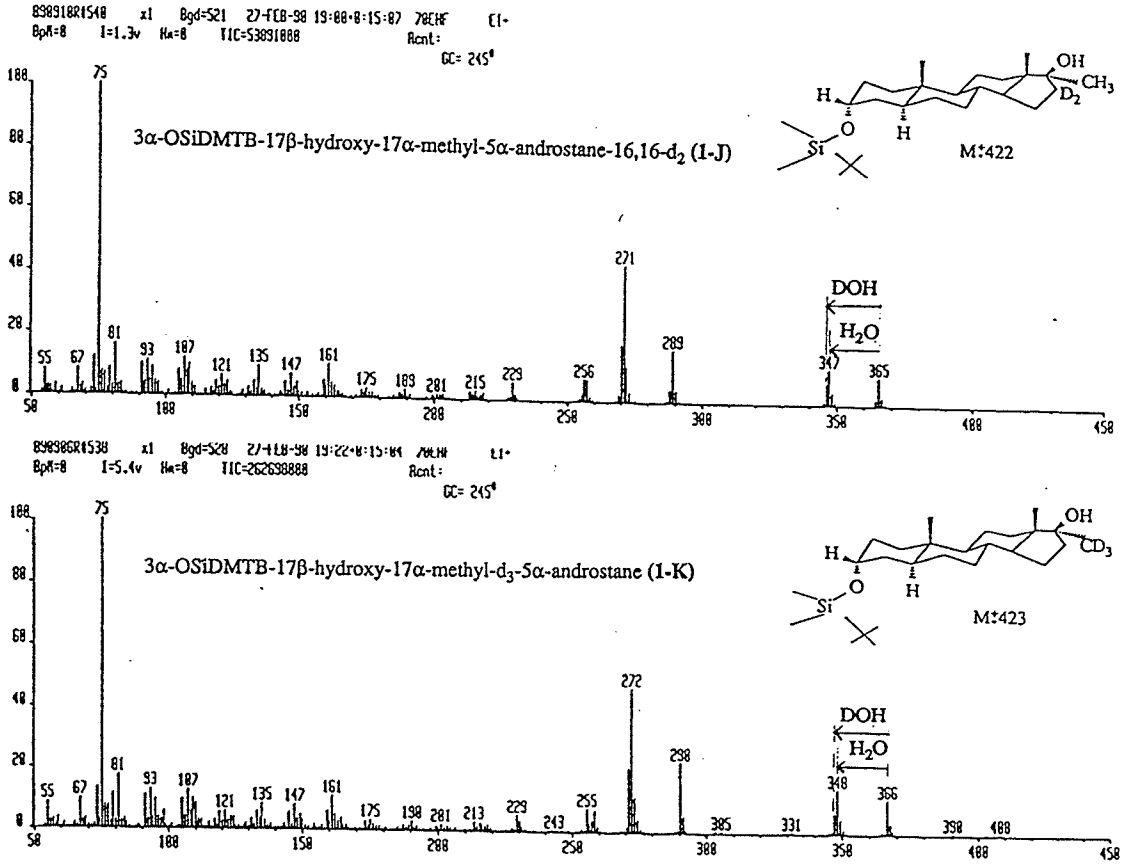
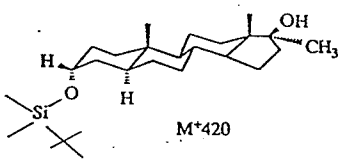


Table 11. Relative abundance (%) in peak clusters for important ion species from 3α--OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane (**1**) and its labeled analogues (**1-A** to **1-K**).

label(s) in compound		FRAGMENT ION				
		$[F_5]^+$	$[F_4]^+$	$[F_3]^+$	$[F_2]^+$	$[F_1]^+$
 M⁺420		$[M - CH_3$ H_2O - TBDMSiOH]	$[F_2$ - $HSiMe_2OH]$ or $[F_3 - H_2O]^+$	$[F_1$ - $HSiMe_2OH]$	$[F_1$ - $H_2O]^+$	$[M$ - *t*-Bu$]^+$
		m/z/relative abundance %				
d_0	(1)	255/2.8 256/0.6	269/56.2 270/12.7 271/ 4.9	287/48.5 288/ 9.8 289/ 1.2	345/16 346/ 4.5 347/ 1.5	363/28 364/ 8.6 365/ 2.4
1α,5α-d_2	(1-A)	255/0.7 256/2.2 257/3.5 258/1.4 259/0.3 260/-	269/ 5.0 270/65 271/19 272/ 8.2 273/ 4.1 274/ 1.6	287/ 1.1 288/44 289/11.5 290/ 1.0 291/ 0.9 292/-	345/- 346/ 0.7 347/29 348/ 7.8 349/ 2.0 350/ 0.8	363/- 364/- 365/41 366/12.4 367/ 3.2 368/ 1.5
1β,5α-d_2	(1-B)	255/1.6 256/4.9 257/9.7 258/3.6 259/1.8 260/-	269/ 4.8 270/26.4 271/11.5 272/ 7.1 273/ 4.4 274/ 1.40	287/- 288/ 8.3 289/ 3.7 290/ 1.25 291/- 292/-	345/- 346/2.49 347/16 348/ 5.70 349/ 2.83 350/ 1.44	363/-- 364/1.1 365/6.4 366/3.8 367/1.3 368/--
2,2,4,4-d_4	(1-C)	255/1.1 256/1.3 257/1.6 258/3 259/4.1 260/1.3 261/- 262/- 263/-	269/ 7 270/ 3.5 271/ 5.3 272/27 273/91 274/20 275/ 6.1 276/ 1.7 277/ 1.5	287/ 3.9 288/ 1.5 289/ 2 290/10.6 291/48 292/11.4 293/ 1.4 294/- 295/-	345/ 2.7 346/ 1.4 347/ 1.4 348/ 5.7 349/31 350/10.0 351/ 3.3 352/- 353/-	363/ 1.5 364/ 1.2 365/ 0.93 366/ 6.2 366/30 368/ 9.9 369/ 2.1 370/- 371/-

cont'd

Table 11 (continued)

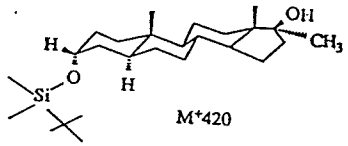
label(s) in compound		$[F_5]^+$	$[F_4]^+$	$[F_3]^+$	$[F_2]^+$	$[F_1]^+$
		m/z/relative abundance %				
3β-d_1	(1-D)	255/-	269/2.0	287/ 0.58	345/ 0.61	363/ 0.52
		256/3.9	270/76	288/37	346/29	364/26
		257/0.97	271/19	289/ 9.2	347/ 8.6	365/ 7.8
		258/-	272/ 5.6	290/ 1.1	348/ 2.4	366/ 2.2
		259/-	273/ 1.0	291/-	349/ -	367/-
		260/-	274/ 0.85	292/-	350/ -	368/-
5α-d_1	(1-E)	255/1.7	269/41	287/31	345/ 0.76	363/ 0.76
		256/1.8	270/19	288/13.1	346/14	364/24
		257/0.68	271/ 6.3	289/ 2.3	347/ 6.7	365/11.1
		258/-	272/ 3	290/-	348/-	366/ 3.6
		259/-	273/ 1.0	291/-	349/-	367/ 0.74
6,6-d_2	(1-F)	255/0.64	269/ 2.9	287/ 1.7	345/-	363/-
		256/0.62	270/14.1	288/ 9.1	346/ 1.2	364/ 1.9
		257/4.0	271/36	289/26	347/11.1	365/18
		258/1.3	272/11.5	290/ 7.5	348/ 5.6	366/ 8.9
		259/-	273/ 4.7	291/ 1.25	359/ 2.1	367/ 3.4
		260/-	274/ 1.5	292/-	350/ 0.5	368/ 0.84
		261/-	275/ 0.6	293/-	351/-	369/ -
7,7-d_2	(1-G)	255/0.8	269/ 4.14	287/ 1.5	345/ 0.97	363/1.0
		256/1.1	270/28	288/14.6	346/ 1.20	364/1.1
		257/4.3	271/42	289/27	347/22	365/26
		258/0.97	272/10.5	290/ 5.5	348/ 6.5	366/7.8
		259/-	273/ 3.9	291/ 0.63	349/ 1.7	367/ 1.9
		260/-	274/ 0.85	292/-	350/-	368/ -
		261/-	275/ 0.60	293/-	351/-	369/ -

cont'd

Table 11 (continued)

label(s) compound		$[F_5]^+$	$[F_4]^+$	$[F_3]^+$	$[F_2]^+$	$[F_1]^+$
		m/z/relative abundance %				
5α,9α,11α-d_3		255/0.8	269/14.3	287/ 9.3	345/1.4	363/ 2.6
	(1-I)	256/1.4	270/33	288/22	346/3.6	364/ 4.9
		257/2.7	271/30	289/16.	347/7.4	365/15
		258/2.6	272/14	290/ 6.1	348/8.7	366/18
		259/1.2	273/ 5.0	291/ 1.2	349/4.7	367/ 9.1
		260/0.5	274/-	292/ 0.3	350/1.4	368/ 2.4
		261/-	275/-	293/-	351/0.4	369/ 0.5
16,16-d_2	(1-J)	255/1.9	269/ 22.0	287/-	345/ 0.66	363/-
		256/6.9	270/18	288/ 3.75	346/ 6.3	364/1.75
		257/6.8	271/43	289/17	347/11	365/8.7
		258/1.4	272/11.0	290/ 3.41	348/ 3.4	366/2.4
		259/-	273/ 3.0	291/-	349/ 0.77	367/ -
17α-CD_3	(1-K)	255/7.4	269/-	287/-	345/-	363/-
		256/2.7	270/ 1.7	288/-	346/ 0.53	364/-
		257/3.6	271/21	289/ 0.72	347/ 6.5	365/-
		258/6.8	272/46	290/22	348/14	366/11.0
		259/1.5	273/11.3	291/ 5.1	349/ 4.5	367/ 3.3
		260/-	274/ 3.7	292/ 0.67	350/ 1.3	368/ 0.97
		261/-	275/ 0.83	293/-	351/-	369/ -

Table 12. Deuterium content (%) of fragment ions from deuterium-labeled 3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane (**1-A** to **1-K**).

label(s) in compound			FRAGMENT ION				
			$[F_5]^+$	$[F_4]^+$	$[F_3]^+$	$[F_2]^+$	$[F_1]^+$
 M⁺420			$[M - CH_3 H_2O - TBDMSiOH]$	$[F_3\text{-}H_2O]^+$ or $[F_2 - HSiMe_2OH]$	$[F_1 - HSiMe_2OH]^+$	$[F_1 - H_2O]^+$	$[M - t\text{-}Bu]^+$
			m/z of principal peaks				
		d_n	**255**	**269**	**287**	**345**	**363**
1α,5α-d_2	**(1-A)**	d_0	11	7	2	-	-
		d_1	32	80	98	2	-
		d_2	47	6	-	98	100
		d_3	11	-	-	-	-
1β,5α-d_2	**(1-B)**	d_0	10	12	-	-	-
		d_1	27	62	82	12	12
		d_2	53	13	18	76	67
		d_3	10	12	-	4	21
		d_4	-	-	-	8	-
2,2,4,4-d_4	**(1-C)**	d_0	-	-	-	-	-
		d_1	-	-	-	-	-
		d_2	20	6	3	4	3
		d_3	35	22	18	15	17
		d_4	45	74	79	81	80

cont'd

Table 12 (continued)

label(s) in compound		d_n	m/z of principal peaks 255	269	287	345	363
3β-d_1	(1-D)	d_0	-	3	2	2	2
		d_1	100	97	98	98	98
5α-d_1	(1-E)	d_0	49	77	83	5	3
		d_1	42	18	17	81	83
		d_3	9	5	-	15	13
6,6-d_2	(1-F)	d_0	13	5	5	-	-
		d_1	9	24	24	8	8
		d_2	78	64	66	73	73
		d_3	-	7	5	15	15
7,7-d_2	(1-G)	d_0	14	6	4	4	3
		d_1	16	41	36	4	3
		d_2	70	53	60	92	94
5α,9α,11α-d_3		d_0	12	18	21	7	7
	(1-I)	d_1	19	38	46	16	11
		d_2	37	30	26	32	36
		d_3	32	10	7	33	36
		d_4	-	3	0.4	11	10
16,16-d_2	(1-J)	d_0	14	3	-	4	-
		d_1	47	34	19	39	18
		d_2	39	63	81	57	82
17α-CD_3	(1-K)	d_0	41	-	-	-	-
		d_1	6	4	-	3	-
		d_2	18	31	3	33	-
		d_3	34	64	97	63	100

Figure 16. Positive ion mass spectra of unlabeled (**2**) and labeled (**2-A to 2-K**) 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstanes .

Figure 16 (continued)

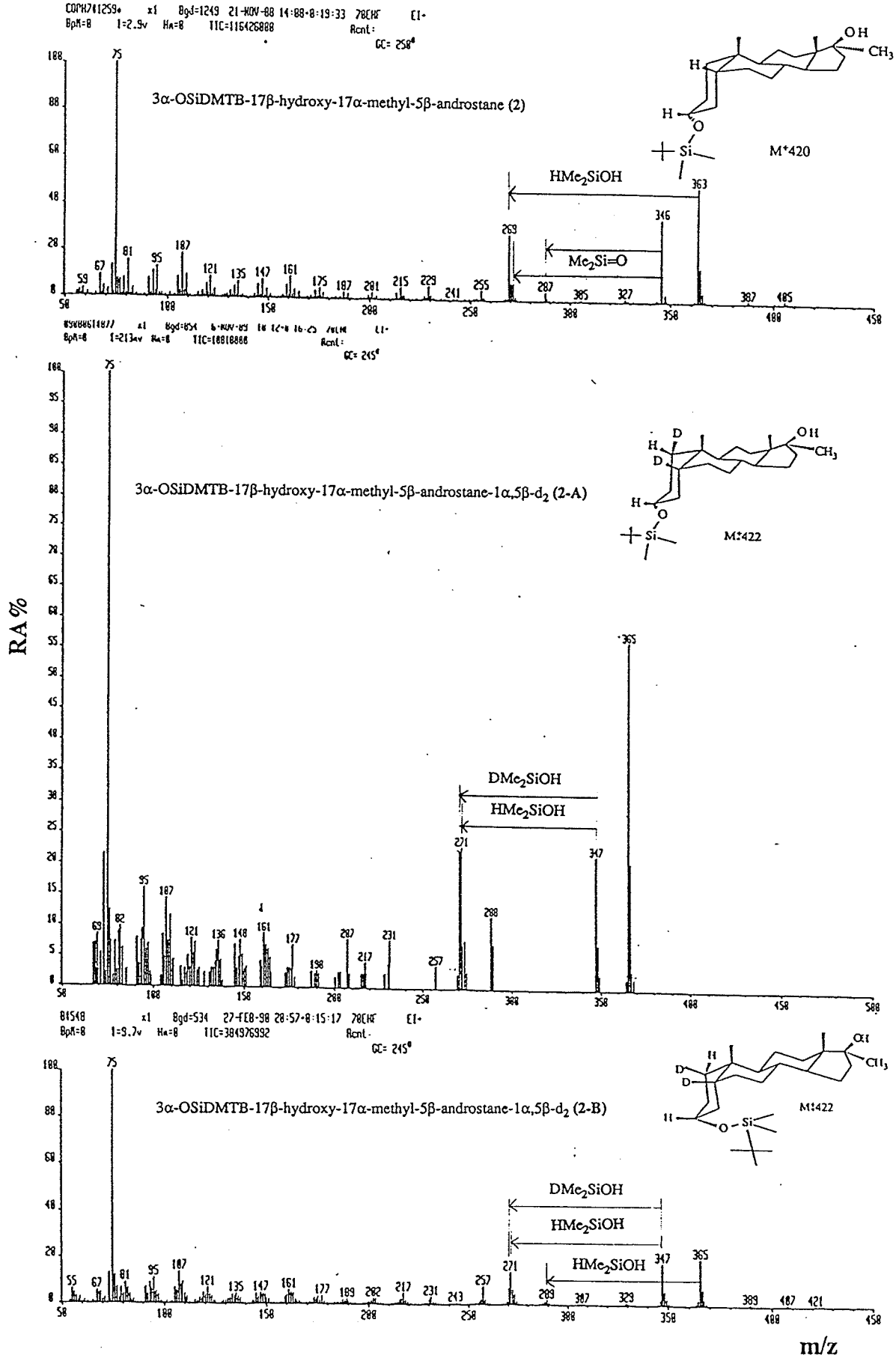


Figure 16 (continued)

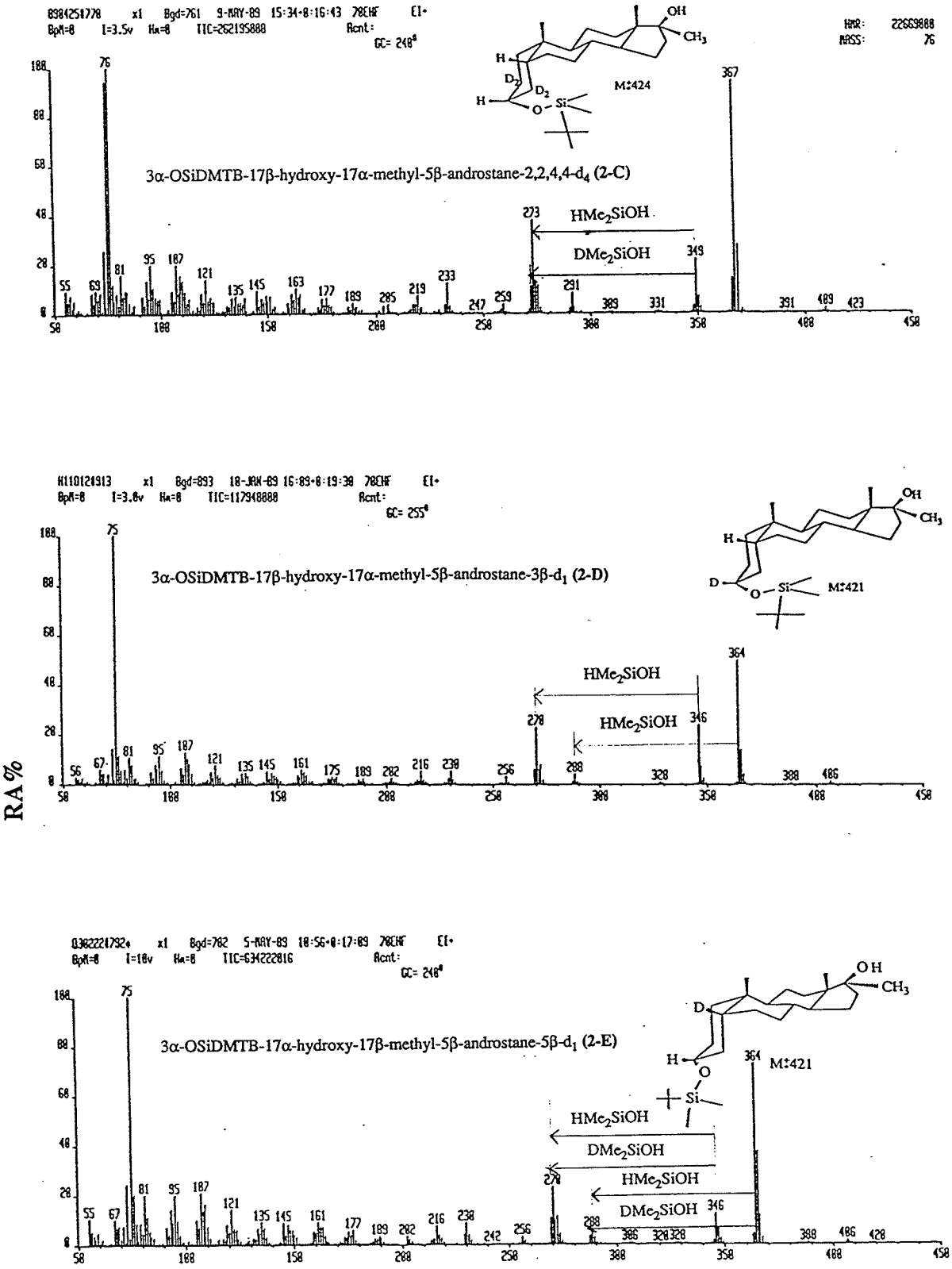


Figure 16 (continued)

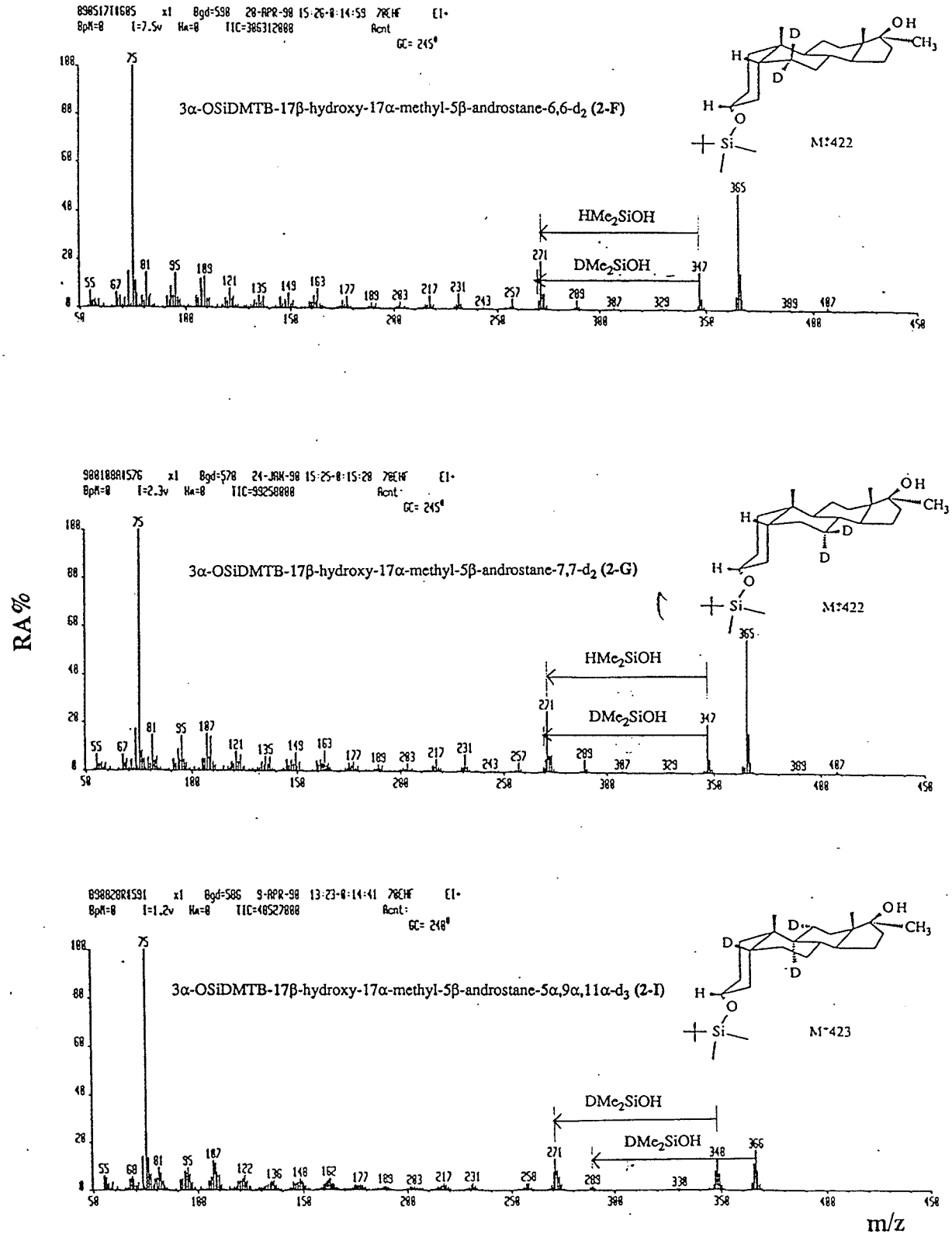


Figure 16 (continued)

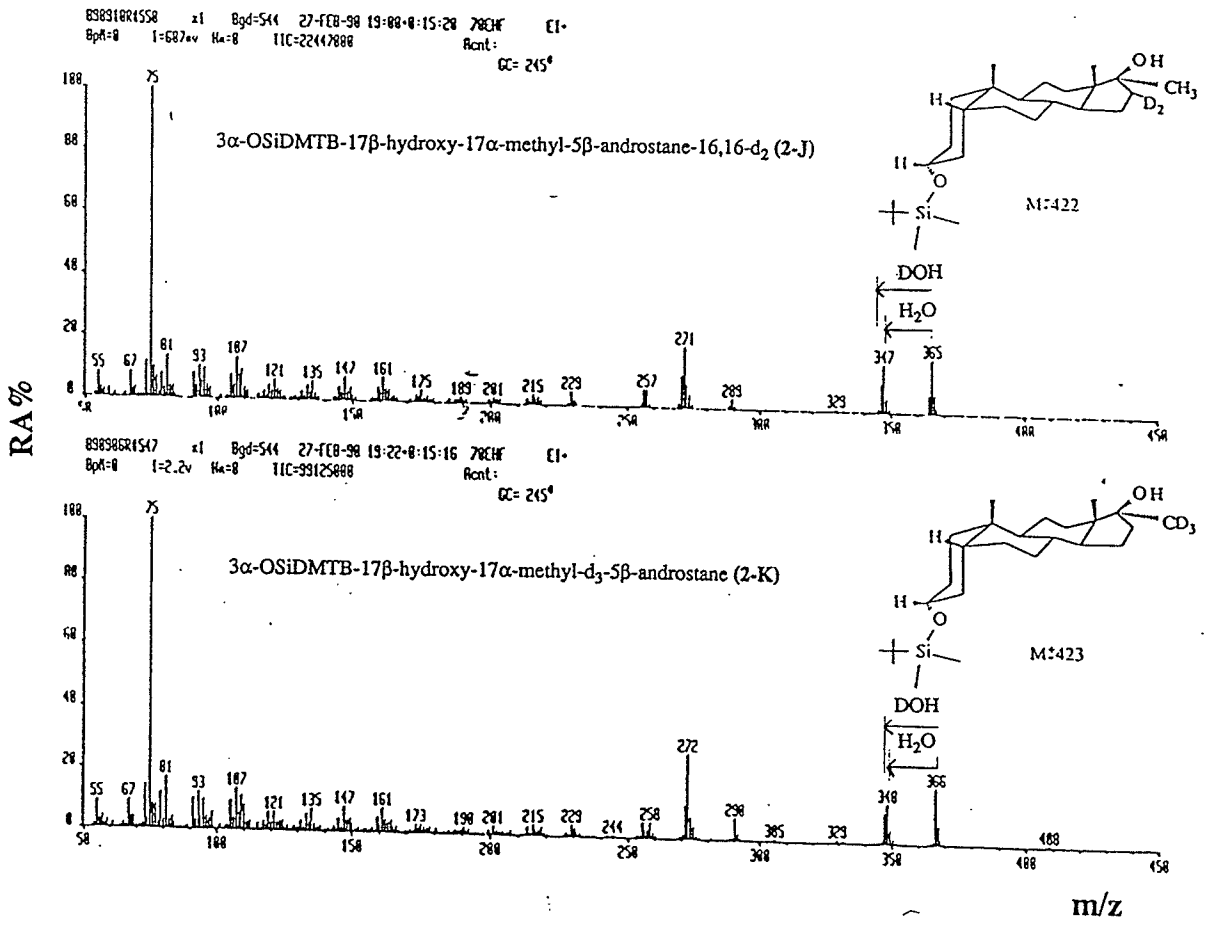
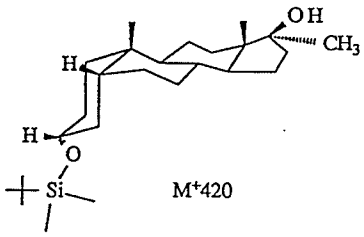


Table 13. Relative peak heights (%) for components of selected ions for deuterium labeled analogues (**2-A** to **2-K**) of 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (**2**).

label(s) in compound		FRAGMENT ION				
		$[F_5]^+$ m/z 255	$[F_4]^+$ m/z 269	$[F_3]^+$ m/z 287	$[F_2]^+$ m/z 345	$[F_1]^+$ m/z 363
 M+420		[M - CH_3 H_2O - TBDMSiOH]	$[F_3$-$H_2O]^+$ or $[F_2$ - $HSiMe_2OH]^+$	[F_1 - $HSiMe_2OH$]	[F_1 - $H_2O]^+$	[M - *t*-Bu]$^+$
		m/z/relative abundance %				
d_0	(2)	255/3.7 256/0.86	269/28.0 270/ 7.2 271/ 7.2* 272/ 1.4*	287/3.5 288/0.9	345/35.0 346/- 347/ 2.9	363/48.5 364/14.5 365/ 3.9
1α,5β-d_2	(2-A)	255/- 256/- 257/3.6	269/ 2.3 270/22.4 271/23.1 272/ 7.1 273/ 7.8* 274/ 2.6*	287/- 288/11.8 289/ 7.2	345/- 346/- 347/21.6 348/ 7.2 349/ 2.4	363/- 364/ 1.6 365/56.6 366/20.7 367/ 2.9 368/ 1.7
1β,5β-d_2	(2-B)	255/0.87 256/1.9 257/7.4 258/2.0 259/0.6	269/ 0.85 270/ 6.7 271/13.4 272/ 6.0 273/ 4.2* 274/ 1.2*	287/- 288/1.0 289/1.6 290/0.6	345/- 345/ 1.8 347/17.3 348/ 5.6	363/- 364/ 1.9 365/19.3 366/ 6.5

cont'd

Table 13 (continued)

label(s) in compound		$[F_5]^+$	$[F_4]^+$	$[F_3]^+$	$[F_2]^+$	$[F_1]^+$
		m/z/relative abundance %				
2,2,4,4-d_4	(2-C)	255/- 256/- 257/0.73 259/1.5 260/3.6 261/0.6	269/- 270/- 271/ 1.4 272/10.8 273/37.7 274/12.3 275/11.3* 276/ 2.4* 277/ 0.7*	287/- 288/- 289/- 290/1.9 291/8.1 292/2.3	345/- 346/- 347/- 348/3.0 349/21.5 350/ 6.6 351/ 2.2	363/- 364/- 365/- 366/13.7 367/93.9 368/(?) 369/27.5 370/ 1.2
3β-d_1	(2-D)	255/- 256/3.0 257/0.6	269/ 5.7 270/22.9 271/ 5.8 272/ 7.8* 273/ 1.7*	287/0.9 288/3.8 289/1.1	345/(?) 346/23.8 347/ 7.0 348/ 2.1	363/ 0.7 364/49.8 365/14.0 366/ 3.8 367/ 0.7
5β-d_1	(2-E)	255/<0.5 256/2.8 257/1.2	269/10.7 270/22.8 271/10.0 272/11.4* 273/ 4.3* 274/ 1.2*	287/3.2 288/5.7 289/2.5 290/0.7	345/ 0.85 346/12.1 347/ 6.2 348/ 2.2 349/ 0.6	363/ 4.1 364/72.6 365/37.4 366/11.7 367/ 2.7
6,6-d_2	(2-F)	255/- 256/0.9 257/3.9 258/0.9	269/- 270/ 3.5 271/19.5 272/ 6.5 273/ 6.5* 274/ 1.6*	287/- 288/0.7 289/3.6 290/1.1	345/- 346/ 1.7 347/15.0 348/ 4.7 349/1.4	363/- 364/ 5.3 365/47.8 366/14.7 367/4.2 368/ 0.7
7,7-d_2	(2-G)	255/0.66 256/0.66 257/3.6 258/0.8	269/ 1.8 270/ 5.0 271/24.7 272/ 6.7 273/ 6.8* 274/ 1.3*	287/- 288/0.67 289/4.8 290/1.3	345/ 1.0 346/ 0.9 347/19.7 348/ 5.4 349/ 1.6	363/ 2.7 364/ 2.4 365/54.9 366/16.0 367/ 4.2 368/ 0.6

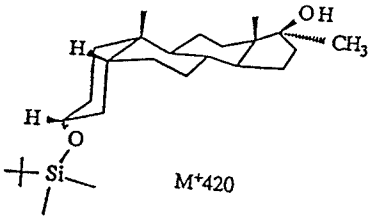
cont'd

Table 13 (continued)

label(s) in compound		$[F_5]^+$	$[F_4]^+$	$[F_3]^+$	$[F_2]^+$	$[F_1]^+$
		m/z/relative abundance %				
5β,9α,11α-d_3	(2-I)	255/0.8 256/1.4 257/2.7 258/2.6 259/1.2 260/0.5	269/ 5.3 270/18.1 271/21.9 272/12.1 273/ 6.5 274/ 3.1* 275/ 1.3*	287/1.7 288/4.1 289/5.0 290/2.0 291/0.6	345/0.8 346/3.4 347/8.3 348/9.3 349/4.3 350/1.2 351/0.4	363/ 2.4 364/11.9 365/29.9 366/34.2 367/14.5 368/ 4.3 369/ 0.7
16,16-d_2	(2-J)	255/0.98 256/5.5 257/5.5 257/1.0	269/ 1.4 270/10.4 271/19.2 272/ 7.3 273/ 4.1* 274/ 0.9*	287/- 288/0.98 289/3.0 290/0.8	345/ 0.9 346/ 8.9 347/15.2 348/ 4.2 367/ 1.3	363/- 364/ 5.7 365/16.9 366/ 5.7
17α-CD_3	(2-K)	255/4.9 256/2.6 257/2.7 258/4.9 259/1.6 260/0.5	269/ 0.57 270/ 1.3 271/10.5 272/27.2 273/ 7.1 274/ 4.0* 275/ 1.0* 276/ 0.5*	287/- 288/- 289/0.5 290/6.7 291/1.9	345-/ 346/ 0.6 347/ 9.6 348/12.6 349/ 4.3 350/ 1.2	363/- 364/- 365/ 0.8 366/18.0 367/ 5.9 368/ 1.4

* could include contribution from $[M - t\text{-Bu} - H_2O - Me_2SiO]^+ = [M - 149]^+$ and $[M - 150]^+$

Table 14. Deuterium content (%) of fragment ions for deuterium labeled 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (**2-A** to **2-K**).

label(s) in compound			FRAGMENT ION				
			$[F_5]^+$	$[F_4]^+$	$[F_3]^+$	$[F_2]^+$	$[F_1]^+$
 M⁺420			$[M - CH_3 - H_2O - TBDMSiOH]$	$[F_3\text{-}H_2O]^+$ or $[F_1 - H_2O - HSiMe_2OH]^+$	$[F_1 - HSiMe_2OH]$	$[F_1 - H_2O]^+$	$[M - t\text{-}Bu]^+$
			m/z of principal peaks				
		d_n	**255**	**269**	**278**	**345**	**363**
(1)		d_0	100	100	100	100	100
			-	-	-	-	-
1α,5β-d_2	(2-A)	d_0	-	5	-	-	-
		d_1	-	52	43	-	-
		d_2	100	43	57	97	100
		d_3	-	-	-	3	-
1β,5β-d_2	(2-B)	d_0	9	4.4	-	-	-
		d_1	18	34	43	10	10
		d_2	73	62	57	90	90
2,2,4,4-d_4	(2-C)	d_0	-	-	-	-	-
		d_1	-	-	-	-	-
		d_2	14	2.8	-	-	-
		d_3	25	20	20	13	7
		d_4	61	69	80	87	93
		d_5		8			
3β-d_1	(2-D)	d_0	-	21	21	-	1
		d_1	100	79	79	100	99
5β-d_1	(2-E)	d_0	-	30	33	5	5
		d_1	83	56	53	75	78
		d_2	7	14	14	16	17

cont'd

Table 14 (continued)

label(s) in compound			m/z of principal peaks				
		d_n	255	269	278	345	363
6,6-d_2	(2-F)	d_0	-	-	-	-	-
		d_1	20	16	17	10	10
		d_2	80	84	83	90	89
7,7-d_2	(2-G)	d_0	-	6	-	5	4
		d_1	16	16	13	3	3
		d_2	84	84	87	92	93
5β,9α,11α-d_3		d_0	12	10	16	4	3
	(2-I)	d_1	19	32	35	16	16
		d_2	37	34	38	37	38
		d_3	32	15	9	35	36
		d_4	-	8	2	8	6
16,16-d_2	(2-J)	d_0	9	5	-	4	-
		d_1	50	36	19	39	27
		d_2	41	59	81	57	73
17α-CD_3	(2-K)	d_0	37	1	-	-	-
		d_1	12	3	-	-	-
		d_2	17	28	-	50	-
		d_3	33	68	100	50	100

Figure 17. Positive EI mass spectra of unlabeled (**3**) and deuterium-labeled (**3-A** to **3-K**) 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstanes. (The m/z 207 peak, in the spectrum of **3-A**, arises from column bleed)

Figure 17 (continued)

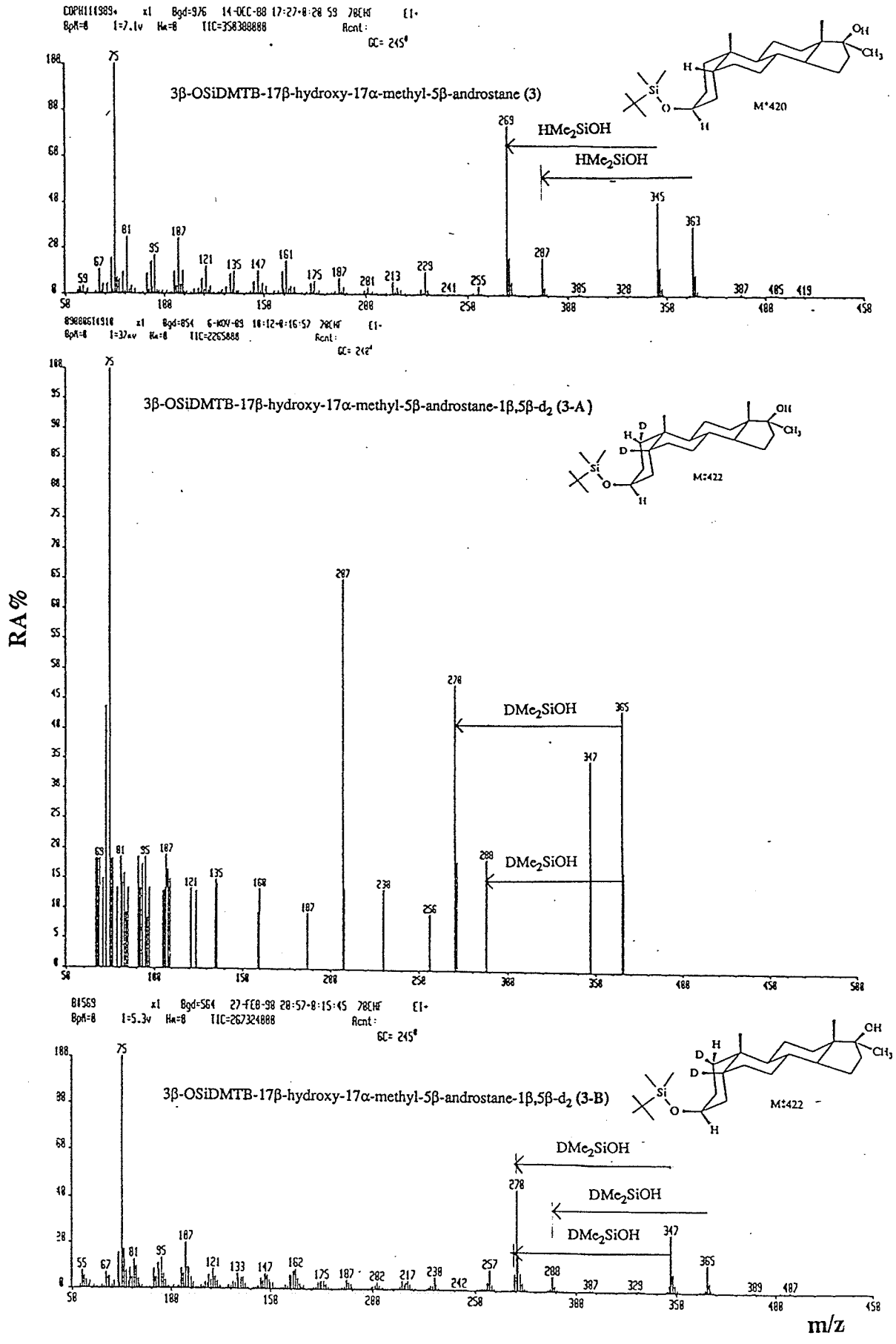


Figure 17 (continued)

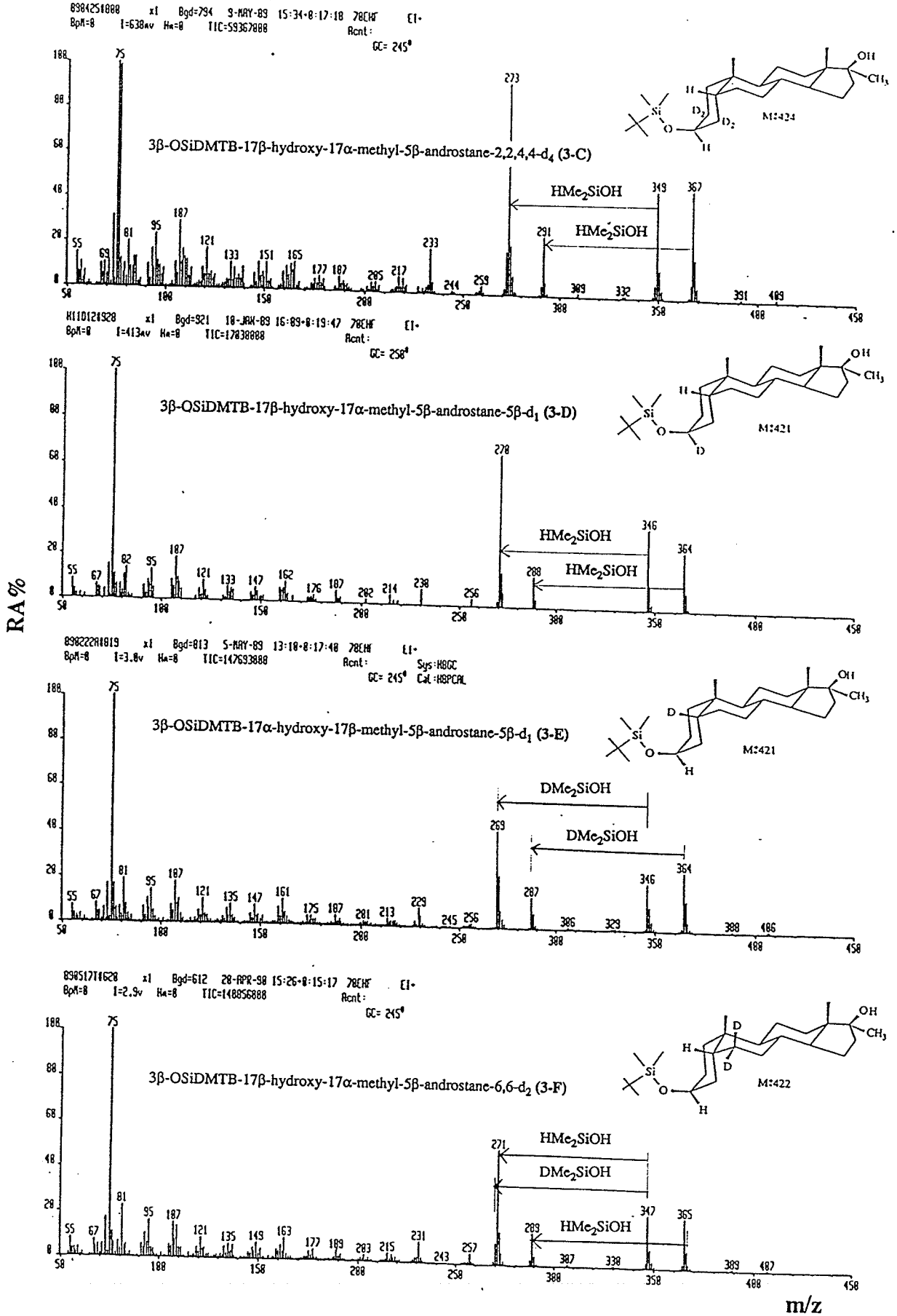


Figure 17 (continued)

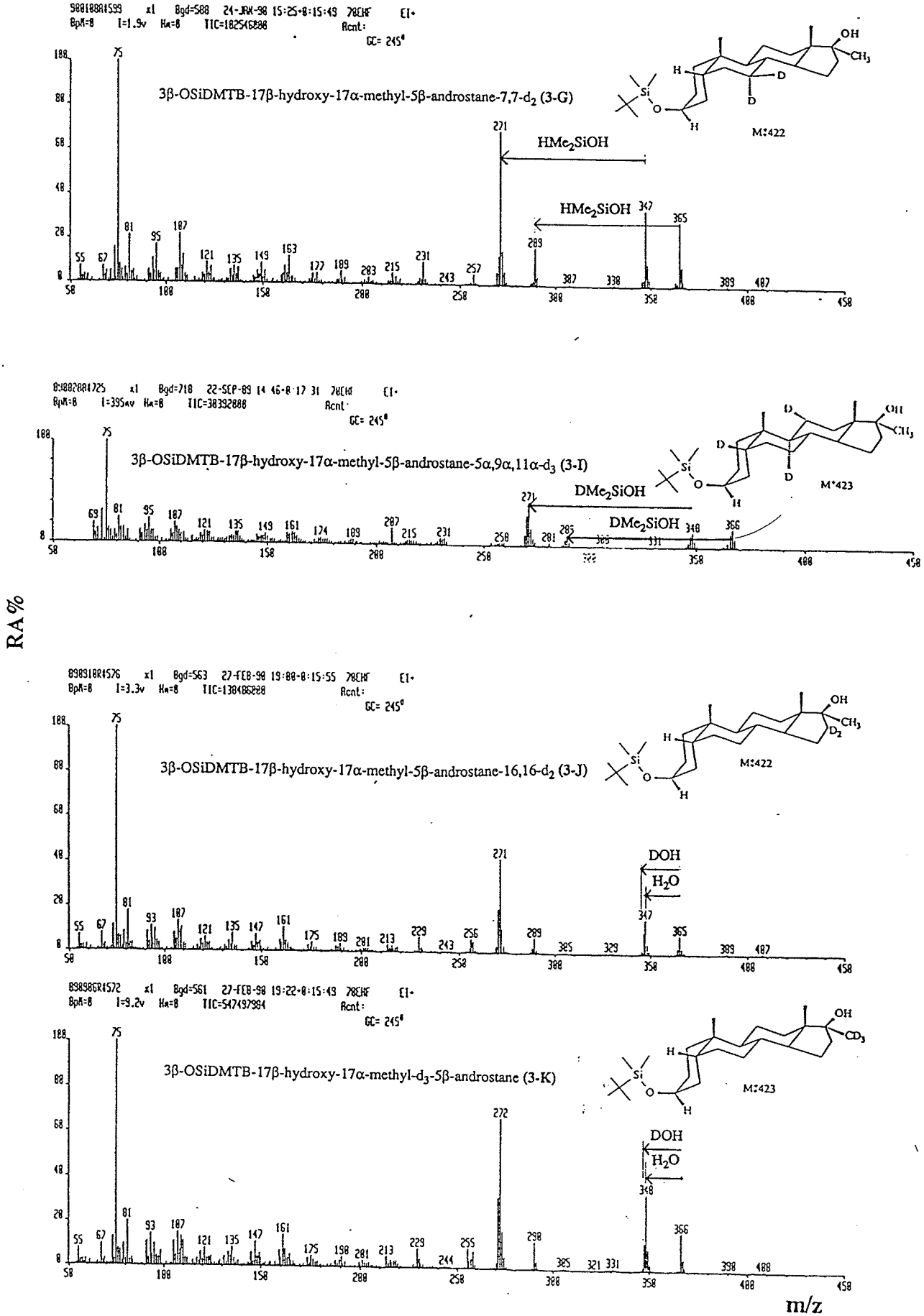
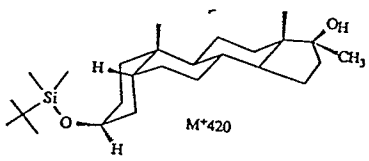


Table 15. Relative abundance (%) in peak clusters for selected ions of deuterium-labeled analogues (**3-A** to **3-K**) of 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (**3**).

label(s) in compound		FRAGMENT ION				
		$[F_5]^+$	$[F_4]^+$	$[F_3]^+$	$[F_2]^+$	$[F_1]^+$
		$[M - CH_3$ H_2O - TBDMSiOH]	$[F_3 - H_2O$ or $[F_2$ - $HSiMe_2OH]^+$	$[F_1$ - $HSiMe_2OH]$	$[F_1$ - $H_2O]^+$	$[M$ - *t*-Bu$]^+$
		m/z/relative abundance %				
d_0	(3)	255/3.5 256/0.6	269/73.9 270/16.0 271/ 5.5 272/ 0.7	287/15.9 288/ 3.2 289/< 0.5	345/40.5 346/11.9 347/ 3.3	363/29.8 364/ 8.7 365/ 2.1
$1\alpha,5\beta\text{-}d_2$	(3-A)	255/- 256/9.3	269/- 270/47.8 271/18.2	287/- 288/18.6	345/- 346/- 347/35.2	363/- 364/- 365/43.7 366/16.6
$1\beta,5\beta\text{-}d_2$	(3-B)	255/0.7 256/3.3 257/8.6 258/2.3 259/0.7	269/ 9.0 270/45.5 271/13.3 272/ 5.3 273/ 2.0 274/ 0.4	287/0.7 288/5.2 289/1.4 290/0.2	345/ 0.7 346/ 2.8 347/22.5 348/ 7.0 349/ 2.2 350/ 0.6	363/- 364/1.0 365/7.4 366/2.5 367/0.6

cont'd

Table 15 (continued)

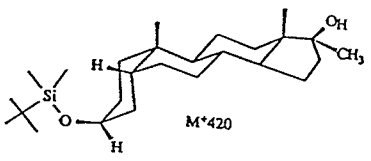
label(s) in compound		$[F_5]^+$	$[F_4]^+$	$[F_3]^+$	$[F_2]^+$	$[F_1]^+$
		m/z/relative abundance				
2,2,4,4-d_4	(3-C)	255/-	269/-	287/-	345-/	363/-
		256/1.0	270/ 0.6	288/-	346/-	364/-
		257/0.9	271/ 2.9	289/ 0.5	347/ 1.1	365/ 1.0
		258/1.7	272/18.9	290/ 4.1	348/ 5.9	366/ 6.4
		259/3.6	273/94.2	291/26.0	349/47.4	367/47.9
		260/0.9	274/21.9	292/ 5.7	350/12.4	368/17.6
			275/ 8.4	293/ 0.9	351/ 3.6	369/ 5.0
			276/ 1.6		352/ 0.8	370/ 0.5
3α-d_1	(3-D)	255/-	269/ 2.2	287/-	345/-	363/-
		256/2.95	270/66.8	288/13.5	346/35.6	364/26.0
			271/15.0	289/ 3.1	347/11.0	365/ 7.6
			272/ 6.1		348/ 2.2	366/ 1.0
5β-d_1	(3-E)	255/1.8	269/42.6	287/13.6	345/ 0.9	363/ 1.2
		256/1.5	270/22.7	288/ 6.4	346/20.5	364/25.8
		257/0.7	271/ 7.4	289/ 1.4	347/10.3	365/13.1
			272/ 3.8		348/ 3.7	366/ 2.9
			273/ 1.5		349/ 0.9	367/ 0.9
6,6-d_2	(3-F)	255/0.6	269/ 1.2	287/-	345/-	363/-
		256/0.9	270/ 9.4	288/ 2.3	346/ 2.4	364/ 1.9
		257/4.5	271/50.8	289/14.0	347/23.3	365/22.0
		258/1.0	272/14.4	290/ 3.9	348/ 8.0	366/ 7.7
			273/ 5.2	291/ 0.5	349/ 2.6	367/ 2.0
			274/ 1.5		350/ 0.6	368/ 0.6
7,7-d_2	(3-G)	255/0.8	269/ 5.5	287/ 1.3	345/ 2.6	363/ 2.2
		256/0.7	270/12.9	288/ 1.8	346/ 2.8	364/ 1.7
		257/4.4	271/69.0	289/16.6	347/34.1	365/29.1
		258/0.9	272/15.2	290/ 3.4	348/ 9.8	366/ 8.5
			273/ 5.7		349/ 2.8	367/ 2.3
			274/ 1.1			

cont'd

Table 15 (continued)

label(s) in compound		$[F_5]^+$	$[F_4]^+$	$[F_3]^+$	$[F_2]^+$	$[F_1]^+$
		m/z/ relative abundance %				
5β,9α,11α-d_3		255/1.5	269/10.5	287/ 1.8	345/ 1.5	363/ 0.9
	(3-I)	256/1.7	270/30.1	288/ 6.6	346/ 4.4	364/ 3.9
		257/2.0	271/42.8	289/11.0	347/11.3	365/13.6
		258/2.2	272/17.3	290/ 4.2	348/13.8	366/17.9
		259/1.8			349/ 6.2	367/ 8.3
16,16-d_2	(3-J)	255/1.5	269/ 2.5	287/-	345/ 1.3	363/-
		256/5.7	270/19.4	288/2.2	346/ 9.2	364/ 2.6
		257/4.5	271/41.4	289/6.4	347/15.0	365/ 8.0
		258/1.0	272/10.2	290/1.3	348/ 4.3	366/ 2.5
			273/ 3.0		349/ 1.2	367/ 0.6
			274/ 0.6			
17α-CD_3	(3-K)	255/8.8	269/ 0.6	287/-	345/-	363/-
		256/3.1	270/ 2.6	288/-	346/ 1.3	364/-
		257/4.3	271/31.7	289/ 0.5	347/12.3	365/ 0.7
		258/7.4	272/67.2	290/12.4	348/34.1	366/16.8
		259/1.7	273/16.7	291/ 3.1	349/ 9.4	367/ 5.0
			274/ 5.1		350/ 2.4	368/ 1.3

Table 16. Deuterium content (%) of fragment ions from deuterium-labeled 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (**3-A** to **3-K**).

label(s) in compound			FRAGMENT ION				
			$[F_5]^+$	$[F_4]^+$	$[F_3]^+$	$[F_2]^+$	$[F_1]^+$
			$[F_2$ - $[M - CH_3$ H_2O - TBDMSiOH]	$[F_1$ - $HSiMe_2OH]$ or $[F_3- H_2O]^+$	$[F_1$ - $HSiMe_2OH]$	$[M$ - $H_2O]^+$	$[M$ - t-Bu$]^+$
			m/z of principal peaks				
		d_n	**255**	**269**	**287**	**345**	**363**
1α,5β-d_2	(3-A)	d_0	-	-	-	-	-
		d_1	100	87	100	-	-
		d_2	-	13	-	100	93
		d_3	-	-	-	-	7
1β,5β-d_2	(3-B)	d_0	12	16	13	3	-
		d_1	18	84	87	10	12
		d_2	70	-	-	84	84
		d_3	-	-	-	4	4
2,2,4,4-d_4	(3-C)	d_o	-	-	-	-	-
		d_1	-	-	-	-	-
		d_2	16	2	2	2	2
		d_3	27	16	13	11	11
		d_4	57	76	85	87	82
		d_5	-	5	-	-	6
3α-d_1	(3-D)	d_0	-	-	-	-	-
		d_1	100	100	100	100	100
		d_2	-	-	-	-	-
		d_3	-	-	-	-	-

cont'd

Table 16 (continued)

label(s) in compound		d_n	m/z of principal peaks 255	269	287	345	363
5β-d_1	(3-E)	d_0	60	79	81	4	4
		d_1	40	21	19	80	79
		d_2	-	-	-	16	16
		d_3	-	-	-	-	-
6,6-d_2	(3-F)	d_0	10	2	-	-	-
		d_1	14	14	15	9	8
		d_2	76	74	85	91	88
		d_3	-	5	-	-	4
		d_4	-	5	-	-	-
7,7-d_2	(3-G)	d_0	-	7	6	7	7
		d_1	15	14	8	5	3
		d_2	75	79	85	88	90
		d_3	-	-	-	-	-
5β,9α,11α-d_3	(3-I)	d_0	19	12	9	5	3
		d_1	18	31	32	14	12
		d_2	22	41	49	36	40
		d_3	23	9	9	37	45
		d_4	18	-	-	-	-
16,16-d_2	(3-J)	d_0	15	4	-	6	-
		d_1	53	31	27	39	26
		d_2	32	60	73	55	74
		d_3	-	2	-	-	-
		d_4	-	3	-	-	-
17α-CD_3	(3-K)	d_0	43	-	-	-	-
		d_1	6	2	-	3	-
		d_2	18	30	4	27	4
		d_3	33	58	96	70	96
		d_4	-	2	-	-	-
		d_5	-	6	-	-	-

Figure 18. Positive ion mass spectra of unlabeled **(4)** and deuterium-labeled **(4-A** to **4-K)** 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane.

Figure 18

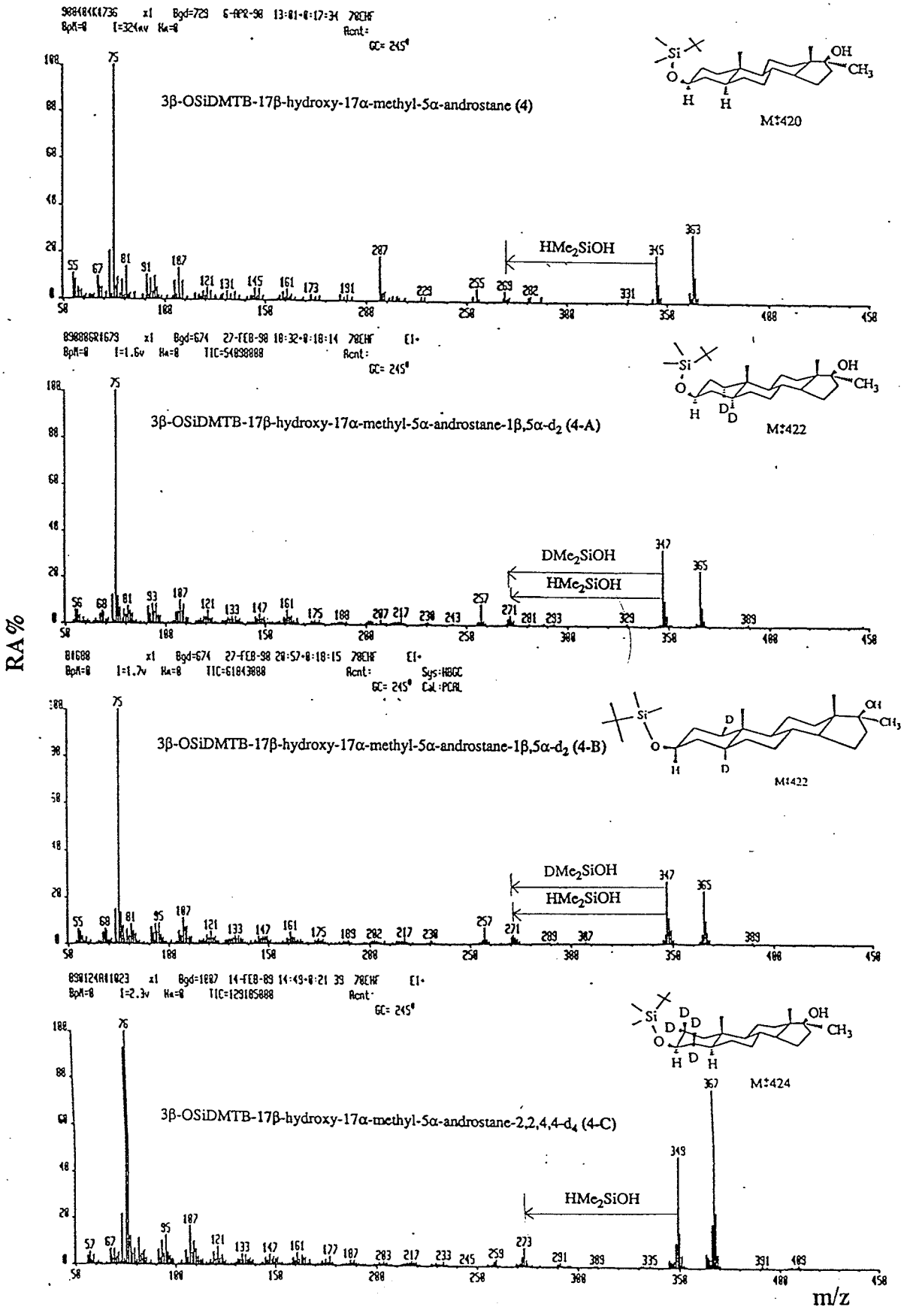

3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane (4)
M+420
HMe2SiOH
3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-1β,5α-d2 (4-A)
M+422
DMe2SiOH
HMe2SiOH
3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-1β,5α-d2 (4-B)
M+422
DMe2SiOH
HMe2SiOH
3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane-2,2,4,4-d4 (4-C)
M+424
HMe2SiOH
RA %
m/z

Figure 18 (continued)

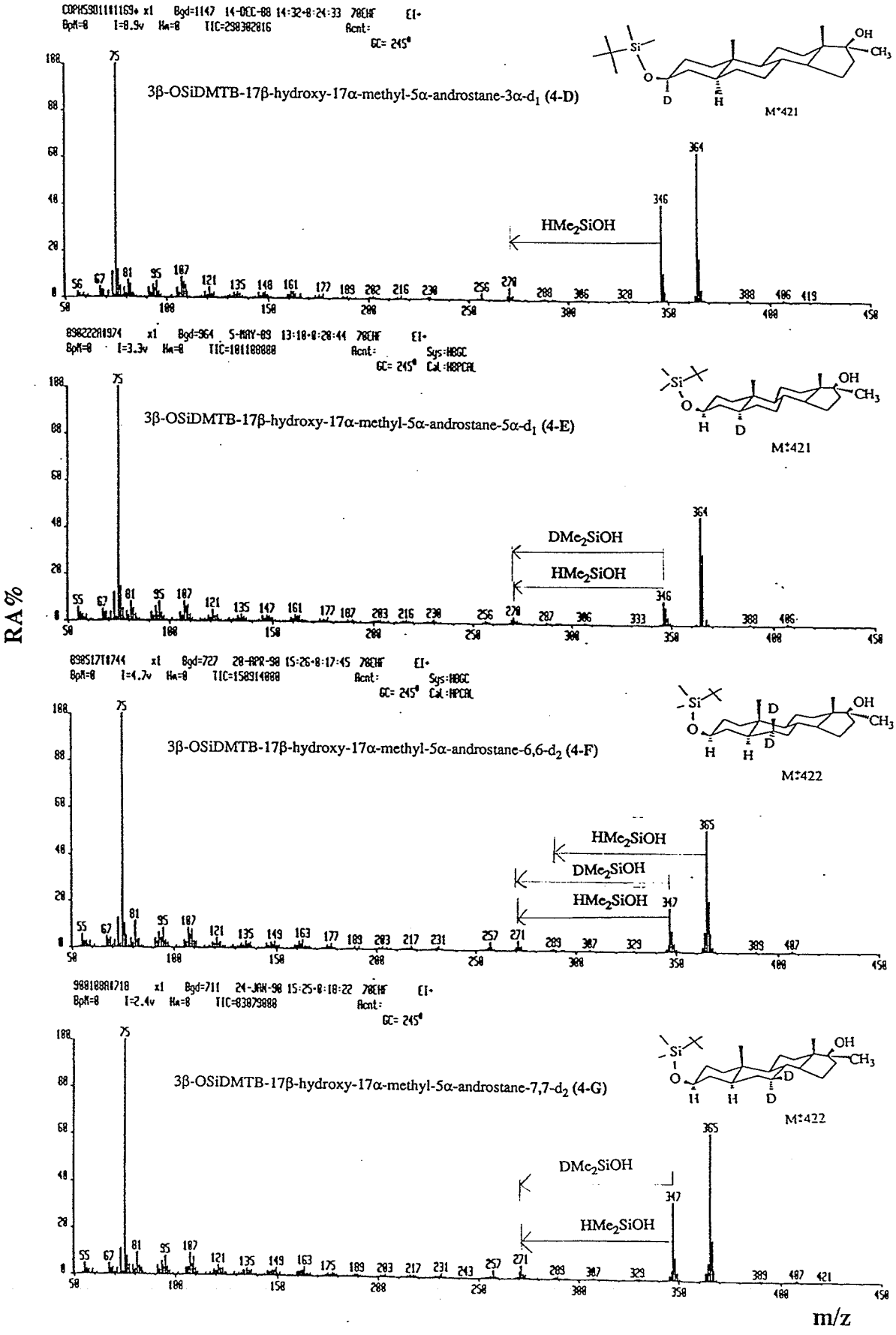
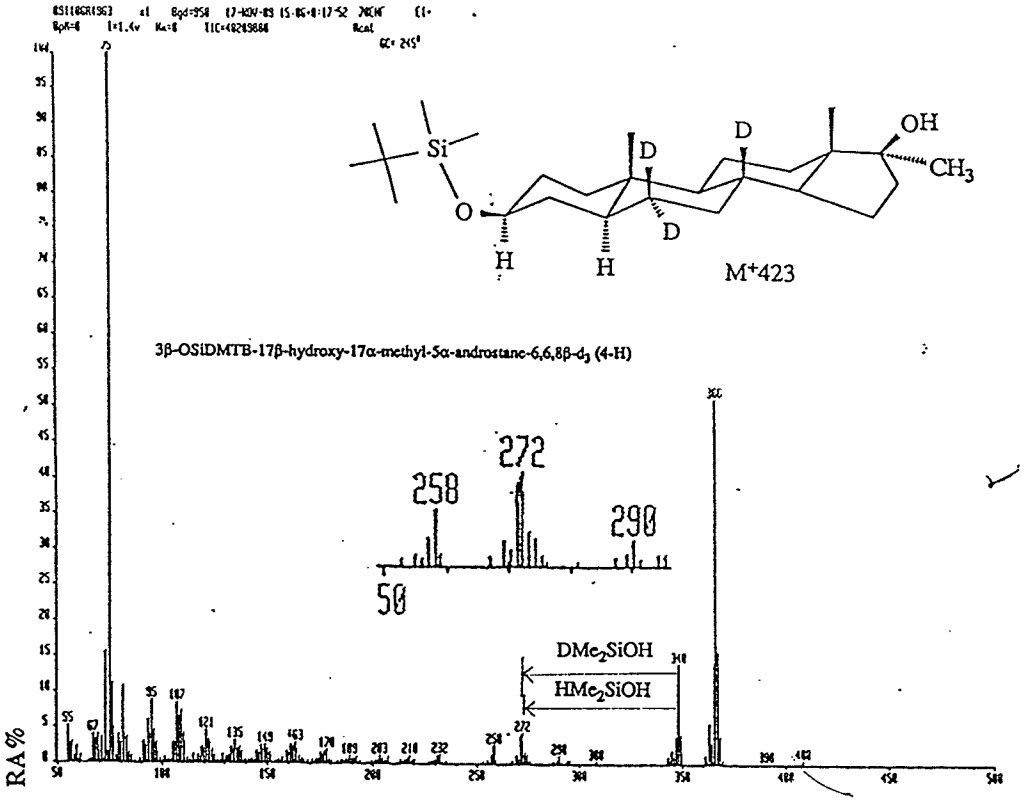


Figure 18 (continued)



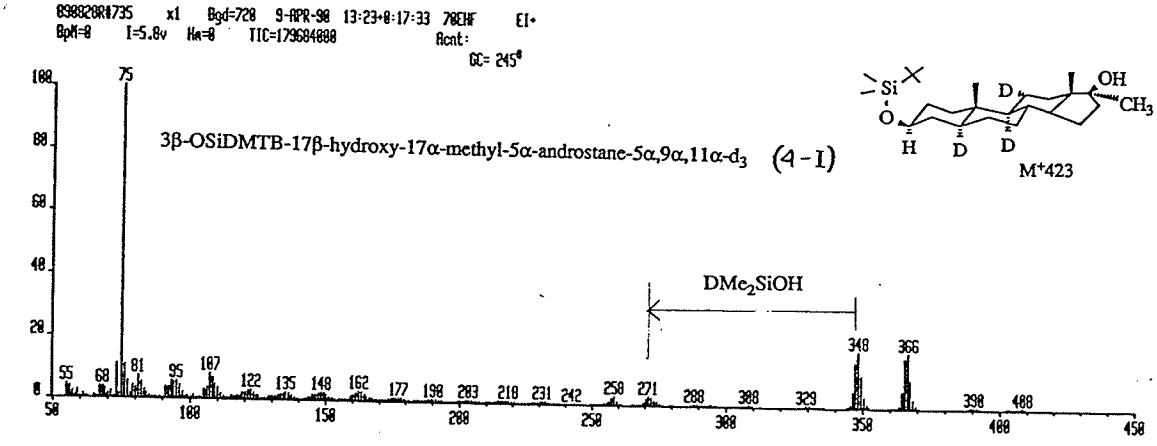


Figure 18 (continued)

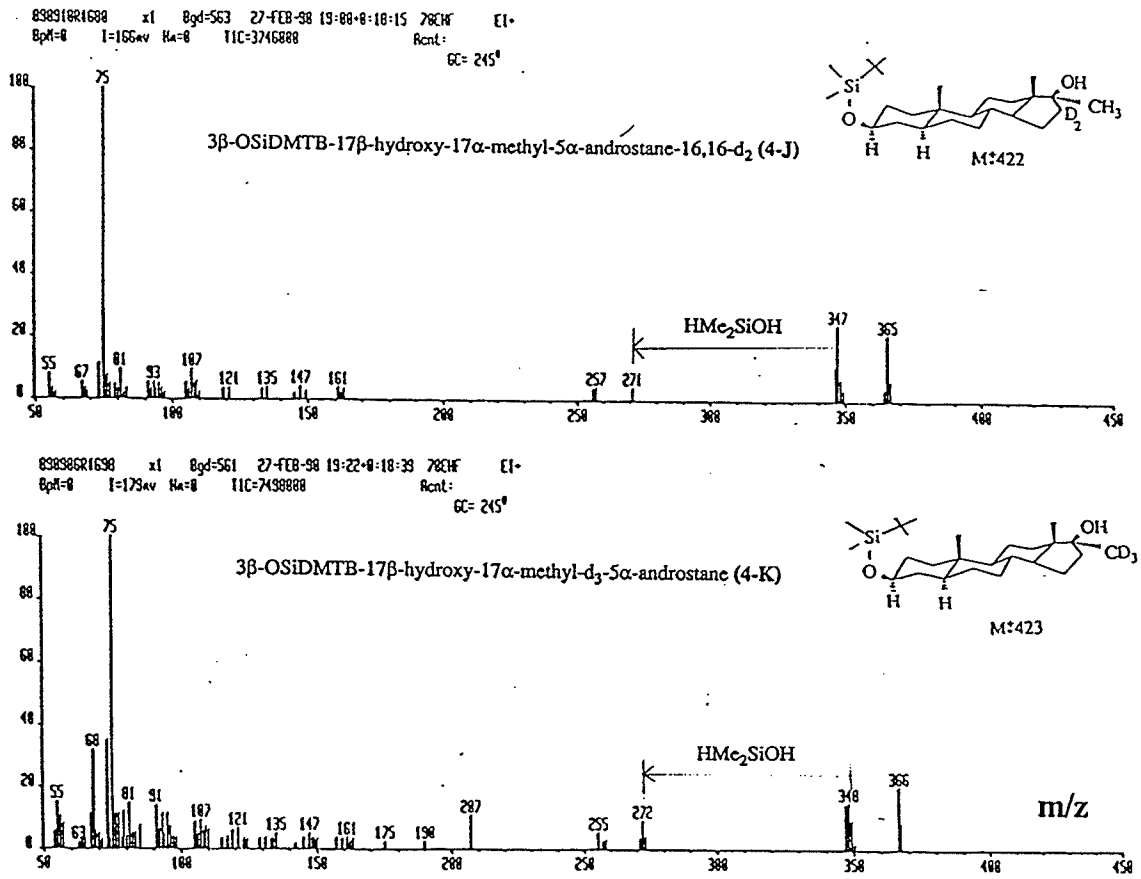
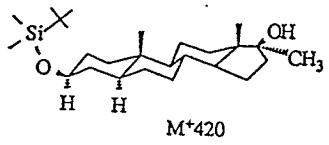


Table 17. Relative abundance (%) in peak clusters for selected ions of deuterium-labeled analogues of 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane (**4**).

label(s) in compound		FRAGMENT ION				
		$[F_5]^+$	$[F_4]^+$	$[F_3]^+$	$[F_2]^+$	$[F_1]^+$
 M⁺420		[M - CH_3 H_2O - TBDMSiOH]	[F_3 - H_2O]$^+$ or [F_2 - $HSiMe_2OH$]$^+$	[F_1 - $HSiMe_2OH$]	[F_1 - H_2O]$^+$	[M - *t*-Bu]$^+$
		m/z/relative abundance %				
d_0	(4)	255/5.2	269/4.2	287/1.9	345/19.8	363/29.0
		256/1.1	270/1.2*		346/ 7.8	364/11.0
			271/1.7*		347/ 2.1	365/ 2.1
1α,5α-d_2	(4-A)	255/0.8	269/-	287/>0.5	345/ 0.7	363/ 1.2
		256/1.1	270/2.5		346/ 0.7	364/ 0.8
		257/8.6	271/4.0		347/32.6	365/23.5
		258/1.7	272/2.3*		348/10.7	366/ 7.6
		259/0.6	273/2.0*		349/ 4.1	367/ 2.1
					350/ 0.5	
1β,5α-d_2	(4-B)	255/0.8	269/-	287/>0.5	345/ 1.7	363/ 1.0
		256/1.7	270/2.9		346/ 3.9	364/ 3.8
		257/6.9	271/3.7		347/26.5	365/22.6
		259/1.9	272/2.7*		348/10.8	366/ 9.6
		260/1.1	273/1.9*		349/ 5.8	367/ 4.6
			274/0.9*		350/ 1.5	368/ 1.7

cont'd

Table 17 (continued)

label(s) in compound		$[F_5]^+$	$[F_4]^+$	$[F_3]^+$	$[F_2]^+$	$[F_1]^+$
		m/z/relative abundance %				
2,2,4,4-d_4	(4-C)	255/-	269/0.9	287/-	345/ 2.8	363/ 5.7
		256/-	270/0.6	288/-	346/ 2.8	364/ 4.6
		257/0.5	271/1.1	289/-	347/ 2.5	365/ 3.6
		258/1.9	272/3.7	290/0.6	348/10.3	366/18.4
		259/2.5	273/7.7	291/1.4	349/47.3	367/76.1
			274/2.6*		350/14.5	368/23.1
			275/1.4*		351/ 5.0	369/ 5.5
			276/0.6*		352/ 1.1	370/ 1.1
3α-d_1	(4-D)	255/-	269/1.5	287/-	345/-	363/ 2.7
		256/2.5	270/5.1	288/0.8	346/41.0	364/63.4
		257/0.5	271/1.5*		347/12.0	365/18.5
			272/1.6*		348/ 3.82	366/ 4.7
					349/ 0.78	367/ 0.8
5α-d_1	(4-E)	255/1.1	269/1.7	287/0.5	345/-	363/-
		256/0.6	270/2.6		346/10.0	364/46.0
			271/1.4*		347/7.3	365/30.3
			272/1.3*		348/3.1	366/-
			273/0.7*		349/1.0	367/ 2.7
6,6-d_2	(4-F)	255/-	269/-	287/-	345/ 0.9	363/ 1.9
		256/0.9	270/1.4	288/-	346/ 3.0	364/ 8.3
		257/3.3	271/3.8	289/0.6	347/18.4	365/51.8
		258/1.0	272/1.6*		348/ 6.8	366/21.4
			273/1.5*		349/ 3.3	367/ 7.7
					350/ 0.8	368/ 1.9
7,7-d_2	(4-G)	255/0.6	269/0.7	287/-	345/ 2.0	363/ 3.5
		256/0.7	270/3.0	288/0.7	346/ 4.1	364/ 7.6
		257/3.1	271/5.6	289/0.9	347/33.4	365/63.0
		258/0.7	272/1.8*		348/ 9.5	366/17.4
			273/1.5*		349/ 3.4	367/ 4.4
					350/ 0.6	368/ 0.7

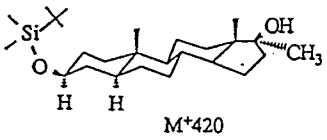
cont'd

Table 17 (continued)

label(s) in compound		$[F_5]^+$	$[F_4]^+$	$[F_3]^+$	$[F_2]^+$	$[F_1]^+$
		m/z/relative abundance %				
6,6,8β-d_3	(4-H)	255/-	269/<0.5	287/>0.5	345/ 1.9	363/ 5.7
		256/-	270/0.7		346/ 1.1	364/ 2.9
		257/1.2	271/3.2		347/ 3.9	365/14.1
		258/2.5	272/4.2		348/13.9	366/51.0
			273/1.5*		349/ 4.1	367/15.8
			274/1.2*		350/ 1.5	368/ 4.0
						369/ 0.5
5,9,11α-d_3	(4-I)	255/0.4	269/3.3	287/0.9	345/ 1.0	363/ 2.9
		256/1.9	270/6.8	288/2.4	346/ 6.3	364/19.0
		257/2.7	271/6.4	289/1.4	347/13.3	365/42.2
		258/2.6	272/4.0	290/0.6	348/13.3	366/44.1
		259/1.0	273/2.4*		349/ 6.3	367/21.3
		260/0.4	274/1.4*		350/ 2.2	368/ 6.1
			275/0.4*		351/ 0.5	369/ 1.4
			276/0.3*			370/ 0.3
16,16-d_2	(4-J)	255/-	269/-	287/<0.5	345/-	363/-
		256/3.6	270/-		346/10.7	364/3.7
		257/4.0	271/4.0		347/25.0	365/21.0
					348/6.6	366/ 6.2
					349/3.1	367/ 6.2
17α-CD_3	(4-K)	255/5.1	269/-	287/<0.5	345/-	363/-
		256/2.1	270/-		346/-	364/-
		257/2.6	271/3.5		347/14.4	365/-
		258/3.0	272/8.9		348/14.9	366/19.8
			273/4.0*		349/ 9.3	367/ 8.5
					350/ 1.7	

* could include contribution from [M - 149] or $[M - 150]^+$

Table 18. Deuterium content (%) of fragment ions from deuterium-labeled 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane (**4**).

label(s) in compound		FRAGMENT ION					
			$[F_5]^+$	$[F_4]^+$	$[F_3]^+$	$[F_2]^+$	$[F_1]^+$
 M+420			[M - CH_3 - H_2O TBDMSiOH]	[F_2 - H_2O]$^+$ or [F_2 - $HSiMe_2OH$]$^+$	[F_3 - $HSiMe_2OH$] or	[F_1 - H_2O]$^+$	[M - *t*-Bu]$^+$
			m/z of principal peaks				
		d_n	255	269	278	345	363
d_0	(4)	100	70	100	100	100	
1α,5α-d_2	(4-A)	d_0	-	-	-	2	5
		d_1	12	43	-	1	2
		d_2	88	57	-	91	91
		d_3	-		-	2	2
		d_4			-	4	-
1β,5α-d_2	(4-B)	d_0	-	-	-	5	3
		d_1	21	50	-	9	11
		d_2	79	50	-	70	68
		d_3	-	-	-	8	9
		d_4	-	-	-	8	6
		d_5	-	-	-		2
2,2,4,4-d_4	(4-C)	d_0	-	7	-	5	6
		d_1	-	3	-	3	3
		d_2	-	7	-	3	2
		d_3	47	26	-	16	18
		d_4	53	50	-	73	71
				7	-	-	-
3α-d_1	(4-D)	d_0	-	24	-	-	4
		d_1	-	76	-	99	96
		d_2	-		-	-	-
					-	1	-

cont'd

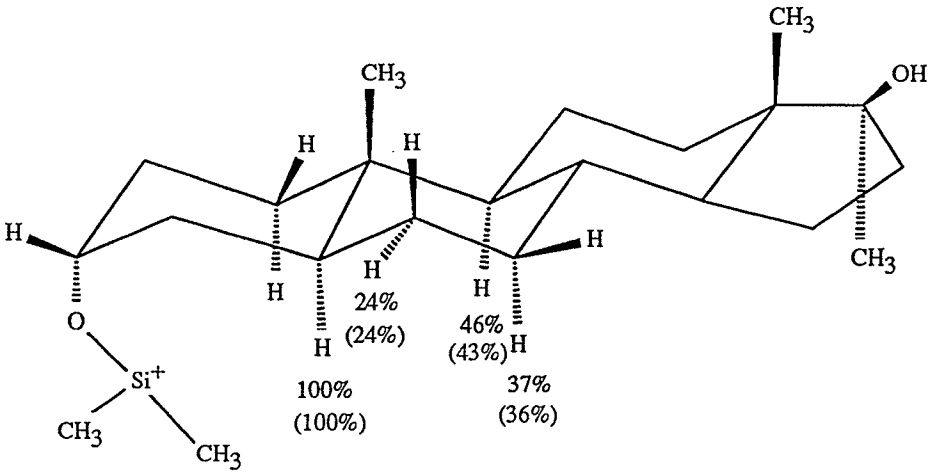
Table 18 (continued)

label(s) in compound		d_n	m/z of principal peaks $[F_5]^+$ 255	$[F_4]^+$ 269	$[F_3]^+$ 278	$[F_2]^+$ 345	$[F_1]^+$ 363
5α-d_1	(4-E)	d_0	-	42	-	-	-
		d_1	100	58	-	70	74
		d_2	-		-	30	26
6,6-d_2	(4-F)	d_0	-	-	-	4	3
		d_1	23	28	-	11	11
		d_2	77	72	-	74	74
		d_3	-		-	5	8
		d_4	-		-	6	3
7,7-d_2	(4-G)	d_0	-	8	-	5	5
		d_1	19	34	49	9	9
		d_2	81	58	51	85	86
6,6,8β-d_3	(4-H)	d_0	-	-	-	3	3
		d_1	-	10	-	19.	19
		d_2	-	42	-	37	37
		d_3	-	48	-	30	32
		d_4	-		-	8	9
5α,9α,11α-d_3		d_0	-	19	-	3	3
	(4-I)	d_1	30	36	-	20	18
		d_2	31	29	-	37	37
		d_3	33	16	-	32	32
		d_4	-		-	9	9
		d_5	-		-		-
16,16-d_2	(4-J)	d_0	-	-	-	-	-
		d_1	53	-	-	33	16
		d_2	47	67	-	67	84
17α-CD_3	(4-K)	d_0	47	-	-	-	-
		d_1	9	-	-	-	-
		d_2	21	30	-	48	-
		d_3	23	70	-	35	89
		d_4	-	-	-	17	11

III.C.1. 3α-OSiDMTB-17β-Hydroxy-17α-methyl-5α-androstane (1).

In the mass spectrum of 3α-OSiDMTB-5α-androstan-17-one Quilliam and Westmore[67,101] attributed the double hydrogen migration in the $HSiMe_2OH$ elimination from $[M - t\text{-Bu}]^+$ to one hydrogen originating from C-5 (5α-H → O-3) plus another from C-4 (4-H → Si). The rearrangement from C-5 has been generally suggested[79], accepted[70], and now is confirmed by the present labeling results, which also establish sources of the second hydrogen as C-6 (*ca* 24%), C-7 (*ca* 37%), and C-9α (*ca* 46%). Table 19 shows the results of the labeling experiments, while Scheme 32 shows the positional origin for HMe_2SiOH loss from the $[M - t\text{-Bu}]^+$ and $[M - t\text{-Bu} - H_2O]^+$ ions.

Within experimental error (±5%), transfer of deuterium from positions 5α, 6, 7, and 9α accounts for all the deuterium eliminated in the neutral species. The stereospecificity of the neutral loss implies that the steroid skeleton remains intact[61-63,67].



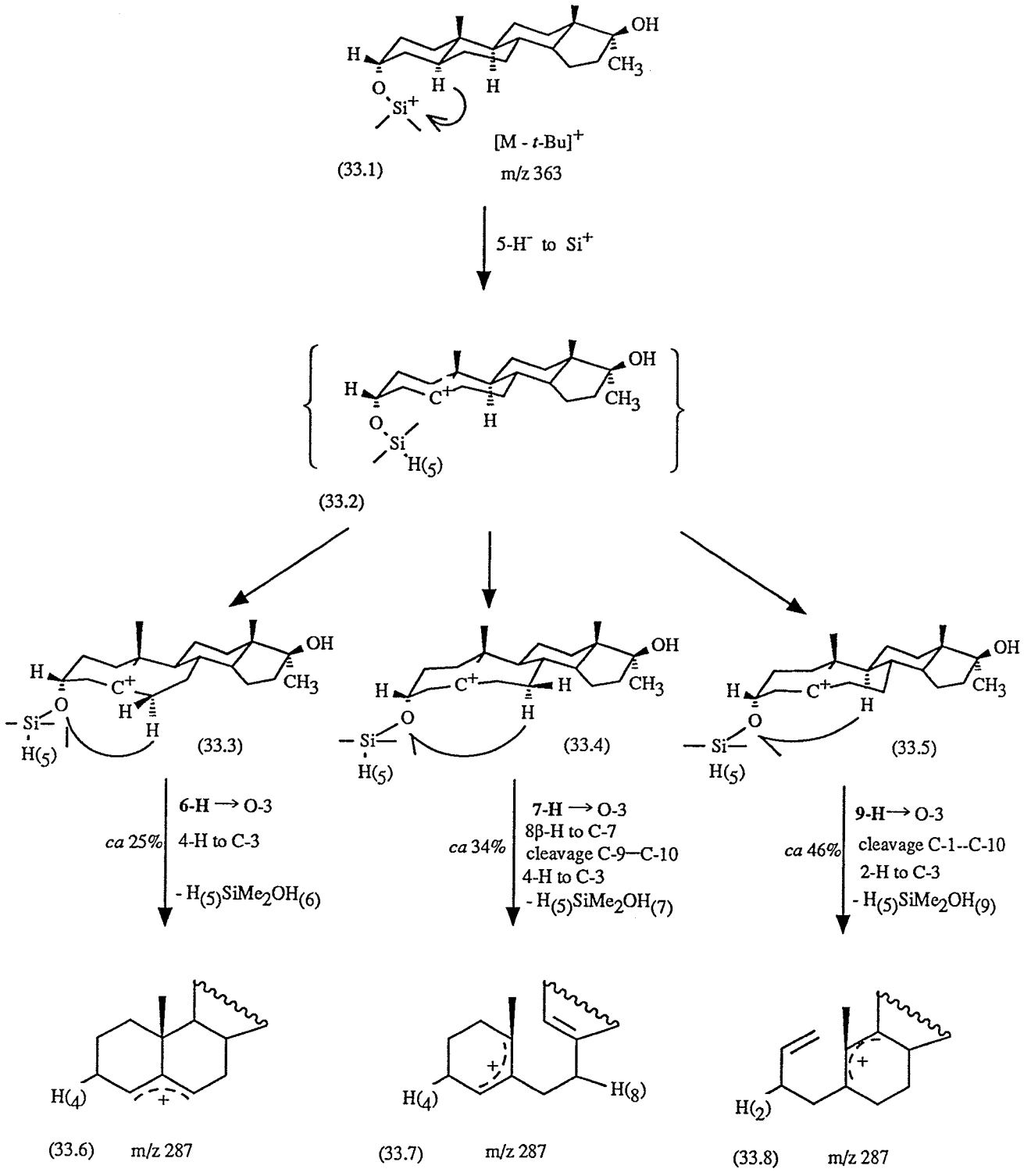
Total hydrogen elimination from the 5α, 6, 7, and 9α sites from:
(a) $[M - t\text{-Bu}]^+ = 100 + 24 + 37 + 46 = 197\%$
(b) $[M - t\text{-Bu} - H_2O]^+ = 100 + 24 + 36 + 43 = 193\%$

Scheme 32. Positional origin (%) of hydrogen transfer from the 5α, 6, 7, and 9α sites, in $HSiMe_2OH$ elimination from the $[M - t\text{-Bu}]^+$ and $[M - t\text{-Bu} - H_2O]^+$ (in parentheses) ions of **1.**

Table 19. Deuterium incorporation (%) into $HSiMe_2OH$ eliminated from the [M - *t*-Bu]$^+$ and [M - *t*-Bu - H_2O]$^+$ ions of labeled 3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane (**1**). (Experimental error ± 5%)

labeled	*parent ion* →	**[M - C_4H_9]$^+$**		**[M - C_4H_9 - H_2O]$^+$**	
position(s)	*deuterium* → *in neutral*	**2d**	**1d**	**2d**	**1d**
1α,5α-d_2	**(1-A)**	0	100	0	100
1β,5α-d_2	**(1-B)**	0	100	0	100
2,2,4,4-d_4	**(1-C)**	0	0	0	0
3β-d_1	**(1-D)**	0	0	0	0
5α-d_2	**(1-E)**	0	100	0	100
6,6-d_2	**(1-F)**	0	25	0	24
7,7-d_2	**(1-G)**	0	37	0	36
5α,9α,11α-d_3	**(1-I)**	46	46	43	43
16,16-d_2	**(1-J)**	0	0	0	0
17α-CD_3	**(1-K)**	0	0	0	0

Because of the unusual stereospecific nature of the 5α-H (100%) rearrangement, I have assumed a hydride transfer from the C-5 site to the positively charged silicon centre of the 3α-OMe_2Si group. The labels do not distinguish between transferred hydrogen as a radical or as hydride, but in view of the behaviour of 3ξ-OSiDMTB-11α-acetyloxy-17β-hydroxy-17α-methyl-5ξ-androstane (**9-12**) isomers under EI, one can reasonably propose that the first itinerent hydrogen must migrate as hydride. Thus, the anticipated transfer of hydride to silicon is supported by the observed migration of the 11α-acetyloxy group to silicon, to form the $[CH_3COOSi(CH_3)]^+$ ion, described in Section III.B.3., where after *t*-butyl radical elimination, a stable positive charge is developed on the silicon atom. Consequently, transfer of the 5α-hydride to Si^+ to give the C-5 carbocation 33.2 (Scheme 33), in a six membered cyclic transition state, is



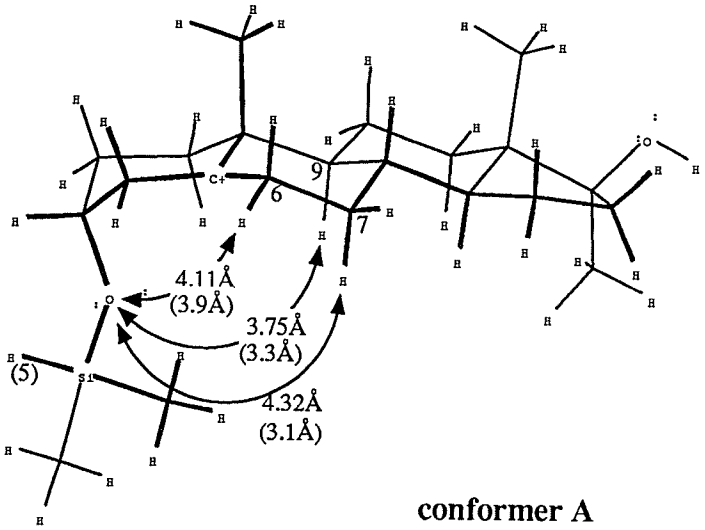
Scheme 33. Mechanisms proposed for elimination of $HSiMe_2OH$ from $[M - t\text{-}Bu]^+$, involving 5α-H, 6-H, 7-H and 9α-H atoms for **1**.

proposed as the initial step in HMe_2SiOH elimination. To the best of our knowledge, it is the first proposal of a hydride transfer to positively charged silicon upon EI.

For the succeeding reactions, in which hydrogen from the 6, 7 or 9α sites is transferred, mechanisms leading to 33.6, 33.7, 33.8. are proposed. The abstraction of 7-H or 9α-H implies a chair structure of ring B, in which 7α-H migrates to $3\alpha\text{-OSiDMH}_{(5)}$ to give 33.7 and 33.8. On the other hand 6-H can undergo facile migration to $3\alpha\text{-OMe}_2\text{SiH}_{(5)}$ as ring B flips to the boat form 33.3. The suggested 1,2 hydrogen shifts, 4-H → C-3 (33.6 and 33.7), 8β-H → C-7 (33.7) and 2-H → C-3 (33.5 - 33.8), always lead to the stable allylic cations (33.6 - 33.8).

To test the feasibility of the hydrogen transfers, the interatomic distances in the transition states were estimated from Dreiding models[176] and also molecular mechanics calculations[186]. Calculations were made with the PCMODEL program described in Appendix II. The results are presented in Table 20.

For the initial [M - *t*-Bu]$^+$ conformer tested (conformer **A**, C-5 trigonal, Scheme 34) the distances between O-3 and 6α, 7α or 9α-H are too large to allow facile migration



conformer A

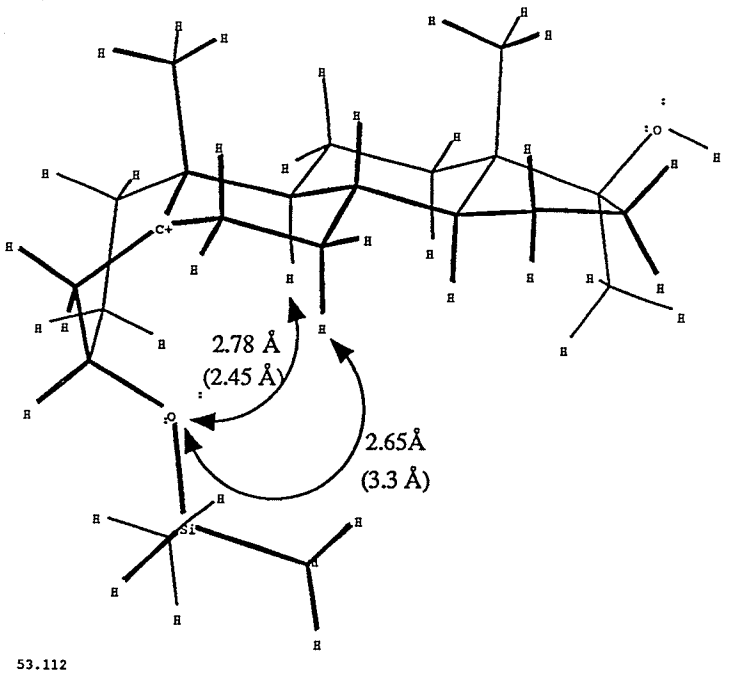
Scheme 34. MM2 calculated geometry of the intial [M - *t*-Bu]$^+$ conformer (C(5)-sp^2) after 5α-hydride transfer to Si$^+$, for 3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane (**1**). (Distances according to PCMODEL program and Dreiding models (parentheses))

Table 20. Minimum distances, energies and torsion angles between O-3 and closest Hs, for conformers **A - E** of 3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane (**1**). (MM2 calculations by PCMODEL)*

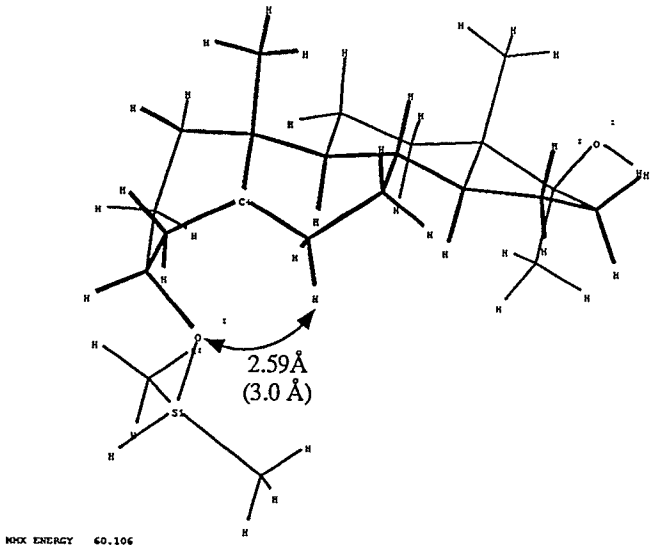
	Distance (Å)	Structure A ring, B ring	MM2 Energy kcal mol^{-1}	Torsional Angle Si-O(3)-C(3)-H(3) (deg)
Conformer A (Scheme 34)				
O-3 - 6α-H_{eq}	4.11[e](3.9)[b]	A chair,B chair	40.2(50.9)[c]	- 47.0
O-3 - 7α-H	4.32[e](3.1)[b]	A chair,B chair	"	"
O-3 - 9α-H	3.75[e](3.3)[b]	A chair,B chair	"	"
Conformers B (Scheme 35[b,e] and Scheme 36[a])				
O-3 - 7α-H	2.65[e](3.15)[a] (3.3)[b]	A $boat_{inv}$,B chair	42.1(53.11)[c]	+ 35.0
O-3 - 9α-H	2.78[e](2.45)[a] (3.1 or 2.45)[b]	A $boat_{inv}$,B chair	"	"
Conformer C (Scheme 35[b,e] and Scheme 36[a])				
O-3 - 6α-H	2.59[e](3.0)[a] (3.0)[b]	A $boat_{inv}$, B boat	49.1(60.11)[c]	+ 38.0
Conformer D (Scheme 38)				
O-3 - 7α-H	2.55[e](2.2)[b]	A $boat_{inv}$,B chair	57.3	+ 30.6
O-3 - 9α-H	2.89[e](2.1)[b]	A $boat_{inv}$,B chair	57.3	+ 30.6
Conformer E (Scheme 38)				
O-3 - 6α-H	2.48[e](1.6)[b]	A $boat_{inv}$, B boat	57.0	+ 29.58
O-3 - 9α-H	2.66[e](2.3)[b]	A $boat_{inv}$, B boat	57.0	+ 29.58

a - interatomic distances determined by plastic Dreiding models[175]
b - interatomic distances determined by a metallic Dreiding model with a ruler (in Å)
c - energy displayed in the minimizaliation mode, as the minimization process is completed
e - interatomic distances determined by MM2 molecular mechanics
*See Appendix II - for explanation

Conformer B



Conformer C

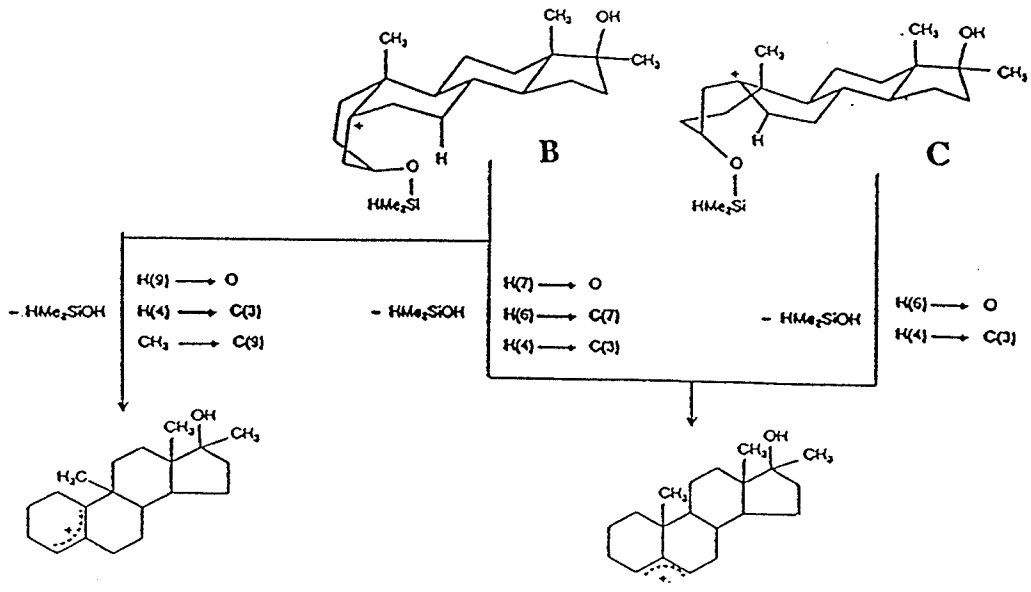


Scheme 35. MM2 calculated geometry of the [M - *t*-Bu]$^+$ ion for conformers **B** and **C**. (Distances determined by the PCMODEL program and Dreiding models (in parentheses))

of hydrogen. Therefore, the calculations were performed for the conformers, for which conformational changes of ring A (conformer **B**; ring A, inverted boat) as well as rings A and B (conformer **C**; ring A, inverted boat and ring B, boat) have occurred, Schemes 35 and 36. The results are summarized in Table 20 and depicted in Scheme 35.

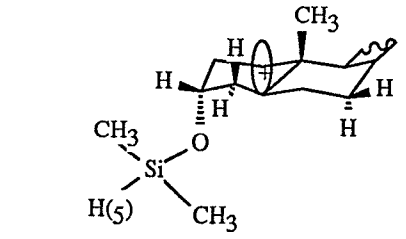
Formation of particularly abundant ions, m/z 287 and m/z 269, in the spectra of **1**, is evidently favoured by the proximity of 3-oxygen and 6α-H or 7α-H or 9α-H atoms. However, according to Dreiding models (Table 20 and Scheme 35 or 36), separation of O-3 and 7α-H or of O-3 and 6α-H, greatly exceeds the expected bonding distance (2.6 Å).

Thus, to explain the observed transfer of hydrogens, Westmore *et al.*[175] assumed that after 5α-H migration to 3α-OMe_2Si^+, the conformational adjustment as C-5 changes from sp^3 to sp^2 hybridization occurs more or less equally in both A and B rings, to yield conformers **B** or **C** for which the pucker of both rings is reduced (Scheme 36). Consequently, with a help of a small "umbrella" vibration at C-5, the O-3 can approach close to other hydrogens (6α-H, 7α-H or 9α-H) in the strain free $[M - t\text{-Bu}]^+$ ion[175].

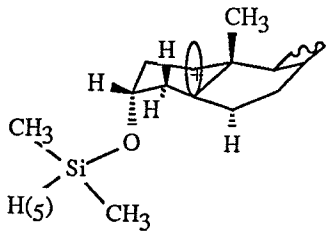


Scheme 36. HMe_2SiOH elimination from conformers **B** and **C**[175].

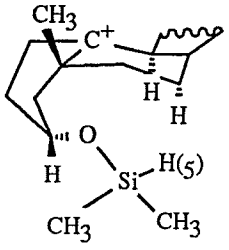
In the second approach, in searching for closer interatomic distances, in particular, between O-3 and 6α-H or between O-3 and 7α-H structures, 37.3 and 37.4 (Scheme 37), are proposed. In this scheme, an alternative reaction, involving a shift of the 19-methyl group to the C-5 site, that might compete with that just described, is considered, as follows. If the vacant p-orbital of the C-5 carbocation is eclipsed with the 19-methyl bond (37.1 or 37.2), then migration of the 19-methyl group can occur to give 37.3 and 37.4, respectively. The rearrangement of the 19-CH_3 group to C-5 requires changes of hybridization at C-5 ($sp^2 \rightarrow sp^3$) and C-10 ($sp^3 \rightarrow sp^2$). In the inverted boat



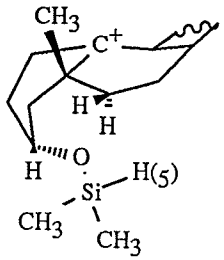
(37.1) ring A, chair
ring B, chair



(37.2) ring A, chair
ring B, boat



(37.3) ring A, inverted boat
ring B, chair



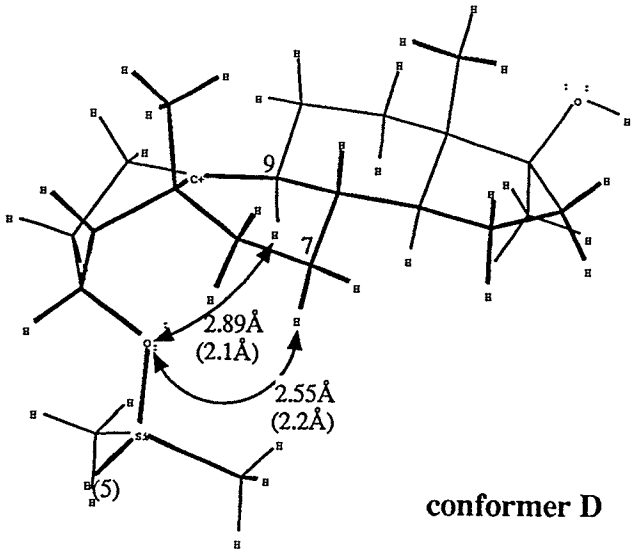
(37.4) ring A, inverted boat
ring B, boat

Scheme 37. Migration of the 19-methyl group in the [M - *t*-Bu]$^+$ ion of 3α-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane (**1**).

conformation of ring A of the new structures (Scheme 38), 3α-O can approach 6α-H (conformer **E**, 2.48 Å, PCMODEL; 1.6 Å, Dreiding model), 7α-H (conformer **D**, 2.55 Å, PCMODEL; 2.2 Å, Dreiding model) or 9α-H (conformer **D** or **E**, 2.66 Å, PCMODEL; 2.3 Å, Dreiding model). Abstraction of tertiary 5α-H or tertiary 9α-H is more energetically favourable than abstraction of secondary hydrogens[30]. Since abstractions of 6α-H, 7α-H or 9α-H are competetive, the factors controlling populations of the conformers are probably delicate balanced. Decomposition mechanisms starting from conformer **D** and **E** are proposed in Scheme 39.

A similar migration, 19-CH_3 → C-5 (sp^2), has been observed in reactions of a number of 5-ene steroids. The migration of the 19-CH_3 group to an initially developed carbocation at C-5, followed by the concerted 1,2-hydrogen shifts 9α-H → C-10, 8β-H → C-9, and 14α-H → C-8, is known as the "Westphalen", Wagner-Meerwein or "backbone" rearrangement[177].

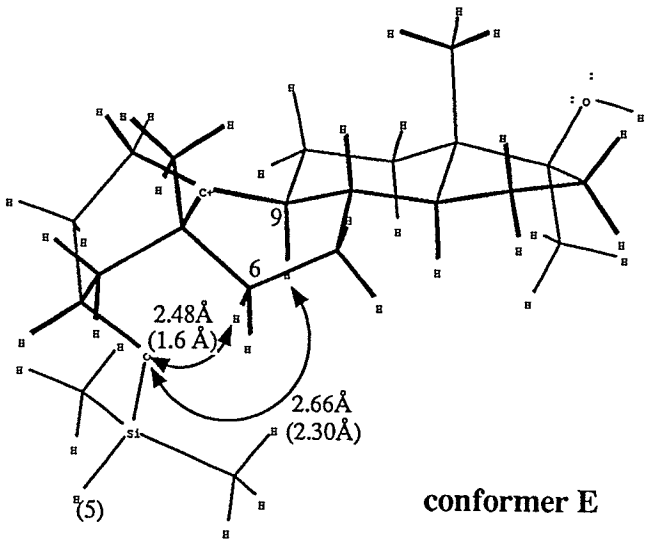
Scheme 40 depicts further migration of the positive charge from C-10 along the steroid backbone, towards C-13, through the tertiary C-9, C-8, and C-14 centers by the hydride shifts, 9α-H → C-10, 8β-H → C-9, and 14α-H → C-8, and the methyl shift 18-CH_3 → C-14, to give the structures 40.1 - 40.6. Elimination of $HSiMe_2OH$ could occur from each intermediate structure, if O-3 comes close enough to any hydrogens. The migrating positive charge could also explain the increased stability of the product ion by formation of the multifunctional ion. Such a concerted 1,2-H shifts involve low activation energy. Similarly, the itinerent positive charge could help in understanding enhanced water elimination from ring D (section III.D., Scheme 33). For example, a positive charge at trigonal C-13 and the pseudoboat form of ring C would reduce the separation between 12α-H and 17β-O to 2.67Å (PCMODEL) or 2.7Å (Dreiding models).



(38.1) **conformer D**

MMX ENERGY 57.291

(A $boat_{inverted}$, B chair)

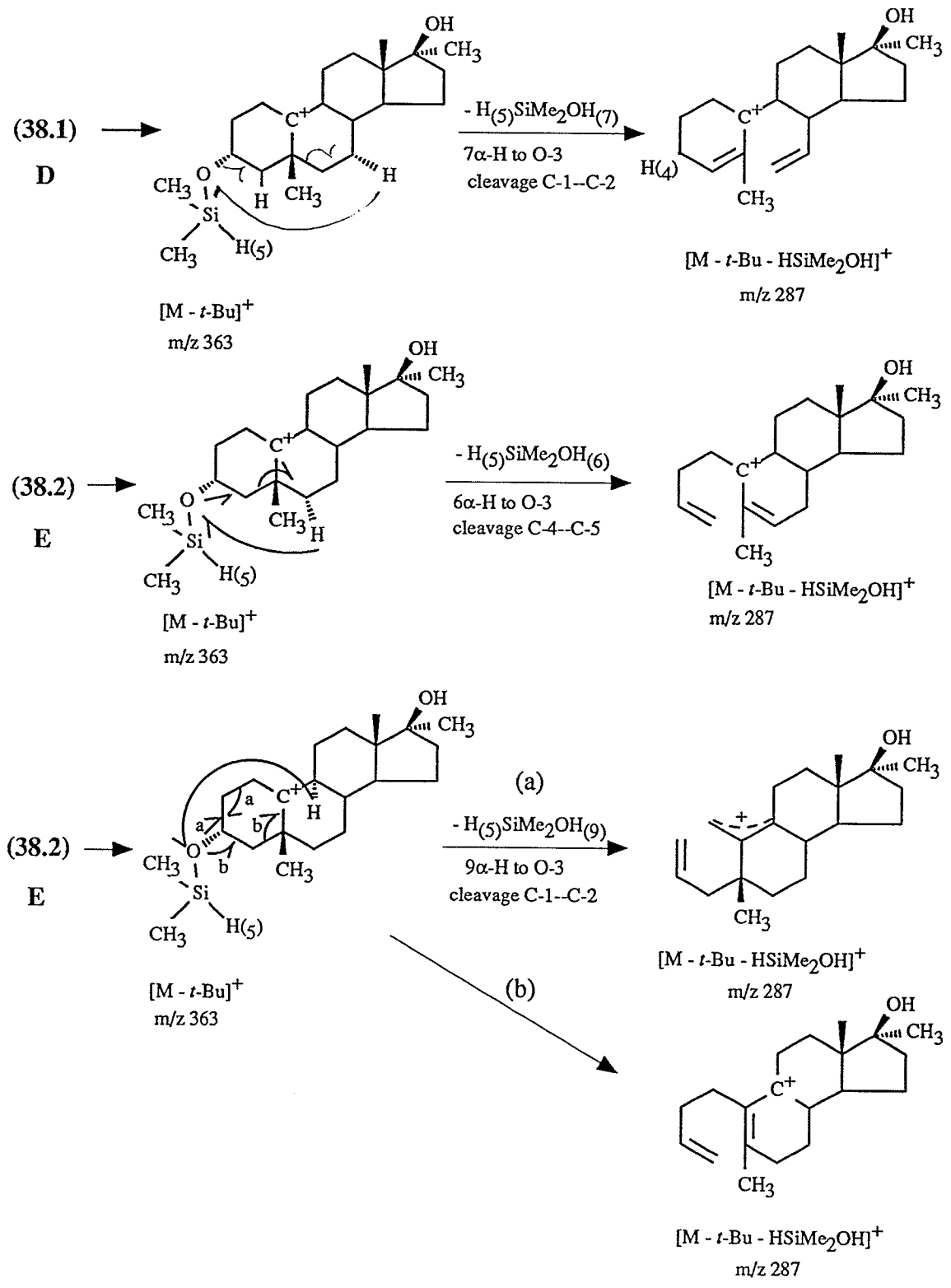


(38.2) **conformer E**

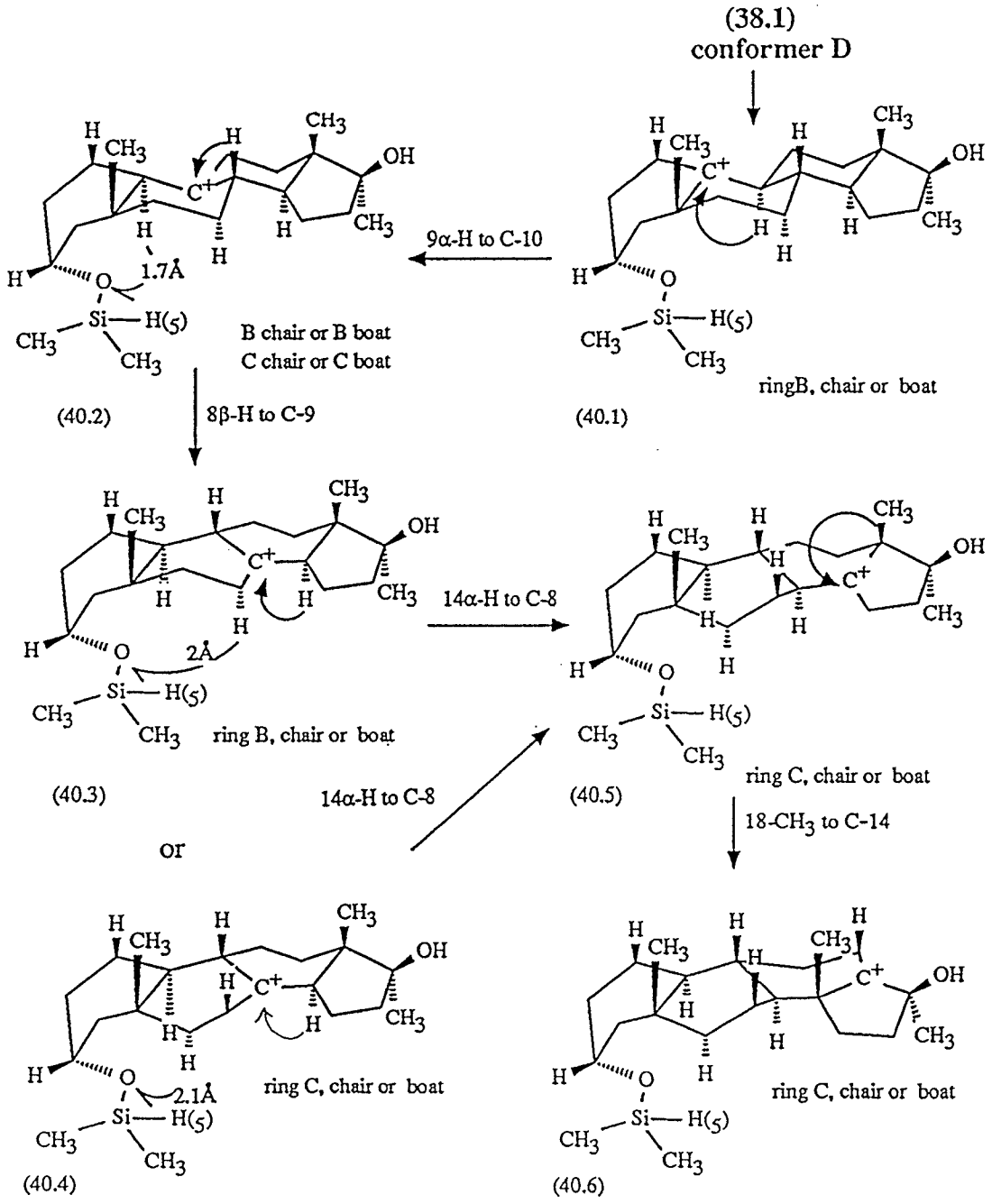
MMX ENERGY 57.011

(A $boat_{inverted}$, B boat)

Scheme 38. MM2 calculated geometry for **D** and **E** conformers of the [M - *t*-Bu]$^+$ ion. (**D**) ring A $boat_{inv}$, ring B chair; (**E**) ring A $boat_{inv}$, ring B boat. (Dreiding models in parentheses)



Scheme 39. Proposed fragmentation mechanisms for conformers **D** and **E** from Scheme 38.



Scheme 40. Structures formed after migration of the positive charge along the steroid backbone. (Distances determined by Dreiding models.)

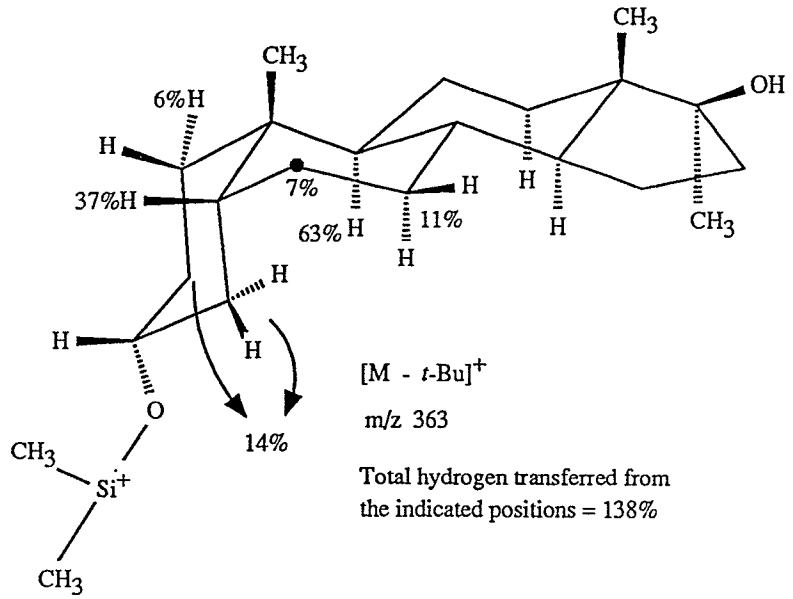
III.C.2. 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (2).

The [M - *t*-Bu - $HSiMe_2OH$]$^+$ ion.

The relative abundance of this ion, formed by elimination of $HSiMe_2OH$ from [M - *t*-Bu]$^+$, varies from 1.6% to 12% (Fig.16), possibly caused by varying source conditions.

The extent of deuterium incorporation into the eliminated neutral species for various labeled molecules are given in Table 21, and shown diagramatically in Scheme 41. Hydrogens atoms from rings A and B contribute 138% (of a maximum of 200%). The most probable sources of the remainder are 12α-H and/or 14α-H of ring C.

As in the previous section, an attempt will be made to explain these results in terms of the geometry of decomposing ions. For convenience, the closest distances between Si or O-3 and selected hydrogens, as determined from Dreiding models and the PCMODEL



Scheme 41. Positional origin (%) of hydrogen transferred in $HSiMe_2OH$ elimination from the [M - *t*-Bu]$^+$ ion of 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (**2**).

Table 21. Deuterium incorporation (%) into $HSiMe_2OH$ eliminated from the [M - *t*-Bu]$^+$ ion of labeled 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane **(2).** (Experimental error ±5%)

label(s)	*deuterium in neutral→*	**2d**	**1d**
1α,5β-d_2	**(2-A)**	-	43 (1α-H,6%)(a)
1β,5β-d_2	**(2-B)**	-	37
2,2,4,4-d_4	**(2-C)**	-	14
3β-d_1	**(2-D)**	-	-
5β-d_1	**(2-E)**	-	37
6,6-d_2	**(2-F)**	-	7
7,7-d_2	**(2-G)**	-	11
5β,9α,11α-d_3	**(2-I)**	-	100 (9α-H, 63%) (b)
16,16-d_2	**(2-J)**	-	0
17β-CH_3-d_3	**(2-K)**	-	0

(a) 1α-H = 43 - 37 = 6%

(b) 9α-H = 100 - 37 = 63%, assuming that 11α-H = 0%

program, are summarized in Table 22 for structures and conformations to be discussed in this section.

Mechanisms of $HSiMe_2OH$ elimination.

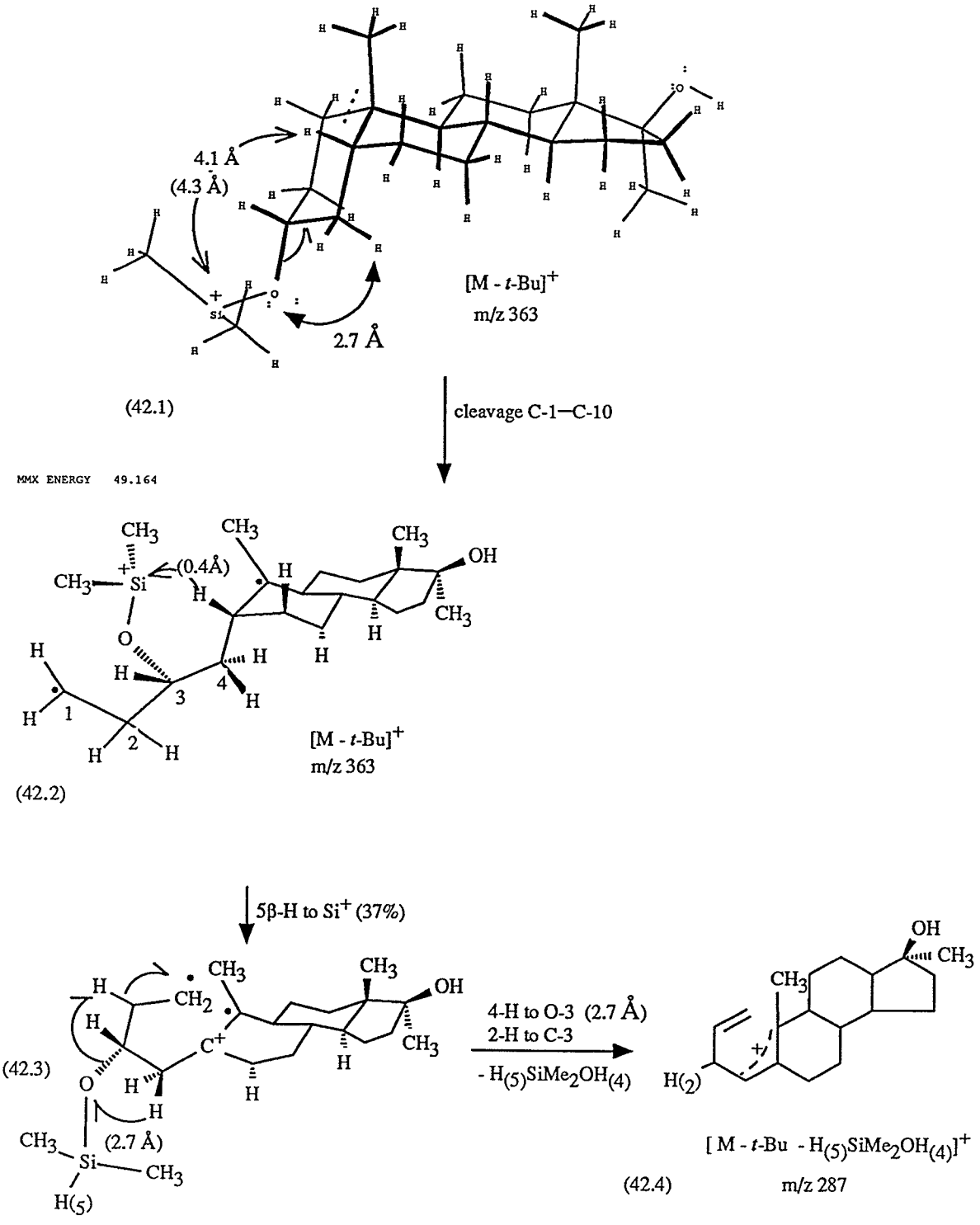
(a) Involvement of 5β-H together with 4-H, 6-H or 7-H.

Table 21 shows that the losses of 4-H (14%), 6-H (7%) and 7-H (11%) total 32%, i.e. within experimental error of the loss of 5β-H (*ca* 37%), which suggests that these losses are correlated. If the steroid skeleton remains intact as in structure 42.1, Scheme 42, the smallest distance between 5β-H and Si^+ is 4.1 Å (Table 22), which greatly exceeds the expected interaction distance (*ca* 2.5 Å). Therefore, to explain the observed losses of 5β-hydride, assumed to be correlated with 4-H, 6-H or 7-H losses in $HSiMe_2OH$ elimination, we propose that fission of the C-1–C-10 bond occurs (Scheme 42), followed

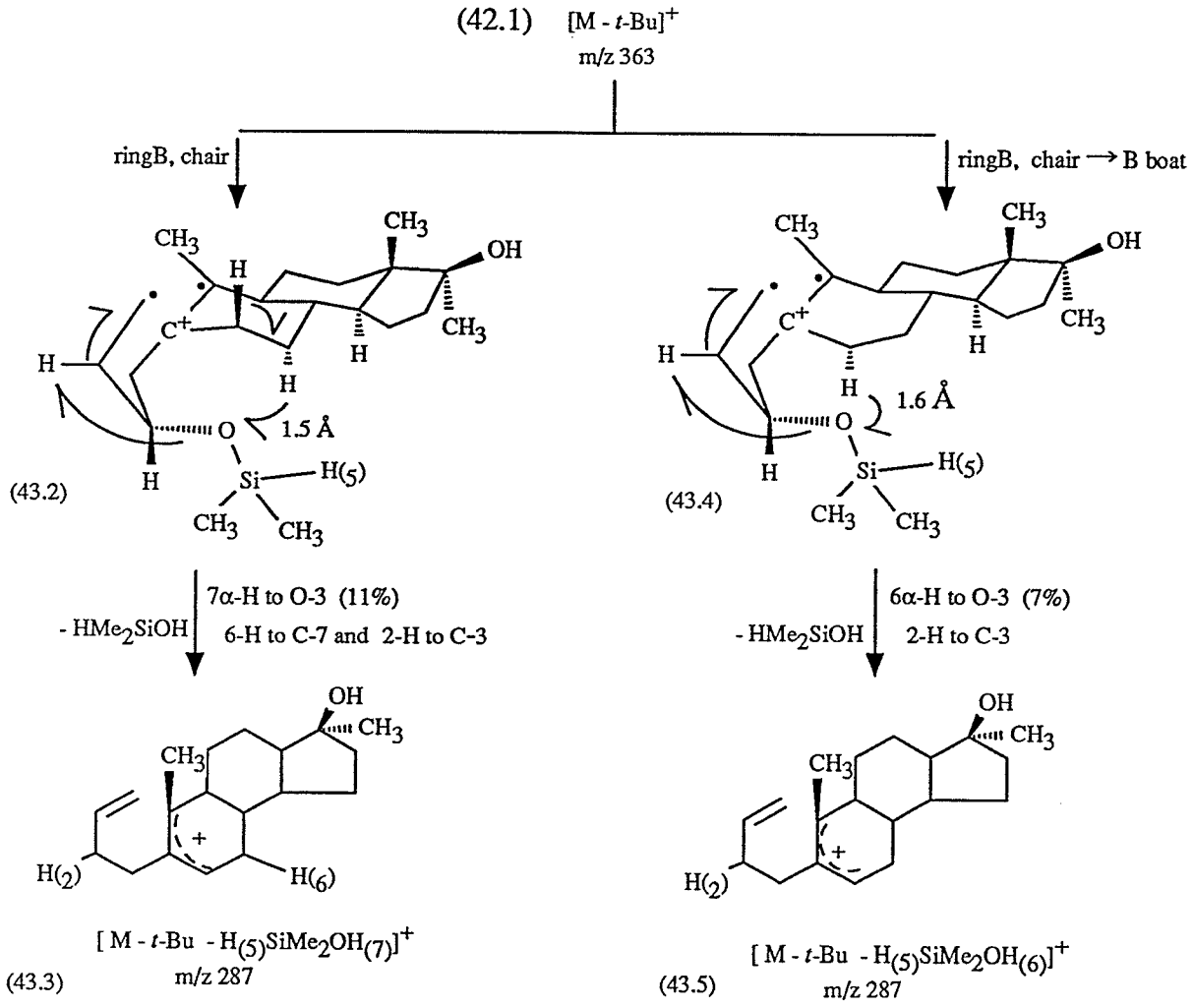
Table 22. Distance of closest approach (Å) between selected atoms for the [M - *t*-Bu]$^+$ and [M - *t*-Bu - H_2O]$^+$ ions of **2** determined by Dreiding models and by PCMODEL.

[M - *t*-Bu]$^+$	Dreiding	PCMODEL	ring conformation	Scheme
5β-H → Si$^+$	4.1	-	intact skeleton	42
After C-1–C-10 cleavage				
5β-H → Si$^+$	0.4	-	A open	42
4β-H → O-3	2.7	-	A open	42
6α-H → O-3	1.6	-	A open, B boat (43.4)	43
7α-H → O-3	1.5	-	A open, B chair (43.2)	47
1α-H → Si$^+$	1.4	2.61	A screw, B chair (44.1)	44
9α-H → O-3	1.1	2.50	A boat$_{inv}$, B chair (44.2)	44
9α-H → Si$^+$	0.9	2.59	A screw, B chair	45
1α-H → O-3	2.5	2.5	A screw, B chair (45.1)	45
12α-H → O-3	1.5	2.94	A boat$_{inv}$, Bchair, C chair	46, 48
14α-H → O-3	1.2	2.83	A boat$_{inv}$, Bchair, C chair	47,48
[M - *t*-Bu - H_2O]$^+$				
9α-H → Si$^+$	0.9	2.59	A screw, B chair, C chair	52
11α-H → O-3	0.6	2.61	A boat$_{inv}$, B chair, C boat	
14α-H → Si$^+$	1.2	-	A boat$_{inv}$, B chair, C chair	53
12α-H → O-3	2.2	-	A boat$_{inv}$, Bchair, C chair	

by transfer of 5β-H$^-$ to Si (Scheme 42 (42.2), 0.4 Å minimum separation; Dreiding model), followed by transfer of a 4-H atom to O-3 (Scheme 42 (42.3), 2.7 Å, Dreiding model) via a four-membered transition state. Alternatively, if ring B alters its conformation, B chair → B boat, rearrangement of 6-H to O-3 (Scheme 43 (43.4), 1.6 Å, Dreiding model) via a six-membered transition state can occur, accompanied by a 1,2 shift of 2-H to C-1. Rearrangement of 7-H to O-3 (Scheme 43 (43.2) 1.5 Å, Dreiding model) can occur, if oxygen approaches the 7α-H. In each case an allylic cation is produced.



Scheme 42. Mechanism proposed for elimination of $H_{(5)}SiMe_2OH_{(4\beta)}$ from the [M - *t*-Bu]$^+$ ion of 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (**2**). (Distance determined PCMODEL (42.1) and Dreiding models (42.1 - 42.3; in parantheses)



Scheme 43. Proposed mechanism for elimination of $H_{(5)}SiMe_2OH_{(6\text{ or }7)}$ from the [M - *t*-Bu]$^+$ ion of 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (**2**). (Distances determined by Dreiding models)

(b) Involvement of 9α-H.

Table 21 shows 63% participation of 9α-H in $HSiMe_2OH$ elimination. From the previous assumption that elimination of hydrogen from sites 4, 6, and 7 is correlated with elimination from the 5β-H position, it follows that the origins of the second hydrogen lost with 9α-D are not rings A or B (though 6% could come from the 1α site). Involvement of hydrogens from ring C and/or D requires conformational changes of ring A. The conformational flexibility of ring A in steroids of the 5β-series was supported by NMR[178]

and mass spectrometry[29,30].

Scheme 44 shows a twist conformation of ring A (44.1) in which H-1α, H-2β, 3α-OSiDMTB, and H-5β are axial. Distances of closest approach between Si or O-3 and the nearest hydrogens are listed in Table 22. Mechanisms for elimination of $HSiMe_2OH$ are proposed in Schemes 44 and 45.

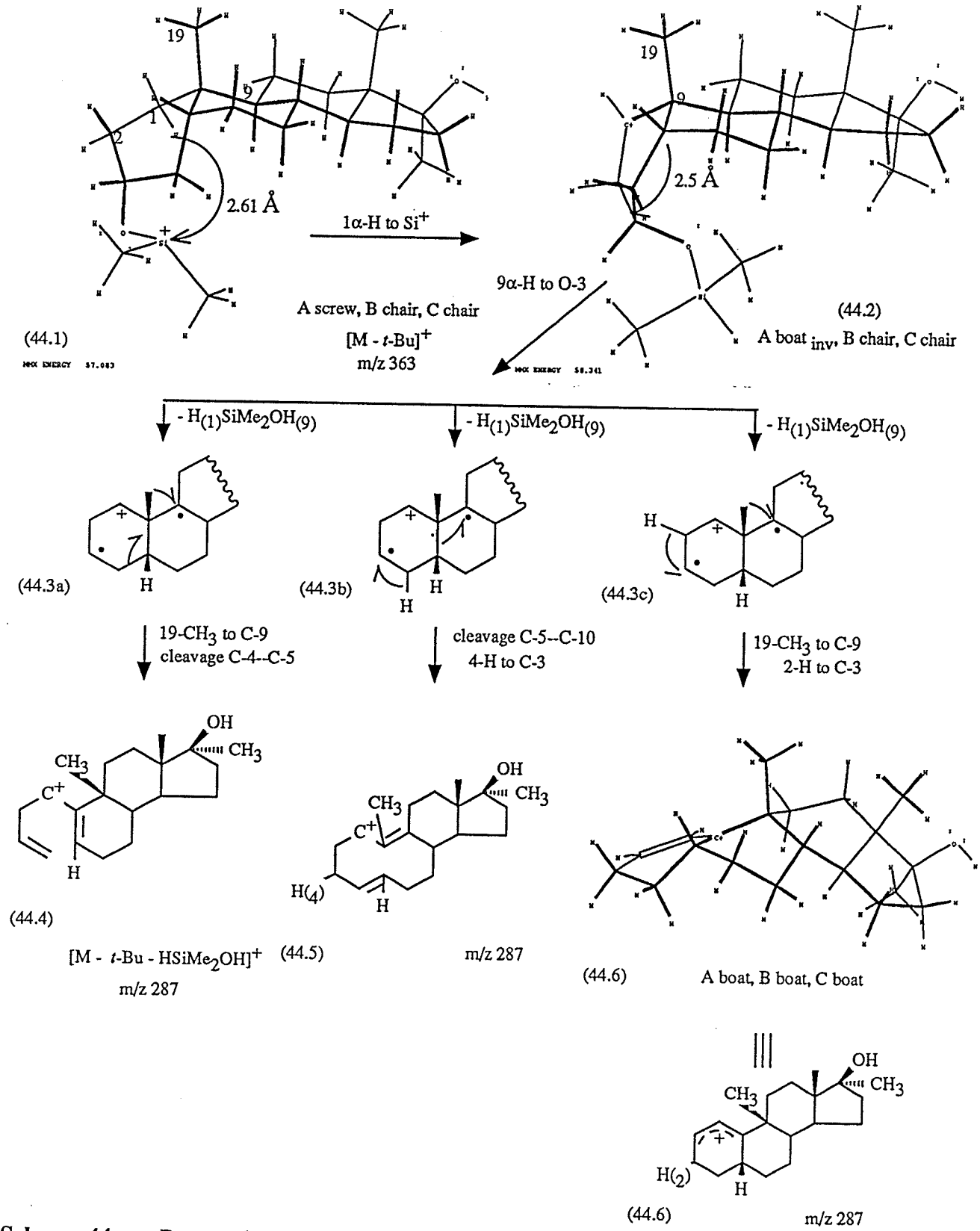
In Scheme 44, abstraction of hydride from the 1α-site by Si^+ (1.2 Å, Dreiding; 2.61 Å, PCMODEL) to give a secondary C-2 carbocation (44.2), followed by 9α-H transfer to 3-oxygen (2.5 Å, Dreiding; 2.43 Å, PCMODEL) could yield, after elimination of HMe_2SiOH, the allylic cations (44.4 - 44.6). The reactions are assisted by a 1,2-shift of the 19-methyl to C-9, or by cleavage of the C-5–C-10 bond (44.3a) and a 1,2 shift of H-4 to C-3 or by a 1,2-shift of 2-H to C-3 (44.3c).

Alternative fragmentations, initiated by transfer of hydride from the 9α site to silicon (1.65 Å separation) followed by abstraction of a H atom from the C-1 site by 3α-O (2.5 Å separation), are shown in Scheme 45. The allyl cation 45.3 could be formed by a 1,2 shift of the 19-methyl group to C-1, accompanied by concerted bond fissions. The allyl cation 45.5 could be formed by a shift of the 19-methyl group to C-9 and a 1,2 shift of H-2 to C-3.

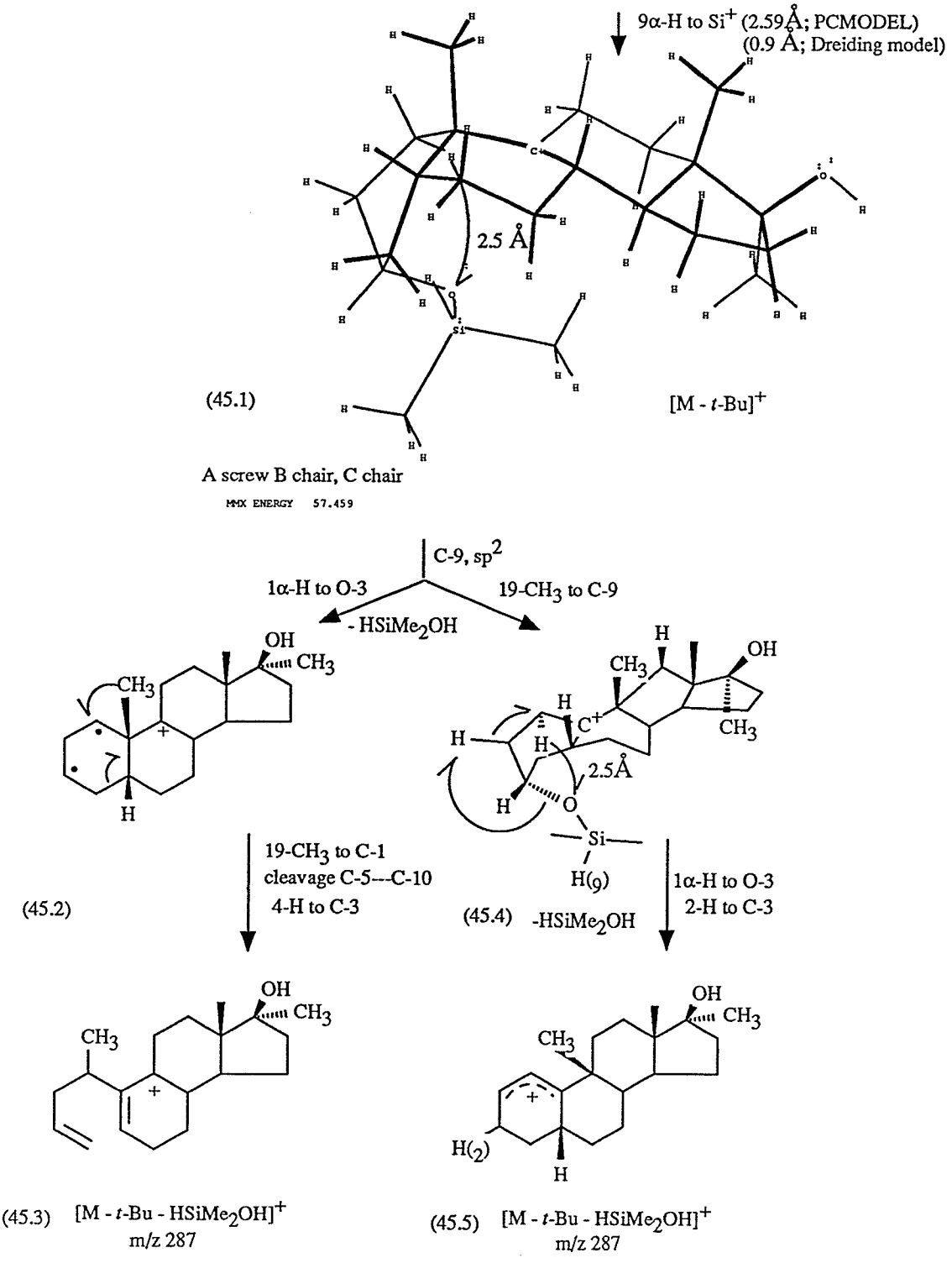
Fragmentation mechanisms involving 12α-H or 14α-H, after transfer of 9α-H, are proposed in Schemes 46 and 47. Dreiding models show that, if C-9 is trigonal, the $[M - t\text{-Bu}]^+$ ion is very flexible, and that 3α-O can come close to 12α-H (1.5 Å separation) or 14α-H (1.2 Å separation). Rearrangement of 12α-H or 14α-H to the $3\alpha\text{-}OSiMe_2H_{(9)}$ group could be assisted by a 1,2-shift of 11β-H to C-12 (Scheme 46), or of 8β-H to C-14 (Scheme 47), followed by:

(a) a concerted electron flow associated with fission of the C-1–C-10 bond (46.3 or 47.3)
or
(b) fission of the C-5–C-10 bond (46.4 or 47.6) accompanied by a 1,2 shift of 4-H to C-3.

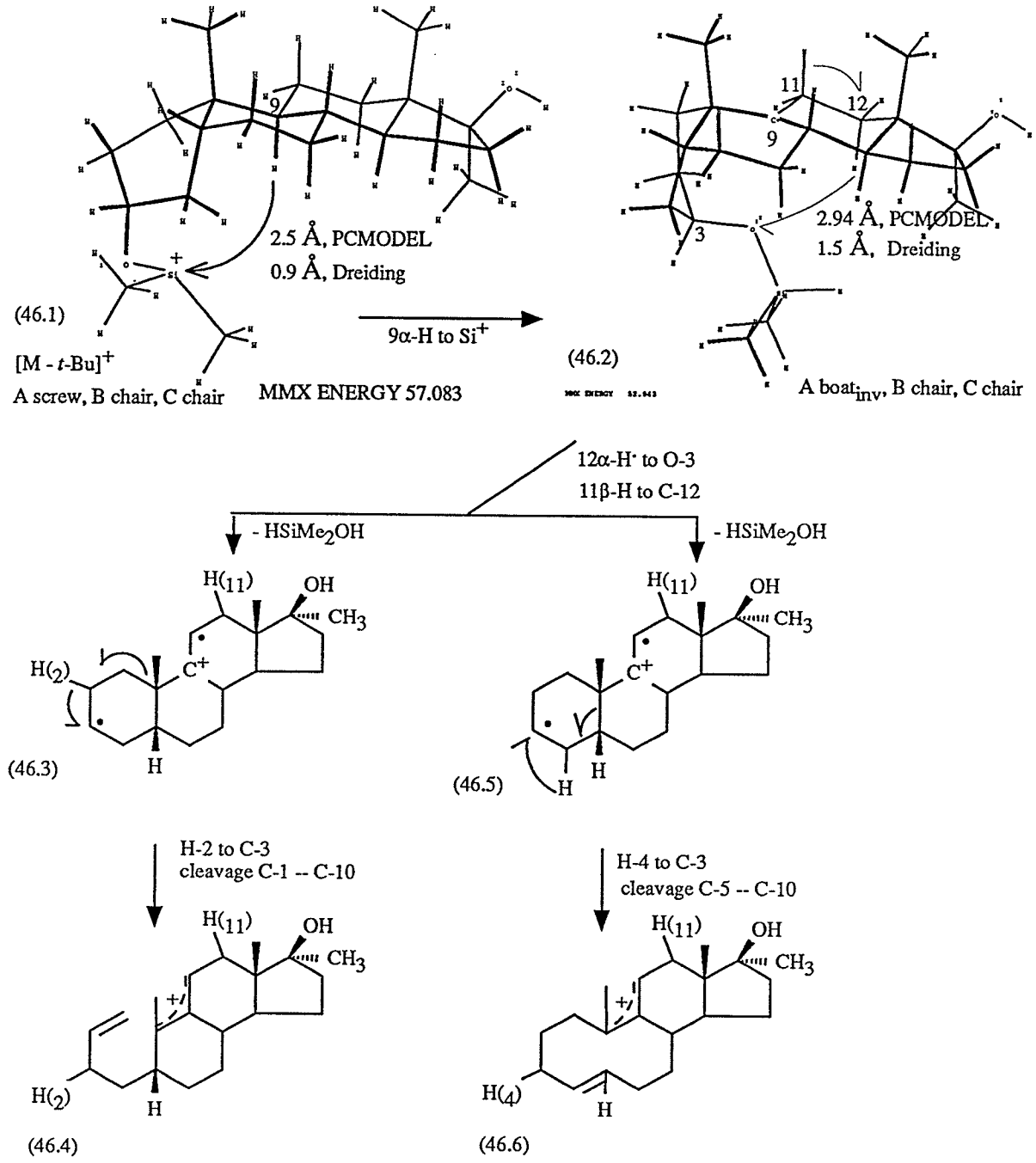
Alternative fragmentation mechanisms, involving transfer of 12α-H or 14α-H *before* transfer of 9α-H are proposed in Scheme 48.



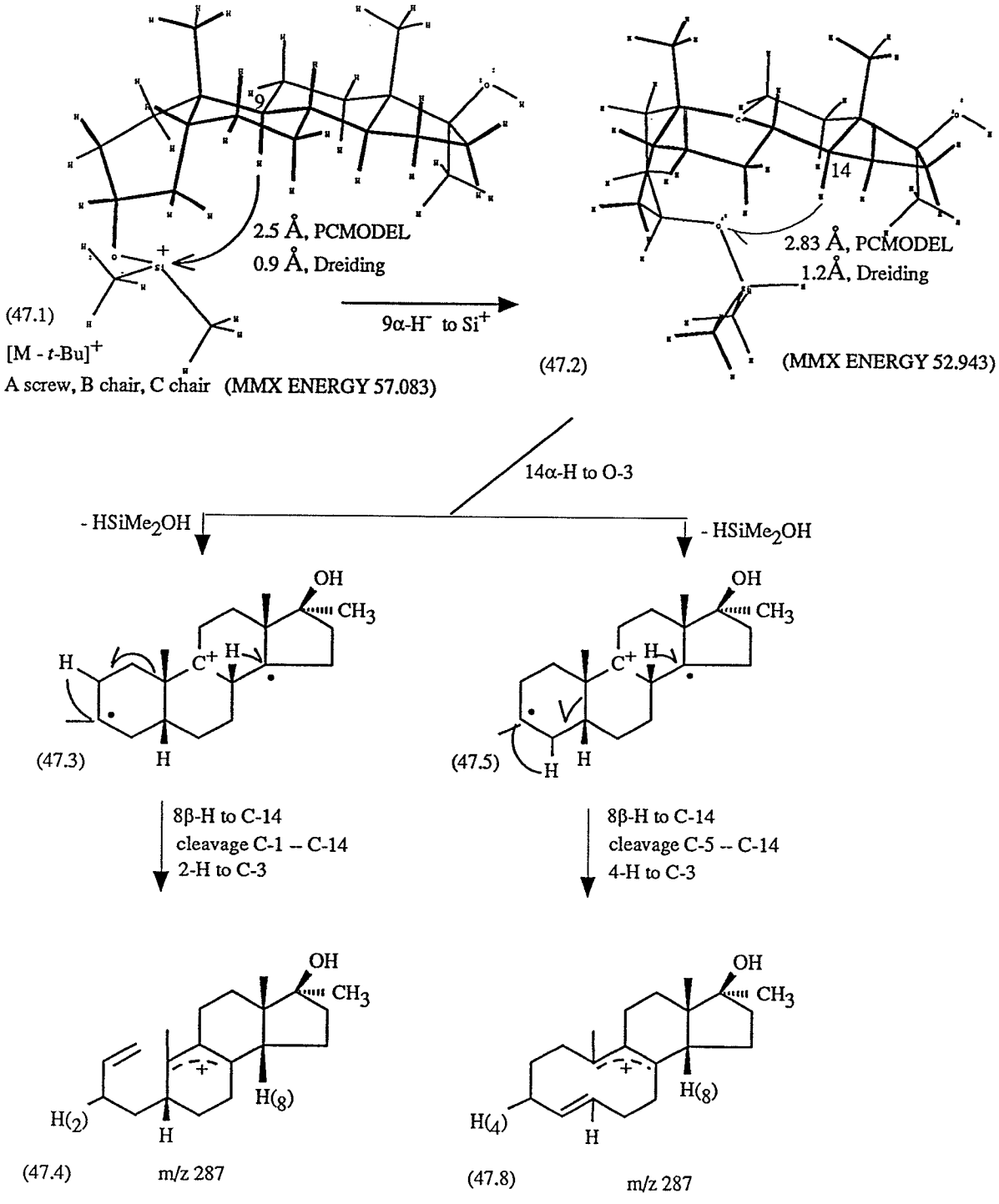
Scheme 44. Proposed mechanism for elimination of $H_{(1\alpha)}SiMe_2OH_{(9\alpha)}$ from the [M - *t*-Bu]$^+$ ion of **2**.



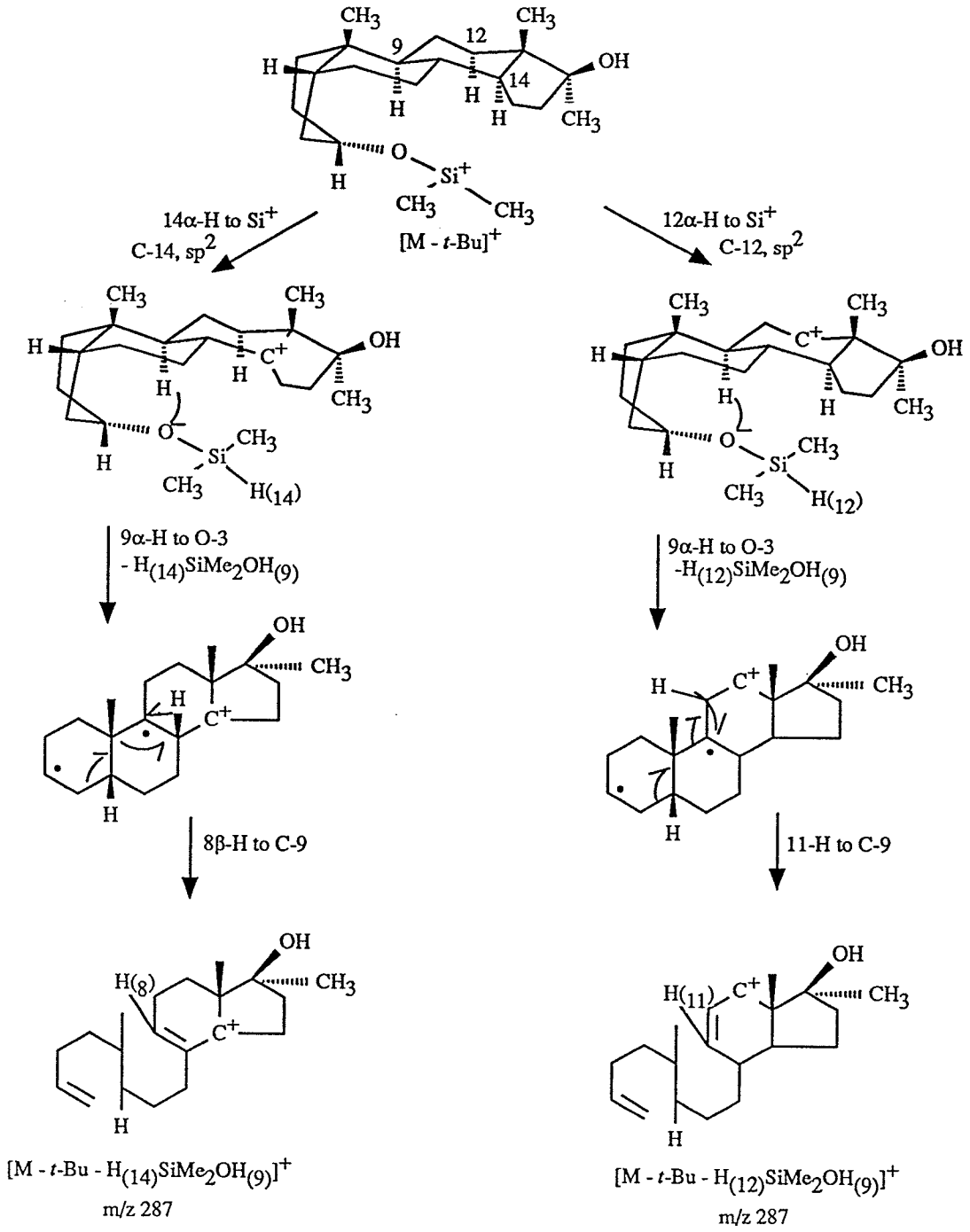
Scheme 45. Alternative mechanism proposed for $H_{(9\alpha)}Me_2SiOH_{(1\alpha)}$ elimination from the $[M - t\text{-}Bu]^+$ ion of **2**.



Scheme 46. Mechanism proposed for $H_{(9\alpha)}SiMe_2OH_{(12\alpha)}$ elimination from the [M - *t*-Bu]$^+$ ion of **2**.



Scheme 47. Mechanism proposed for $H_{(9\alpha)}SiMe_2OH_{(14\alpha)}$ elimination from the [M - *t*-Bu]$^+$ ion of **2**.



Scheme 48. Alternative mechanisms of $HSiMe_2OH$ elimination from the $[M - t\text{-}Bu]^+$ ion of **2**, involving loss of 9α-H together with 14α-H or 12α-H.

The $[M - t\text{-Bu} - H_2O - HSiMe_2OH]^+$ ion.

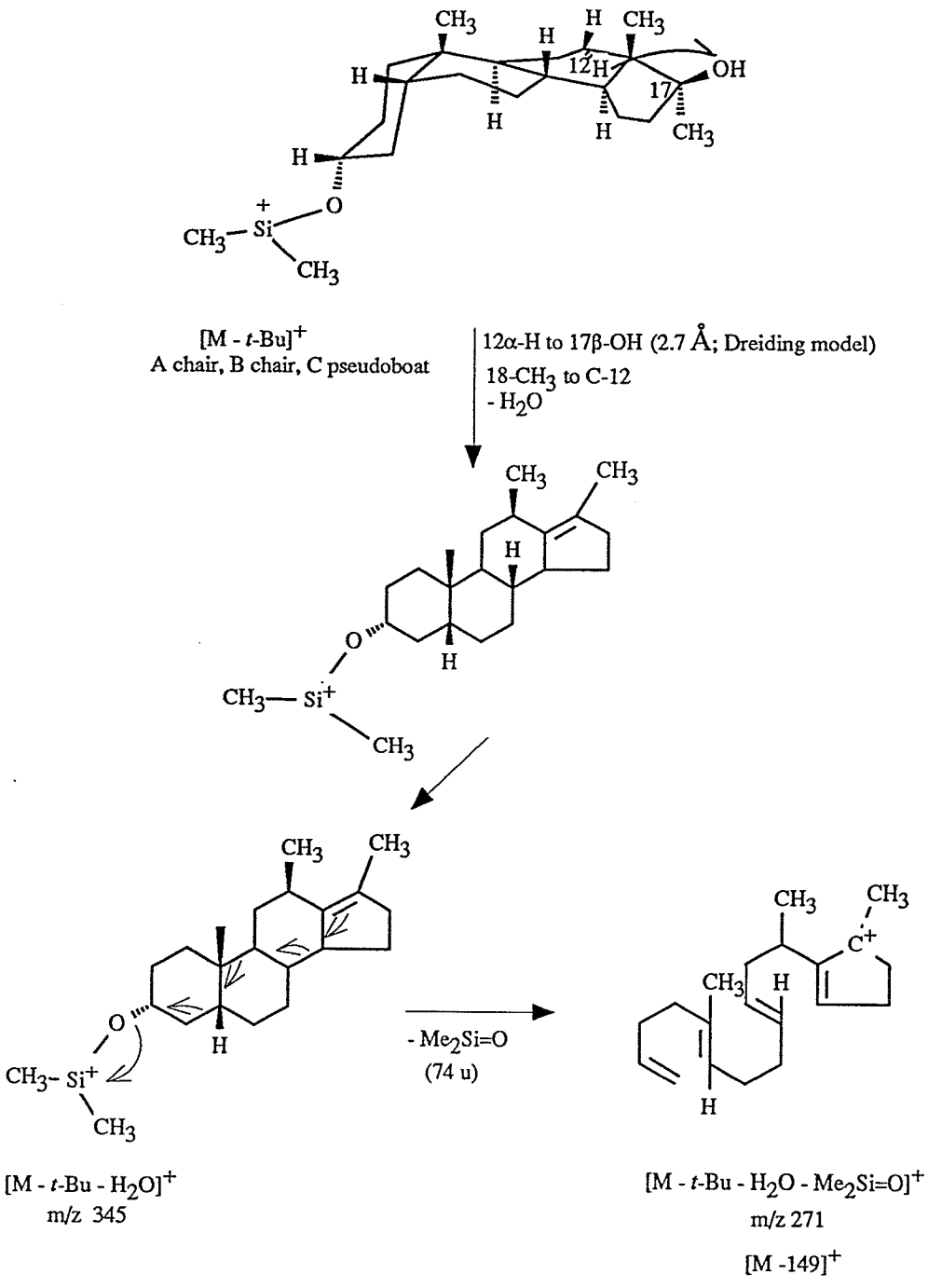
The relative abundance of the $[M - t\text{-Bu} - H_2O - HSiMe_2OH]^+$ ion is four times higher in the spectrum of **2** (28%; 5β-H,3α-OTBDMSi, Fig. 16) than of **4** (7%; 5α-H,-3β-OSiDMTB; Fig. 18). Both compounds have an equatorial 3-OTBDMSi group, but differ at the A/B ring junction.

In the mass spectra of **2** (5β-H,3α-OSiDMTB) and its labeled analogues an ion 2 u heavier than $[M - t\text{-Bu} - H_2O - HSiMe_2OH]^+$ is of sufficient abundance to interfere with analysis of the extent of deuterium incorporation. The heavier ion may arise by elimination of $Me_2Si{=}O$ (74 u) from $[M - t\text{-Bu} - H_2O]^+$ (Scheme 49) or by elimination of the $C_4H_9SiMe_2O\cdot$ radical from $[M - H_2O]^+$ (Scheme 50). The $Me_2Si{=}O$ elimination was supported by observation of the appropriate metastable ion decomposition (B/E scan) of 3α-OSiDMTB-17α-hydroxy-17β-methyl-5β-androstane (**6**). For both proposed mechanisms, elimination of H_2O occurs from a species having a pseudoboat form of ring C. Expulsion of $Me_2Si{=}O$ or of $t\text{-}BuMe_2SiO\cdot$ then occurs. Although the involvement of 12-H is implicated, such a transfer has not been confirmed by deuterium-labeling.

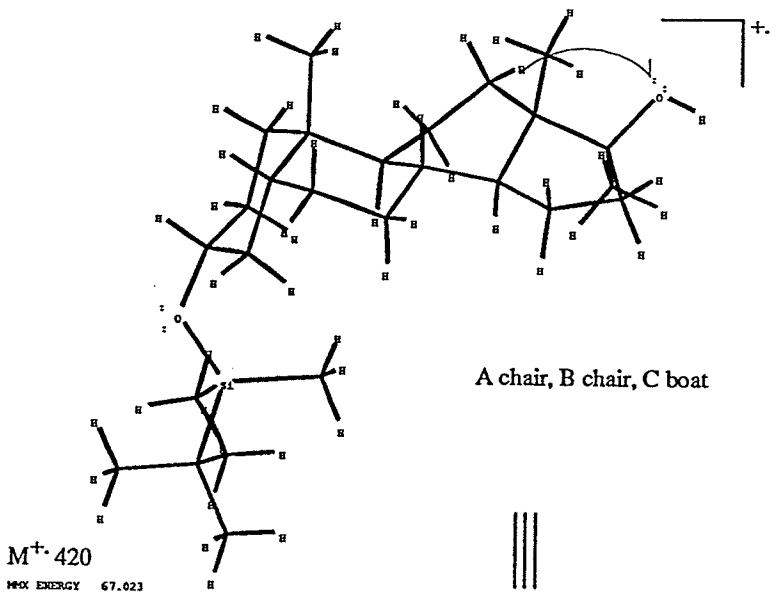
The extents of deuterium incorporation into the eliminated HMe_2SiOH species, after correction for loss of $Me_2Si{=}O$, are given in Table 23 for various labeled molecules, while Scheme 51 diagramatically summarizes the positional origin of the transferred hydrogens. The total transfer of H from the 2, 4, 6 and 7 sites is *ca* 34%, in close agreement with loss of 5β-H (33%). Since these results are very similar to those discussed above for HMe_2SiOH elimination from $[M - t\text{-Bu}]^+$ then similar fragmentation mechanisms are assumed, and will not be discussed further.

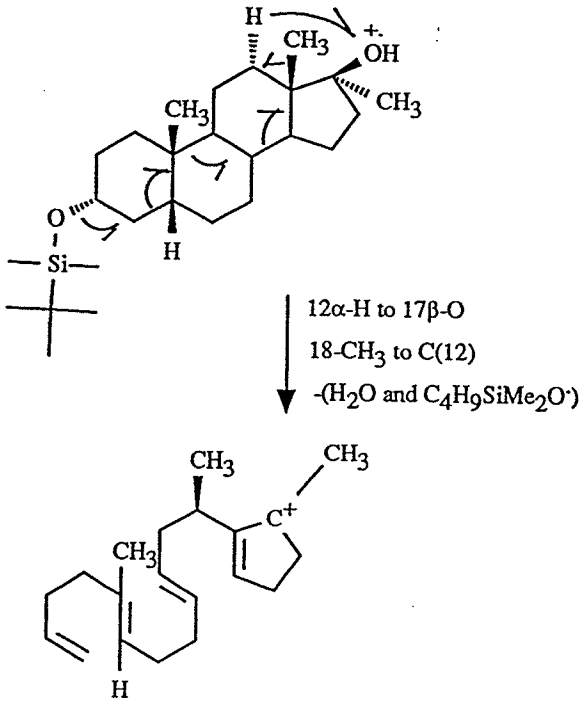
<u>Involvement of 9α-H together with 1α-H or 11α-H(?)</u>

Table 23 shows 41% loss of 9α-H, 20% loss of 1α-H, and possibly, 4% loss of 11α-H. If the losses of 1α-H and 11α-H are correlated with losses of 9α-H, then a further 41 - (20 + 4) = 17% of hydrogen eliminated together with 9α-H originates from undeter-



Scheme 49. A mechanism for $[M - 149]^+$ ion formation via an "unzip" sequence, when 12α-H is not available for transfer to Si^+.



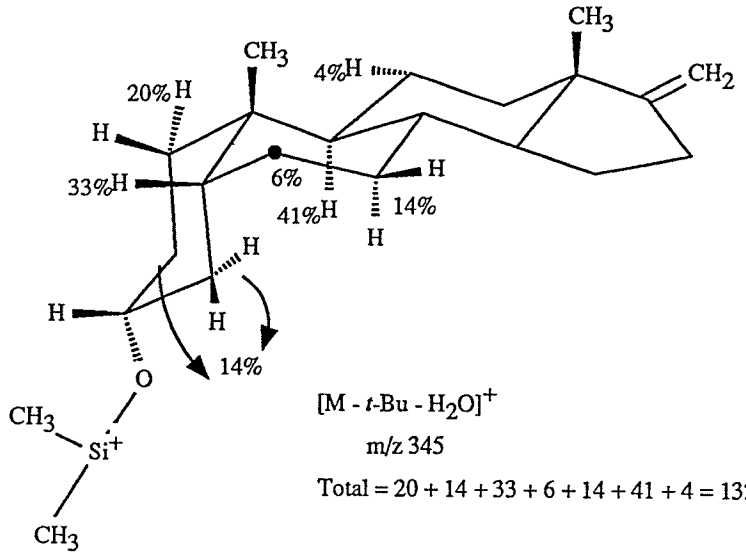


Scheme 50. Alternative mechanism leading to $[M - 149]^+$ by an "unzip" fragmentation for 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (2).

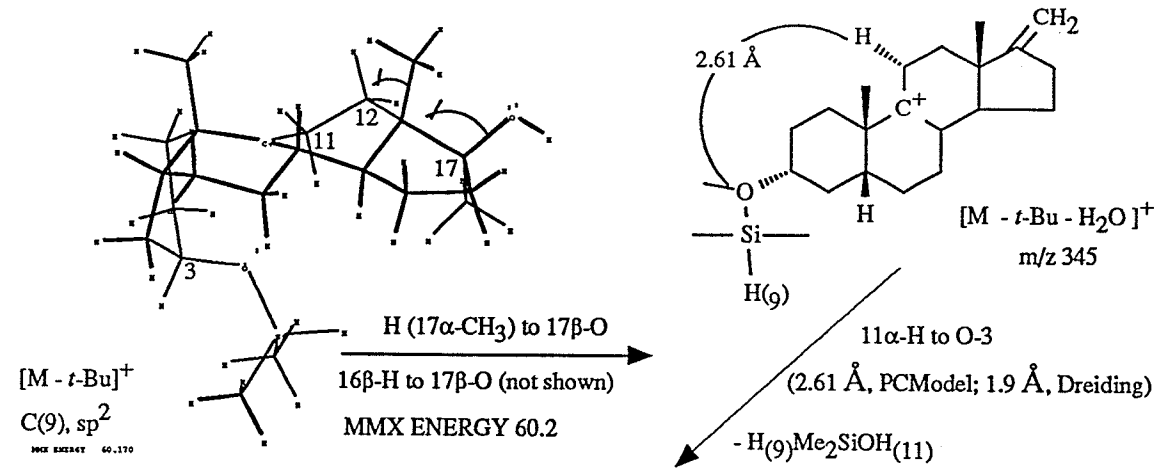
Table 23. Deuterium incorporation (%) into $HSiMe_2OH$ eliminated from the [M - *t*-Bu - H_2O]$^+$ ion of deuterium-labeled 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (**2**). (Experimental error ±5%)

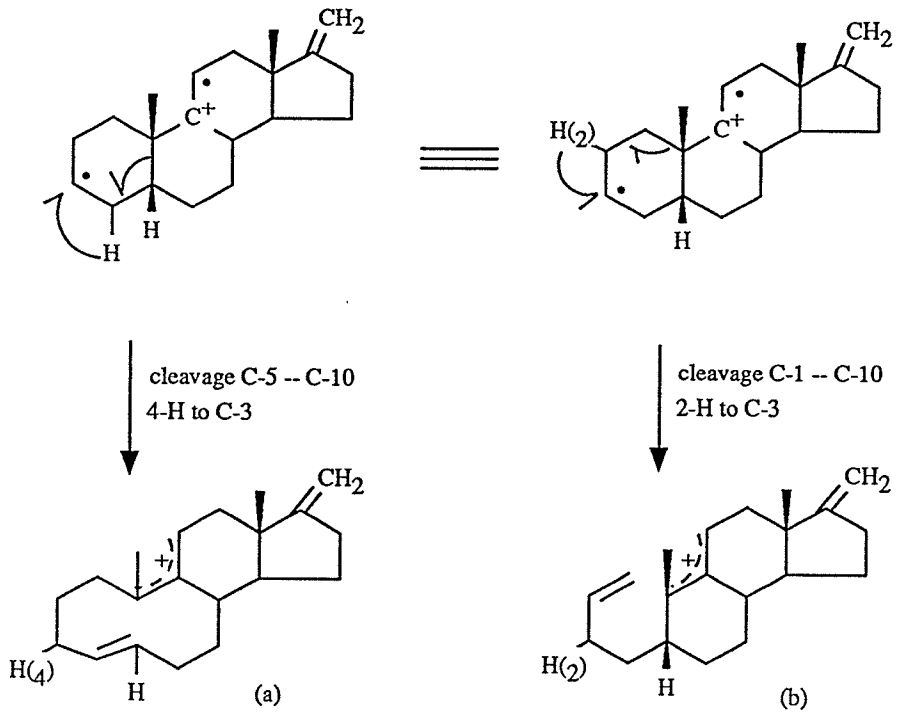
Label(s)	*deuterium in neutral*→	2d	1d	0d
1α,5β-d_2	**2-A**	-	53 (1α-D, 20%)[a]	47
1β,5β-d_2	**2-B**	-	33	67
2,2,4,4-d_4	**2-C**	-	14	86
3β-d_1	**2-D**	-	-	100
5β-d_1	**2-E**	-	33	67
6,6-d_2	**2-F**	-	6	94
7,7-d_2	**2-G**	-	14	86
5β,9α,11α-d_3	**2-I**	4	70 (9α-H; 41%)[b]	26
16,16-d_2	**2-J**	-	0	100
17β-CH_3-d_3	**2-K**	-	0	100

a 1α-H = 53 - 33 (5β-H) = 20% (±5%)
b 9α-H = 70 + 4 - 33(5β-H) = 41% (±5%)

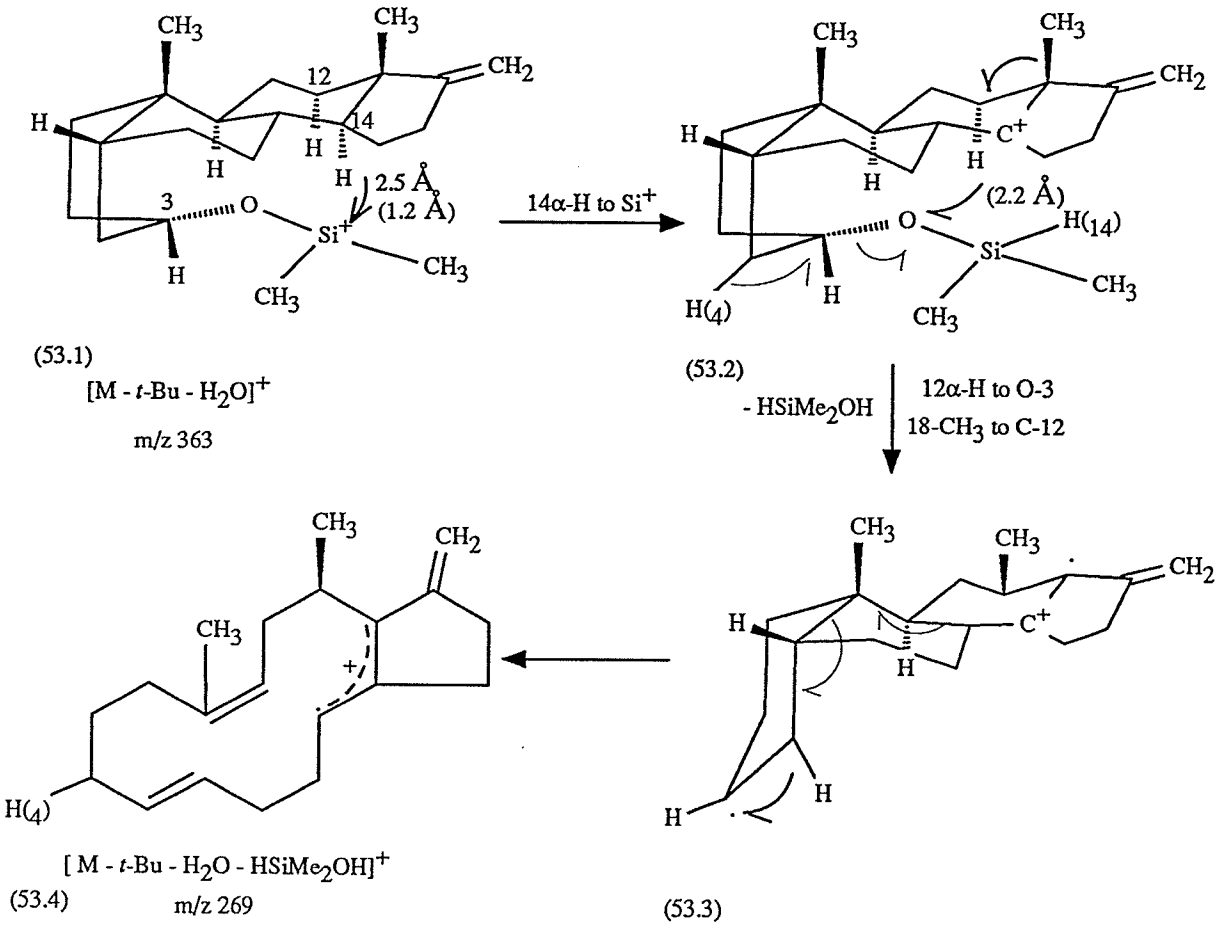


Scheme 51. Positional origin (%) of hydrogen transferred in HMe_2SiOH elimination from the [M - *t*-Bu - H_2O]$^+$ ion of 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (**2**).





Scheme 52. Proposed mechanism of $H_{(9)}SiMe_2OH_{(11)}$ elimination from the [M - *t*-Bu - H_2O]$^+$ ion of 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (**2**).

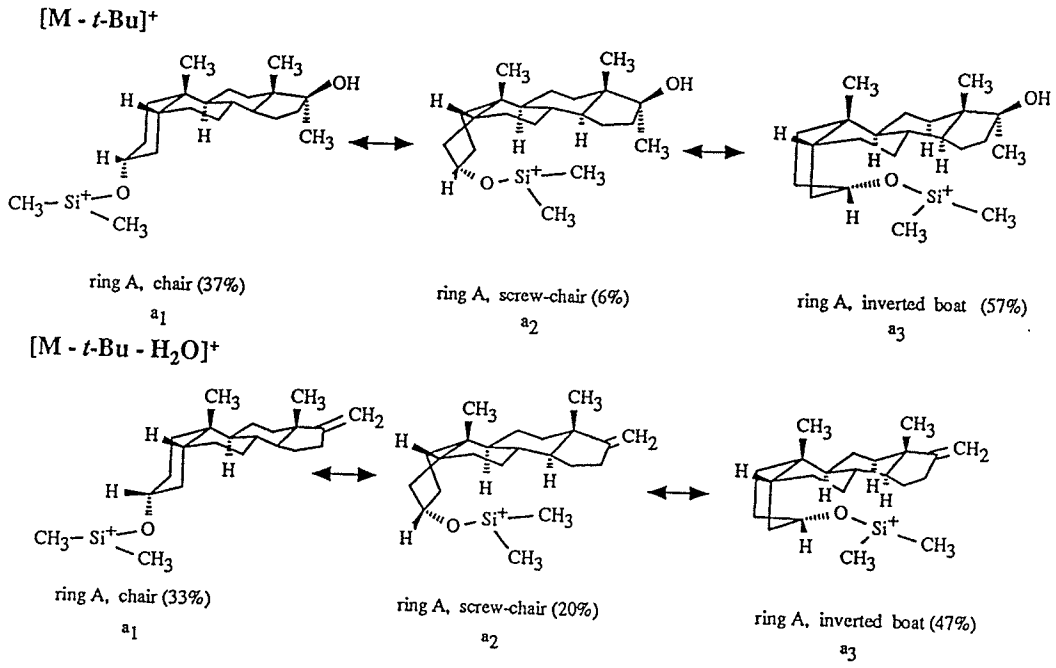


Scheme 53. Proposed mechanism of $H_{(14)}SiMe_2OH_{(12)}$ loss from the [M - *t*-Bu - H_2O]$^+$ ion of 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (**2**). (Distances determined by PCMODEL program and Dreiding model (in parentheses))

mined sites. A mechanism for loss of 9α-H together with 1α-H is depicted in Scheme 45, and one for loss of 9α-H together with 11α-H is depicted in Scheme 52.

Involvement of other hydrogens.

If the first hydrogen lost is assumed to come from 5β-H (33%) and 9α-H (41%) then the remaining 100 - (33 + 41) = 26% comes from undetermined sites. A further (100 - (14 + 6 + 14 + 20 + 4)) = 42% of the second hydrogen lost is of undetermined origin. Likely candidates for transfer are hydrogens from the 12α and 14α sites. A proposed mechanism is shown in Scheme 53, in which fragmentation is initiated by transfer of hydride from the 14α site to Si^+ (minimum separation 2.5 Å, PCMODEL, ring A, inverted boat) followed by abstraction of 12α-H by O-3, and 1,2 shifts of the 18-methyl angular group to C-12 and of 4-H to C-3, and then concerted electron shifts to give an allylic ion (53.4).



Scheme 54. Proposed conformational equilibria of the [M - *t*-Bu]$^+$ and [M - *t*-Bu - H_2O]$^+$ ions for 3α-OSiDMTB-17β-hydroxy-17β-methyl-5β-androstane (2).

To explain the elimination of $HSiMe_2OH$ from either $[M - t\text{-Bu}]^+$ or $[M - t\text{-Bu} - HSiMe_2OH]^+$ fragment ions, we assume that hydride transfer to Si^+ occurs from conformations a_1, a_2, and a_3 in the amounts shown in Scheme 54. These ions need not decompose from their lowest energy conformation after energization by EI. Thus, abstraction of 6-H or 11α-H implies a boat conformation of ring B or C (Schemes 43 and 52). The energy imparted to the molecular ion determines the rates of the various fragmentation processes.

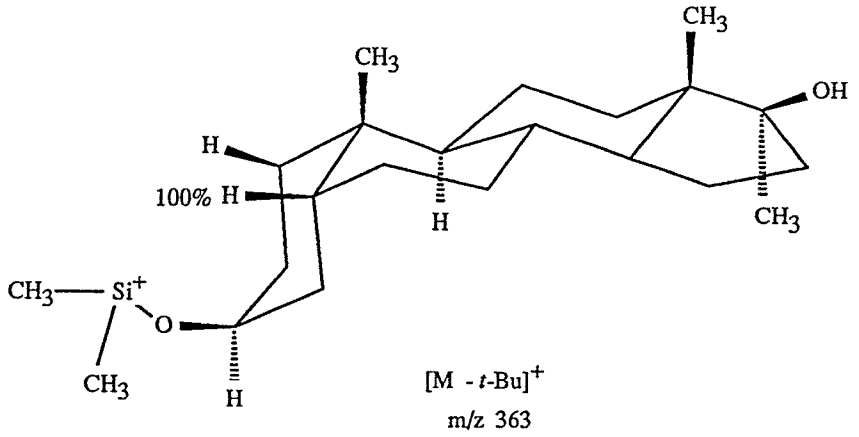
III.C.3. 3β-OSiDMTB-17β-Hydroxy-17α-methyl-5β-androstane (3).

Elimination of $HSiMe_2OH$ from the $[M - t\text{-Bu}]^+$ ion.

The $[M - t\text{-Bu} - HSiMe_2OH]^+$ ion, in the mass spectrum of **3**, is much more abundant (*ca* 16% RA; Fig. 17) than in that of its 3α-OSiDMTB epimer (*ca* 3.5% RA; Fig. 16), **2**. Table 24 shows the results, based on Table 16, of deuterium-labeling experiments, while the positional origin of the transferred hydrogens is shown diagramatically in Scheme 55.

Table 24. Deuterium incorporation (%) into $HSiMe_2OH$ eliminated from the $[M - t\text{-Bu}]^+$ and $[M - t\text{-Bu} - H_2O]^+$ ions of labeled 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (**3**). (Experimental error ± 5%)

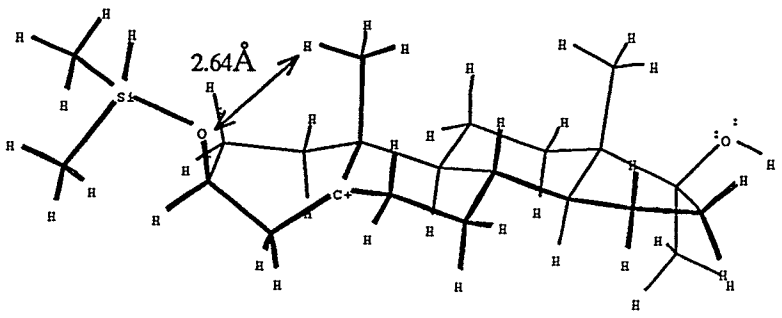
	parent ion →	$[M - t\text{-Bu}]^+$	$[M - t\text{-Bu} - H_2O]^+$		
label(s)	*deuterium in neutral*→	**1d**	**2d**	**1d**	**0d**
1α,5β-d_2	(A-3)	100	0	100	0
1β,5β-d_2	(B-3)	100	16	100	0
2,2,4,4-d_4	(C-3)	0	0	0	100
3α-d_1	(D-3)	0	0	0	100
5β-d_1	(E-3)	95	0	100	0
6,6-d_2	(F-3)	0	0	0	100
7,7-d_2	(G-3)	0	0	0	100
5β,9α,11α-d_3	(I-3)	100	0	90	10
16,16-d_2	(J-3)	0	0	0	100
17α-CH_3-d_3	(K-3)	0	0	0	100



Scheme 55. Positional origin (%) of hydrogen transfer in $HSiMe_2OH$ elimination from the $[M - t\text{-}Bu]^+$ ion of **3.**

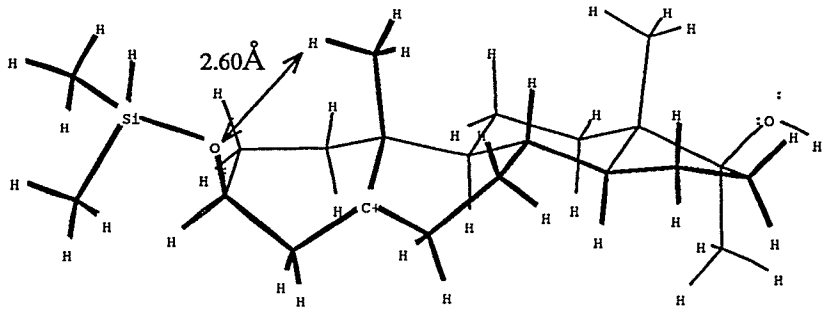
One hydrogen (*ca* 100%) originates from the 5β position, but the second does not come from any of the labeled sites (1α, 2, 3β, 4, 6, 7, 9α, 11α, 16 or 17α-CD_3) . We postulate that the second hydrogen rearranges from the angular 19-CH_3 group, because this is the only one that comes close to the 3β-$OSiMe_2H_{(5)}$ group, without opening of ring A or B. This statement is based on calculated distances between 3β-oxygen and neighbouring hydrogens for the two conformers given in Scheme 56 (see also Table 25). The MMX energies, also shown in Table 25, favour the chair form of ring B, but since the energy difference (ΔE = *ca* 6 kcal mol^{-1}) is small, then both conformers can be present in the ion source. For the favoured **A** conformer the closest 3β-O and 19-H separation is 2.64 Å, while for the **B** conformer, it is 2.60 Å (Scheme 56). Since the sum of the van der Waals radii of hydrogen (1.2 Å) and oxygen (2.4 Å) = 2.6 Å then hydrogen transfer might be possible for both conformers.

Mechanisms for $H_{(5)}SiMe_2OH_{(19)}$ elimination from these conformers are proposed in Schemes 57 and 58. In both schemes, the reaction is initiated, as discussed previously for the similar 5α-H,3α-OSiDMTB cis-diaxial structure of (**1**), by migration of hydride from the tertiary 5β site to positively charged silicon, to form a stable tertiary



(torsional angle H(2β)–C(2)–C(3)–O(3β) = -16.97°).

Conformer **A** (ring A, boat; ring B, chair)



(torsional angle H(2β)–C(2)–C(3)–O(3β) = -22.05°)

Conformer **B** (ring A, boat; ring B, boat)

Scheme 56. MM2 calculated geometry for two conformations of the [M - *t*-Bu]$^+$ ion of 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (**3**).

Table 25. Closest distances (Å) between 3β-O and neighboring hydrogens for the $[M - t\text{-Bu}]^+$ conformers of **3** depicted in Scheme 56.

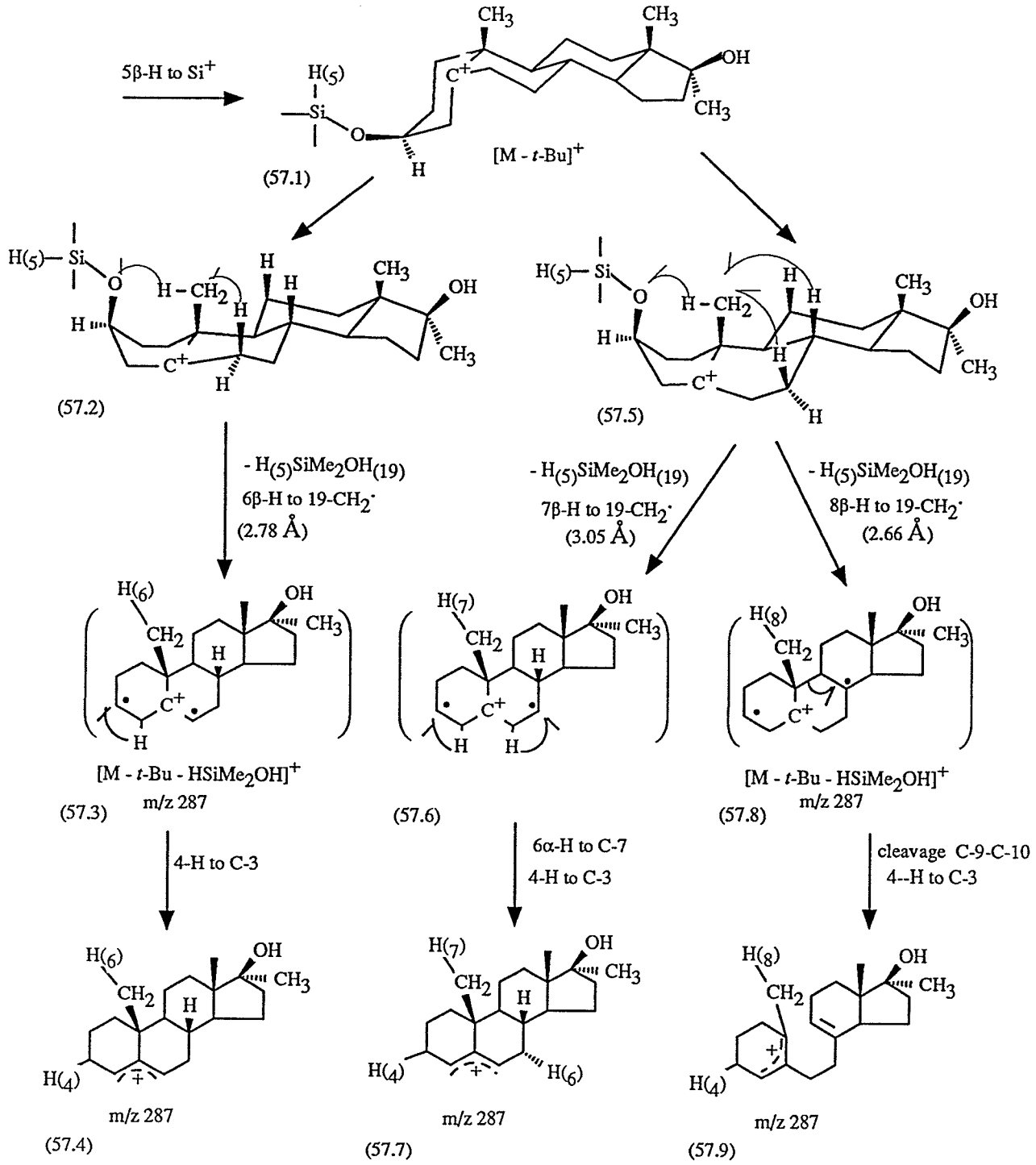
separation considered	structure of rings A and B	distance (Å)	MMX energy (kcal mol^{-1})	torsional angle $H_{2\beta}$-C(2)-C(3)-$O_{3\beta}$
O(3) - H(19-CH_3)	A boat, B chair[c]	2.64	43.1[a](54.01)[b]	-16.97°
O(3) - H(19-CH_3)	A boat, B boat[c]	2.60	49.0[a](60.29)[b]	-22.05°
O(3) - 2β-$H_{eq.}$	A boat, B boat	2.52		
O(3) - 6β-H_a	A boat, B chair	3.79		
O(3) - 7β-H_a	A boat, B boat	4.76		
O(3) - 8β-H_a	A boat, B chair	5.66		
O(3) - 8β-H_a	A boat, B boat	5.67		
O(3) - 11β-H_a	A boat, B boat	6.12		

a MMX energy of the last five iterations displayed on the background minimization screen (see Appendix II)

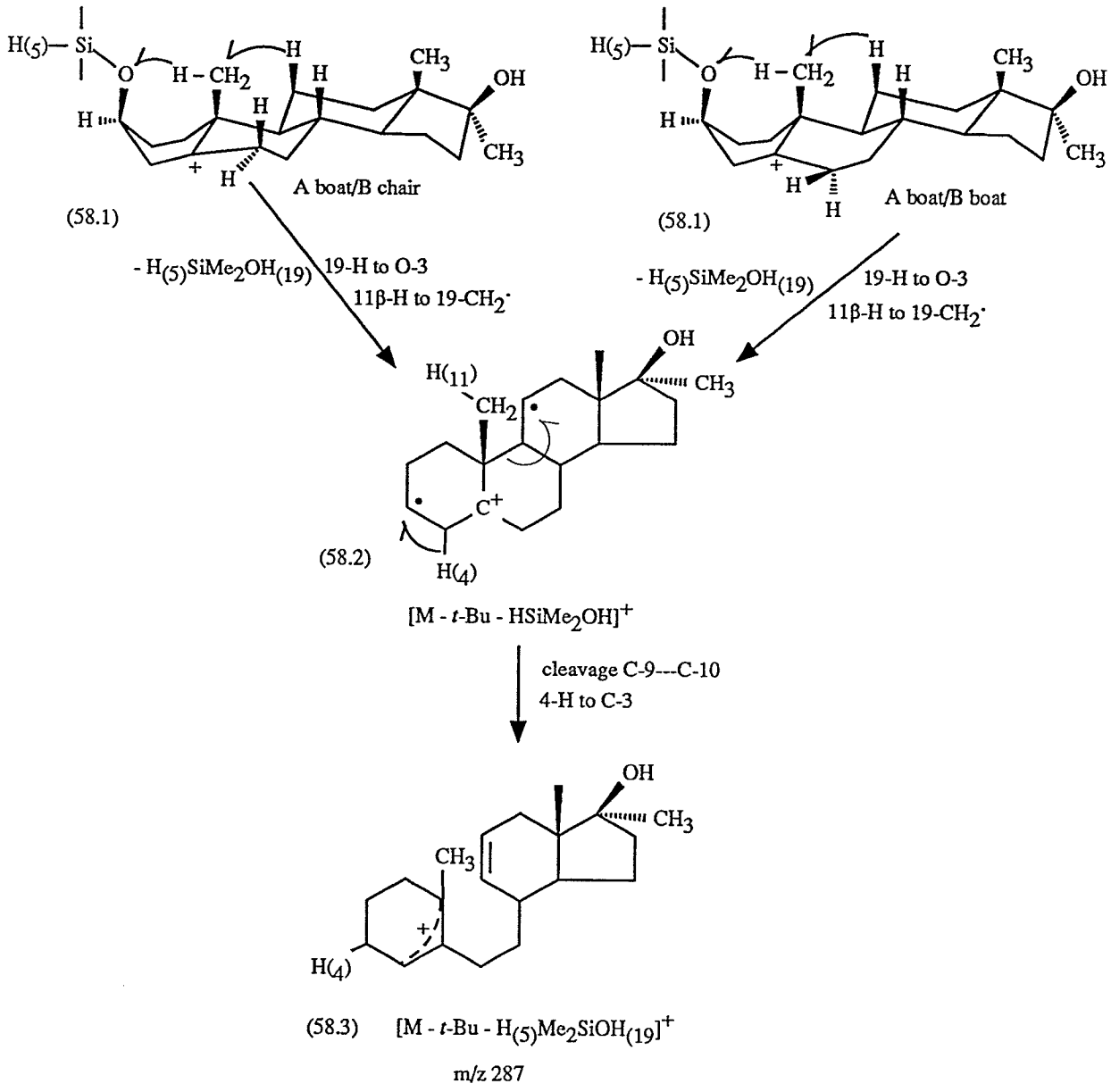
b MMX energy of (a) displayed in the minimization mode, as the minimization proces is completed (see Appendix II)

c $\Delta E = 49.0 - 43.1 = 5.9$ kcal mol^{-1}

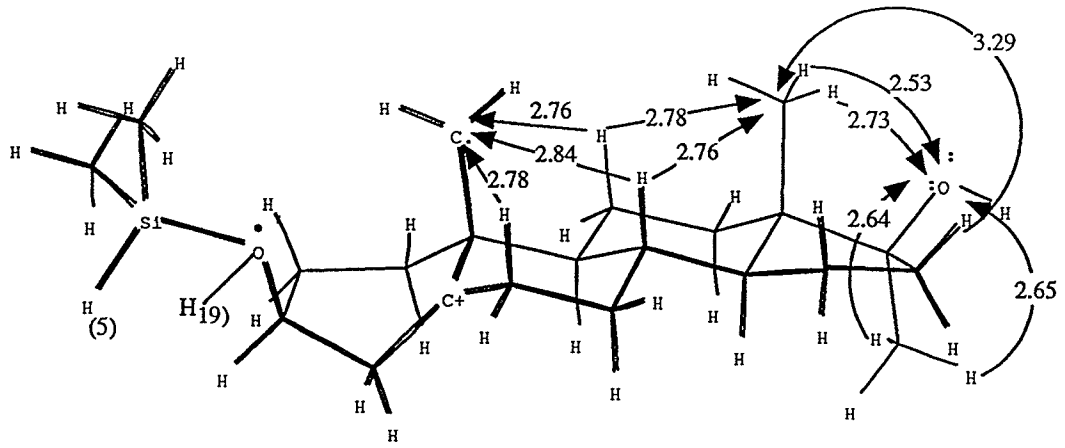
carbocation. The trigonal C-5 site permits ring A to adopt a low energy boat form, with an axial 3β-$OSiMe_2H_{(5)}$ substituent, in which the O-3 atom is close enough to the 19-CH_3 group for H transfer to occur. The newly formed 19-CH_2 radical site can accept a hydrogen from neighboring sites. Suitable sites are 6β, 7β or 8β (Scheme 57) or 11β (Scheme 58). The calculated distances between carbon of the 19-CH_2 radical and all four axial hydrogens of rings B and C, for conformer A, structures 57.2 and 57.5, are depicted in Scheme 59 and summarized in Table 26.. The 6β-H should be the most active, because it is adjacent to the C-5 positive charge. Its transfer to the 19-CH_2 radical would form the stabilized radical ion 57.3. Thus, the 19-CH_3 group may serve as an "antenna" to emit a hydrogen and then to receive another from the steroid skeleton. That is, the 19-methyl group acts as a catalyst.



Scheme 57. Proposed mechanism of $H_{(5)}SiMe_2OH_{(19)}$ elimination from the [M - *t*-Bu]$^+$ ion of 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane **(3).**



Scheme 58. Proposed mechanism of $H_{(5)}SiMe_2OH_{(19)}$ elimination from the [M - *t*-Bu]$^+$ ion of 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (3).



MMX Energy 61.7 $[M - t\text{-Bu}]^+$ intermediate

Scheme 59. Structure of the $[M - t\text{-Bu}]^+$ ion of **3**, having the 19-CH_2 radical centre, determined by PCMODEL, showing separations between 19-C, 18-C, 17-O and neighbouring hydrogens.

Table 26. Closest distances (Å) between 19-C and neighboring hydrogens for 3β-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (**3**).

	A boat/B chair		**A boat/B boat**	
	Sch. 57	**Sch. 59**	**Scheme 59**	
	19-$\underline{C}H_3$ (sp^3)	**19-$\underline{C}H_2$· (sp^2)**	**19-$\underline{C}H_3$ (sp^3)**	**19-$\underline{C}H_2$· (sp^2)**
2β-H_e - C	2.90	2.98	3.00	2.90
6β-H_a - C	2.85	2.78	eq.	eq.
7β-H_a - C	eq.	eq.	3.11	3.05
8β-H_a - C	2.90	2.84	2.75	2.66
11β-H_a - C	2.76	2.76	3.01	2.93

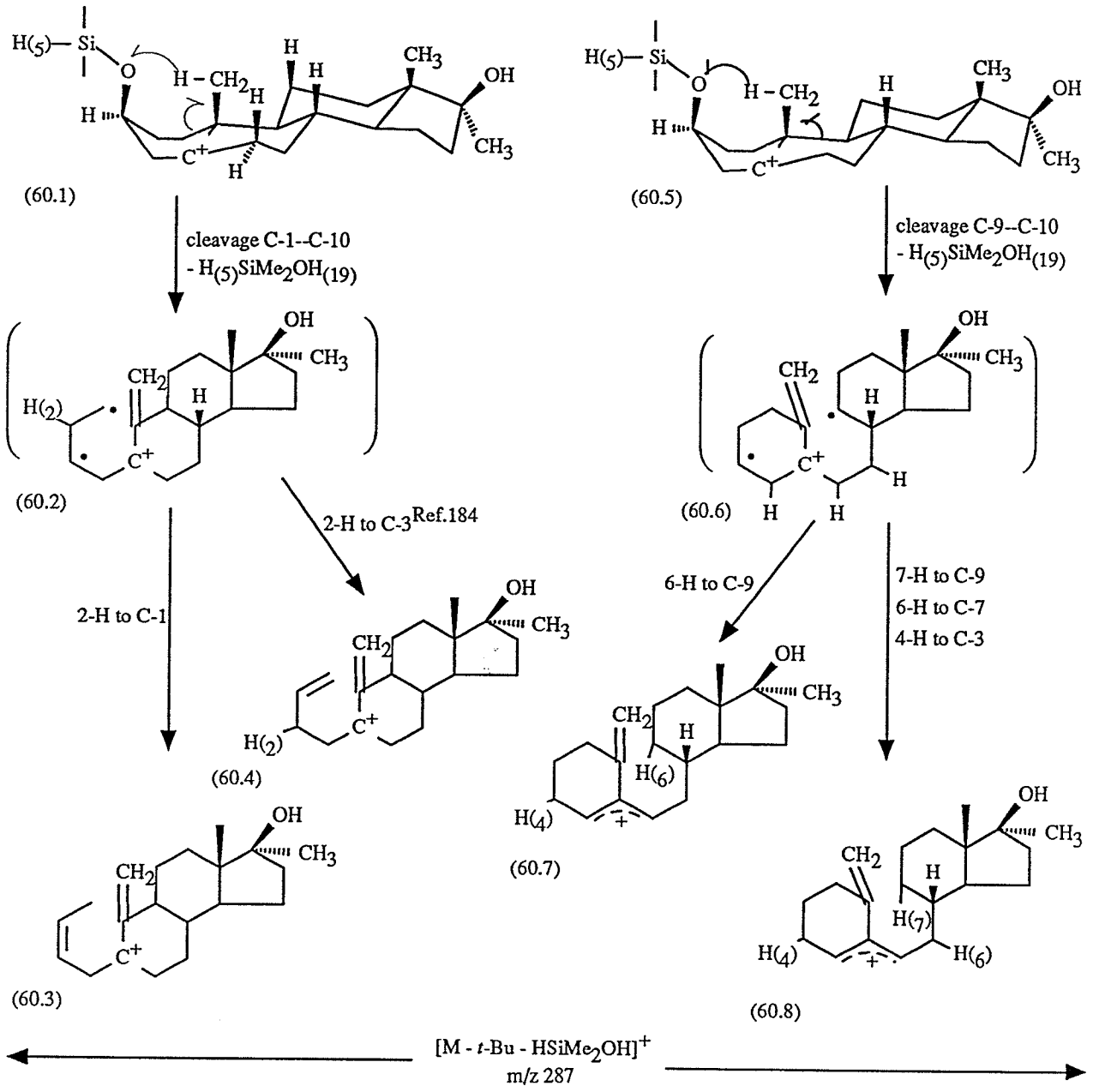
Although the distances between the 19-CH_2 radical and these hydrogens seem to be large, H-transfer has been observed at a distance of 3.29 Å , i.e. for transfer of the 16β-H to the 18-CH_2 radical[163] site, or at 3.11 Å or 3.32 Å for transfer of the 16β-H or 16α-H, respectively, to the C-13 radical site[179].

In an alternative proposal, Scheme 60, the 19-CH_2 radical site triggers fission of one of neighbouring bonds, i.e. C-1–C-10 or C-9–C-10. Fission of the C-1–C-10 bond, with 1,2 shifts of H-2 to C-1 or to C-3, would produce the allylic cations 60.3 or 60.4, respectively. On the other hand, fission of C-9–C-10 bond, with migration of 6-H to C-9 and 4-H to C-3, or migration of 7-H to C-9 followed by 1,2 shifts of 6-H to C-7 and 4-H to C-3, would produce the allylic cations 60.7 and 60.8, respectively.

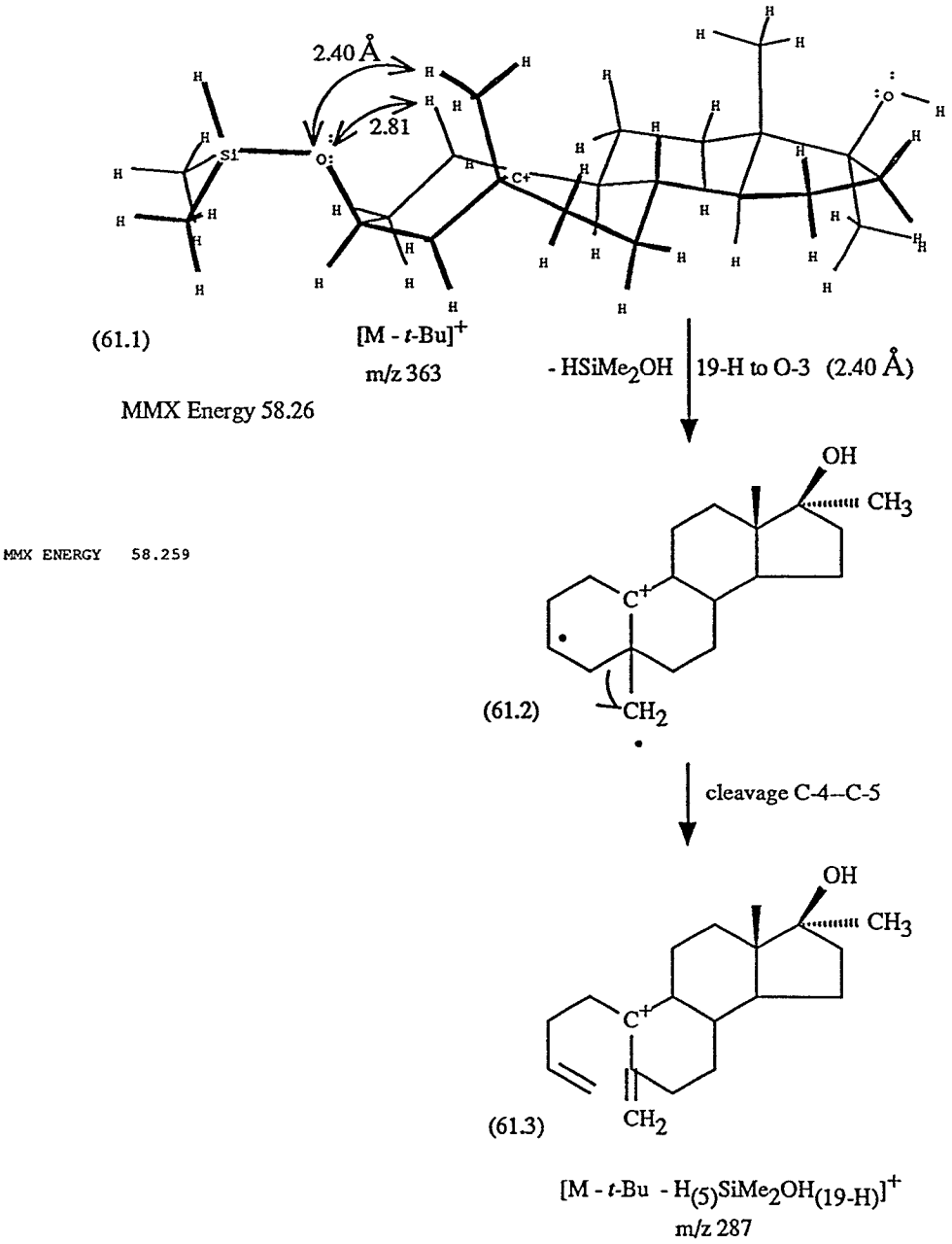
Yet another mechanism is depicted in Scheme 61. After transfer of 5β-H to Si^+ and migration of 19-CH_3 to C-5, the 3β-oxygen can abstract a hydrogen from the 19-CH_3 group (2.35 Å separation, Dreiding model) to give the allylic cation 61.1. A similar 1,3-hydrogen transfer of a methyl hydrogen to the etheral oxygen was reported in the mass spectra of cis-1,3,5-trimethoxycyclohexane and other ethers[180-182]. The involvement of the hydrogens of the 19-CH_3 group is inferred by their steric availability but has not been confirmed by deuterium labeling.

Elimination of $HSiMe_2OH$ from the $[M - t\text{-Bu} - H_2O]^+$ ion.

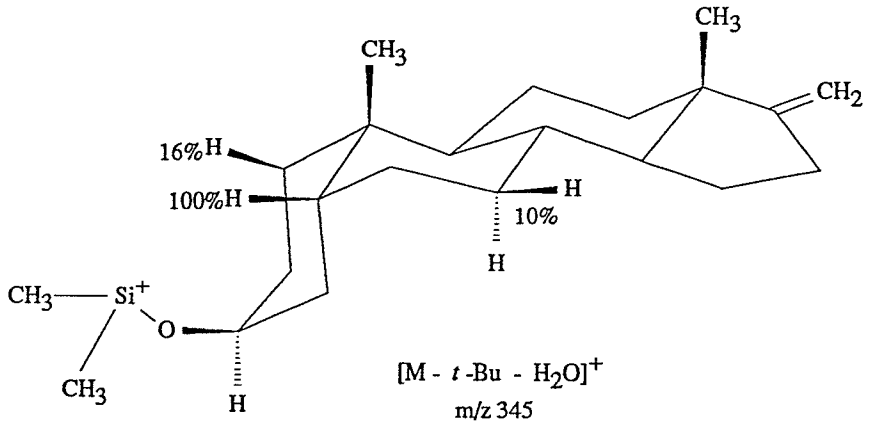
Elimination of $HSiMe_2OH$ from $[M - t\text{-Bu} - H_2O]^+$ involves quantitative, or near quantitative, loss of the axial 5β hydrogen (100%), and a small loss of hydrogen from the 1β (*ca* 16%) and C-7 (*ca* 10%) positions (Table 24 and Scheme 62). No other hydrogens from labeling positions are lost. The preferred abstraction of 5β-H is revealing. Since loss of 1β-H and 5β-H both occur, we propose that this reaction may occur from the conformation shown in Scheme 63, in which ring A is in the chair form, and the axial 1β-H is suitably positioned for abstraction. The minimum separation between Si^+ and



Scheme 60. Proposed mechanism of $H_{(5)}SiMe_2OH_{(19)}$ elimination from the [M - *t*-Bu]$^+$ ion of 3β-OSiDMTB-17α-methyl-17β-hydroxy-5β-androstane (**3**), involving fission of C-1–C-10 or C-9–C-10 bonds.



Scheme 61. Proposed mechanism of $HSiMe_2OH$ elimination from the $[M - t\text{-}Bu]^+$ ion of **3** involving 19-H, after 1,2 shift of 19-CH_3 from C-10 to C-5. (Distances determined by PCMODEL)

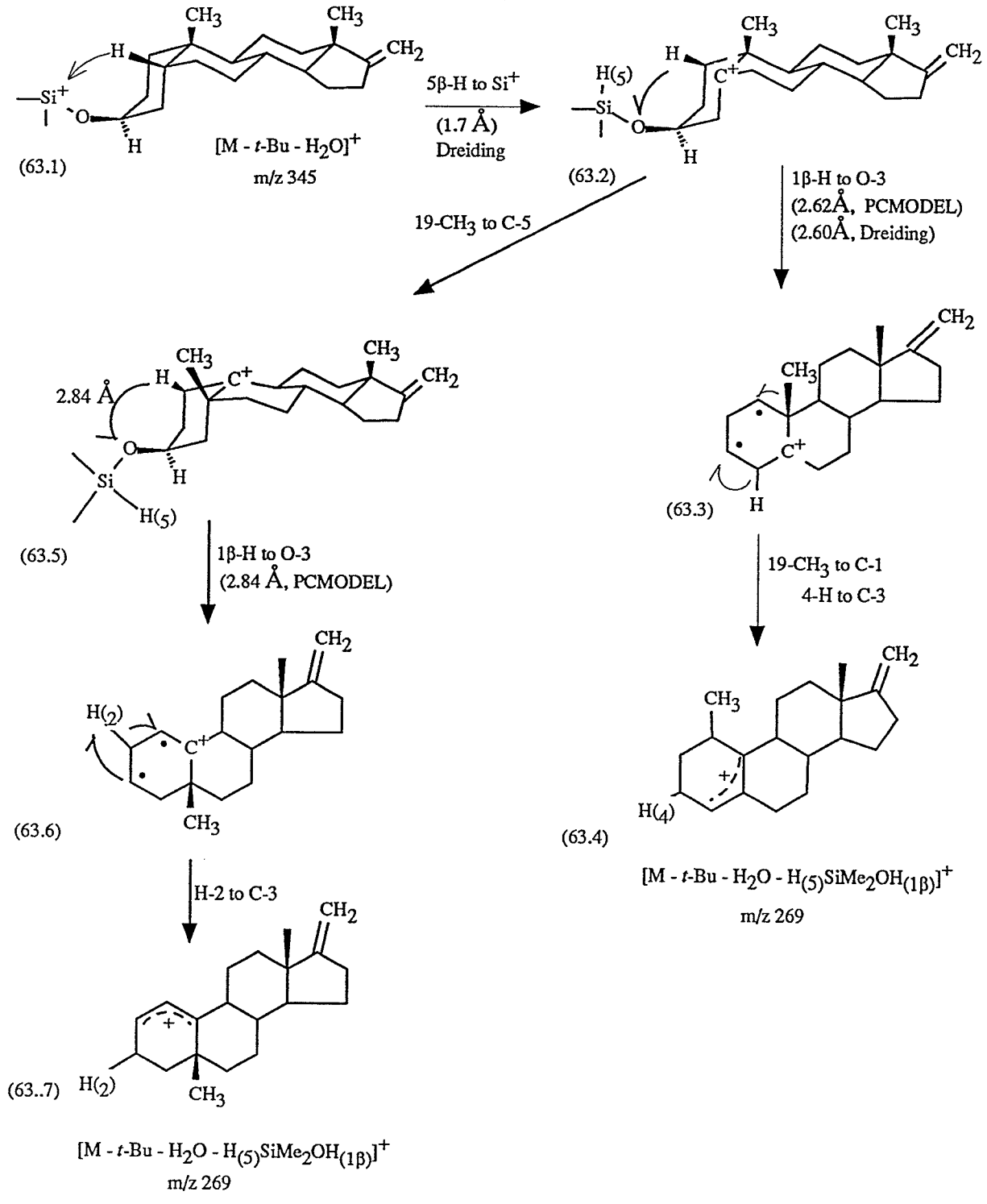


Scheme 62. Positional origin of hydrogen loss in $HSiMe_2OH$ elimination from the $[M - t\text{-Bu} - H_2O]^+$ ion of **3**.

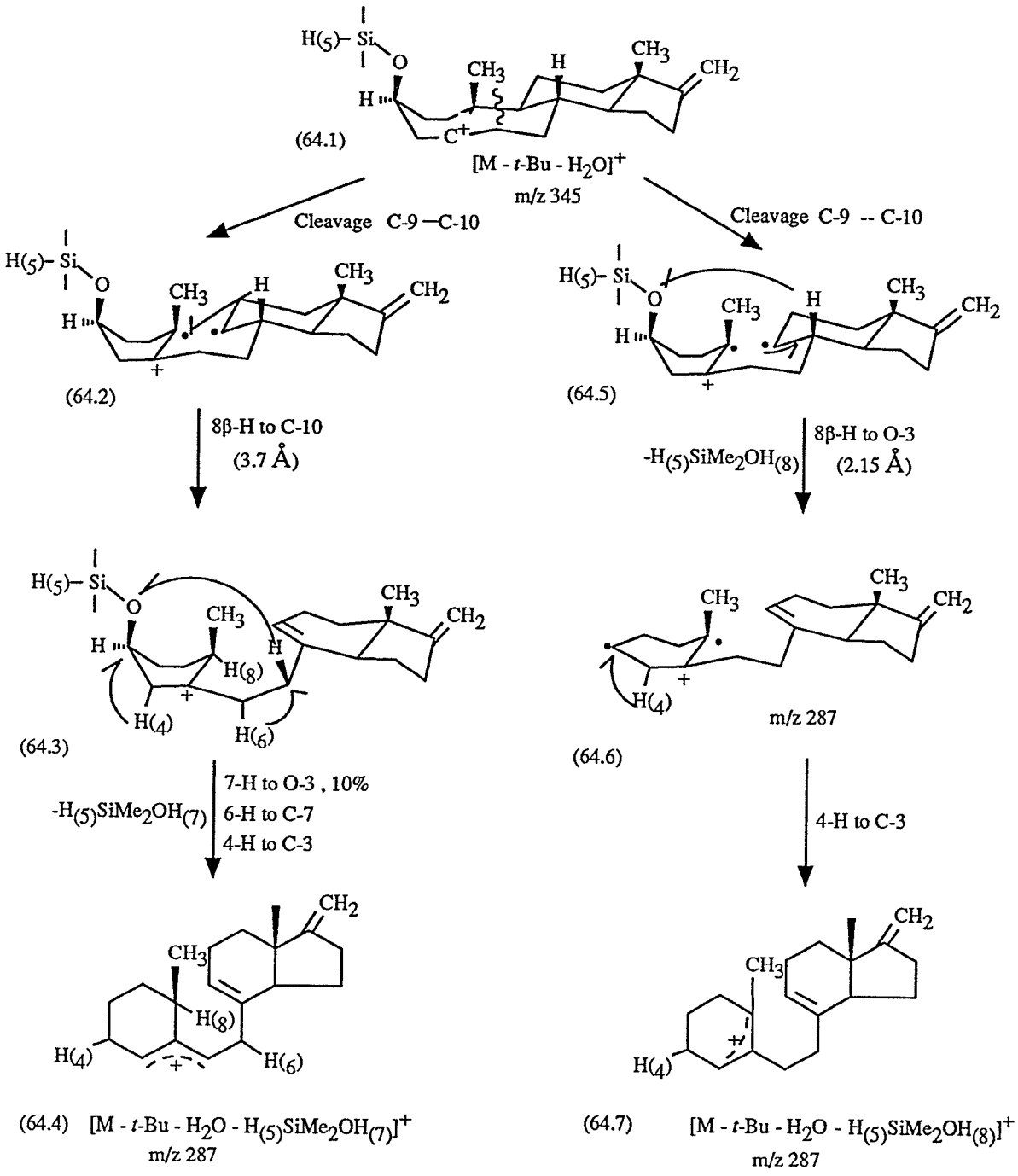
5β-H is 1.7 Å (Dreiding model) and between 1β-H and 3β-O is 2.62 Å (PCMODEL or Dreiding model). Mechanisms for this reaction are proposed in Scheme 63. One mechanism assumes a 1,2 shift of the 19-methyl group to C-5 (63.2 → 63.5), while the second one assumes a 1,2 shift of the 19-CH_3 to C-1 (63.3 → 63.4).

Alternative mechanisms involving fission of the C-9–C-10 bond and transfer of 7-H or 8β-H to O-3 are proposed in Scheme 64. This fragmentation may promote migration of 8β-H to the C-10 radical site (64.2) and formation of the C-8–C-9 double bond (64.3), which, in turn, would activate the rearrangement of 7-H to 3β-oxygen. Next, 1,2-shifts of hydrogens 6-H to C-7 and 4-H to C-4 would produce the allylic cation (64.4).

A Dreiding molecular model also shows that, after fission of the C-9–C-10 bond, 8β-H can approach 3β-O within a bonding distance of 2.15 Å. Scheme 64 (64.5) depicts transfer of 8β-H to the 3β-$OSiMe_2H_{(5)}$ residue. In the next step formation of the C-8–C-9 double bond, followed by a 1,2-shift of 4-H to C-3 leads to formation of the allylic cation 64.7.



Scheme 63. Proposed mechanism of $HSiMe_2OH$ elimination from the $[M - t\text{-Bu} - H_2O]^+$ ion of **3**, involving transfer of 1β-H and 5β-H atoms. (Estimated distances are shown)



Scheme 64. Proposed mechanisms of $H_{(5)}SiMe_2OH_{(7)}$ and $H_{(5)}SiMe_2OH_{(8)}$ elimination from the $[M - t\text{-Bu} - H_2O]^+$ ion for 3β-OSiDMTB-17α-methyl-17β-hydroxy-5β-androstane **(3)**. (Distances determined by Dreiding models)

III.C.4. 3β-OSiDMTB-17β-Hydroxy-17α-methyl-5α-androstane (4).

The [M - *t*-Bu - $HSiMe_2OH$]$^+$ ion.

This ion is formed by elimination of $HSiMe_2OH$ from [M - *t*-Bu]$^+$. In contrast to the mass spectra of isomers **1-3**, where it is a prominent ion (Figs. 15, 16, and 17), its abundance is here very small (1.9% RA; Fig. 18). Presumably, the double hydrogen rearrangement required for $HSiMe_2OH$ elimination from the [M - *t*-Bu]$^+$ ion does not readily occur.

The [M - *t*-Bu - H_2O - $HSiMe_2OH$]$^+$ ion.

This low abundance ion (4.2% RA, Fig. 18) can be generated mainly by loss of $HSiMe_2OH$ from [M - *t*-Bu - H_2O]$^+$. The origin of the hydrogens incorporated into $HSiMe_2OH$ was determined from the parent isotope cluster peaks, [M - *t*-Bu - H_2O]$^+$, and the product isotope cluster peaks, [M - *t*-Bu - H_2O - $HSiMe_2OH$]$^+$, given in Table 16. The results, presented in Table 27 and Scheme 65, show that abstraction occurs from the 2,4 (14%), 5α (58%), 6 (12%), and 7 (28%) sites. The sum of the transfers from 2,4, 6ξ and 7ξ (*ca* 54% (±5%)) is consistent with the observed loss from the 5α-site (*ca* 58% (±5%)). Thus, we may deduce that losses of hydrogen atoms from the C-2,4, C-6 and C-7 sites could be correlated with loss of 5α-H in the double hydrogen transfer.

To see whether the abstraction of the secondary 7(α?) or 6(α?) hydrogens, presumably by 3-oxygen, could involve the chair (transfer of 7-H) or boat (transfer of 6-H) conformations of ring B the minimum interatomic separations were evaluated (Table 28). These separations obtained greatly exceed the required bonding distance (~ 2.6 Å).

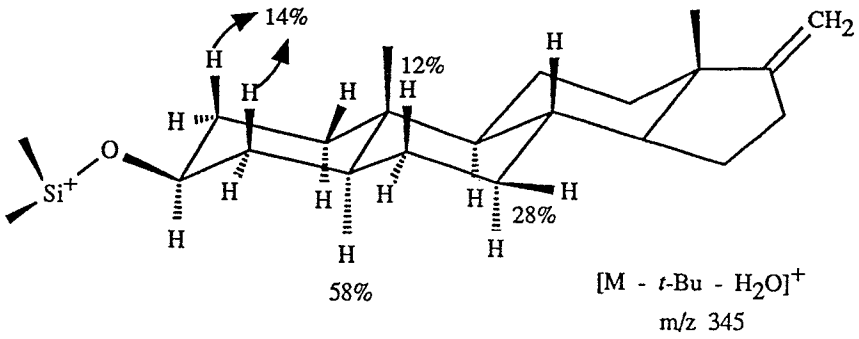
To explain the hydrogen transfers, fission of the C-1–C-10 bond is invoked, after which the separation between the 5β-H and Si^+ becomes *ca* 0.7 Å (Dreiding model). The proposed fragmentation mechanisms are shown in Scheme 66. Thus, C-1–C-10 bond fission, transfer of hydride from the C-5 site to Si^+ gives the carbocations (66.3-5).

Further, these intermediates lead to the allylic cations (66.6-8).

Table 27. Deuterium loss (%) in $HSiMe_2OH$ elimination from the $[M - t\text{-Bu} - H_2O]^+$ ion of labeled 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane **(4)**. (Experimental error ±5%)

label(s)		% D loss	
1α,5α-d_2	**(A-4)**	47	(i.e. 0% from 1α)
1β,5α-d_2	**(B-4)**	49	(i.e. 0% from 1β)
2,2,4,4-d_4	**(C-4)**	14	
3β-d_1	**(D-4)**	0	
5α-d_1	**(E-4)**	58	
6,6-d_2	**(F-4)**	12	
7,7-d_2	**(G-4)**	28	
5α,9α,11α-d_3	**(I-4)**	56	(i.e 0% from 9α)
16,16-d_2	**(J-4)**	0	
17β-CH_3-d_3	**(K-4)**	0	

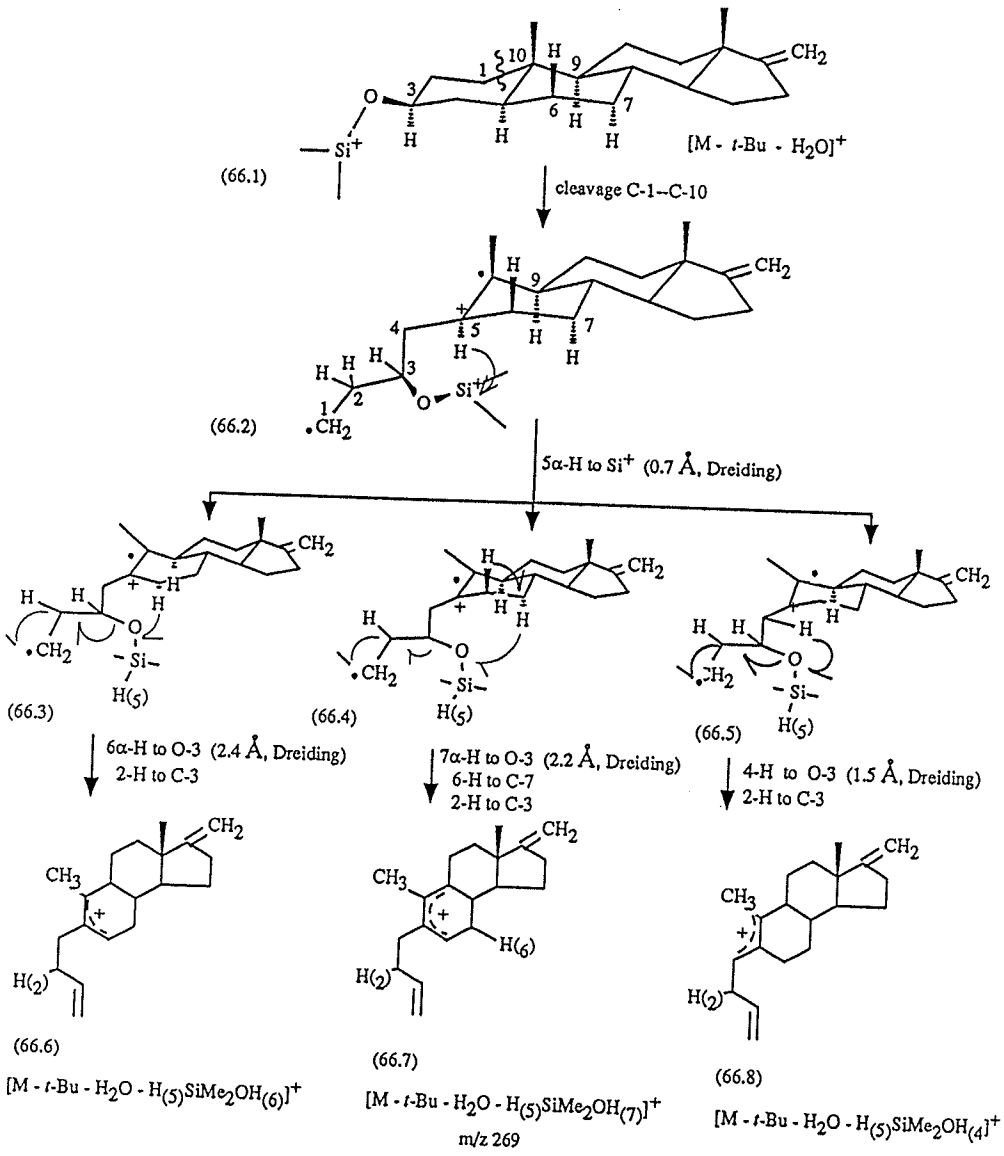
Total hydrogen loss = Σ(5α-H + 2,4-ξ-H + 6ξ-H + 7ξ-H) = 58 + 14 + 12 + 28 = 112% (±5)



Scheme 65. Positional origin (%) of hydrogen transferred in $HSiMe_2OH$ elimination from the $[M - t\text{-Bu} - H_2O]^+$ ion of 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane **(4)**.

Table 28. Minimum separations (Å) between 3β-O and neighboring hydrogens in the $[M - t\text{-Bu} - H_2O]^+$ ion of 3β-OSiDMTB-17β-hydroxy-17α-methyl-5α-androstane **(4)**.

	Conformation	Interatomic distances		
atoms considered	**C(5) sp^2**	**PCMODEL**	**Dreiding model**	**After fission of C(1)-C(10) bond (Dreiding model)**
O-3 — 4β-H	A chair, B boat	2.60	2.7	-
O-3 — 6α-H	A $boat_{inv}$, B boat	4.48	4.7	2.4 (A open, B boat)
O-3 — 6β-H	A boat, B chair	3.74	4.1	-
O-3 — 7β-H	A $boat_{inv}$, B chair	5.21	5.4	2.2 (A open, B chair)
O-3 — 7β-H	A boat, B chair	4.76	4.7	-
O-3 — 9α-H	A $boat_{inv}$, B chair		4.1	1.5



Scheme 66. Mechanisms proposed for elimination of $HSiMe_2OH$ from the [M - *t*-Bu - H_2O]$^+$ ion, for **4**, involving 5α-H together with 6-H, 7-H or 4-H.

III.C.5. 3ξ-OSiDMTB-17α-Hydroxy-17β-methyl-5ξ-androstanes (5-8) and their deuterium-labeled analogues (5-E to 8-E).

The mass spectra of the compounds, **5-8** and **E-5** to **E-8**, are presented in Fig. 19 and the RA% values of peaks of selected ion types are summarized in Table 29. The deuterium compositions (%) for the labeled compounds are given in Table 30. In each spectrum, the molecular ion is not detectable and the m/z 75 ions form the base peak.

As usual, the initial fragmentation is loss of the *t*-butyl radical. Howewer, the $[M - C_4H_9]^+$ ions of compounds **5-7** are of very low abundance. For all four compounds, the $[M - t\text{-Bu} - H_2O]^+$ ions are prominent. The ease of water elimination from the $[M - t\text{-Bu}]^+$ ion may be due to the closer proximity of the pseudoaxial 17α-OH group to the axial 12α and 14α hydrogens, but confirmation of this must await deuterium labeling studies. For **5**, **6**, and **7**, two ions, i.e. $[M - t\text{-Bu} - H_2O]^+$ and $[M - t\text{-Bu} - H_2O - HSiMe_2OH]^+$, are prominent. In addition, for **6**, the $[M - t\text{-Bu} - H_2O - Me_2Si{=}O]^+$ ion, $[M - 149]^+$, (m/z 271) is also prominent.

In the high mass region, for the labeled isomers **5-E**, **6-E**, and **7-E**, there are only two abundant ions, i.e $[M - t\text{-Bu} - H_2O]^+$ and $[M - t\text{-Bu} - H_2O - DMe_2SiOH]^+$. The incorporation of hydrogen, from the C-5 site, into $HSiMe_2OH$ lost from $[M - t\text{-Bu} - H_2O]^+$ is shown in Scheme 67, based on the results of Table 29. For isomer **8-E**, $[M - t\text{-Bu}]^+$ and $[M - t\text{-Bu} - H_2O]^+$ are prominent. In addition, peaks at m/z 271 and m/z 272 are prominent in the spectra of compound **6** and its labeled analog **6-E**, respectively. These ions are produced by successive losses of H_2O and $Me_2Si{=}O$ from $[M - t\text{-Bu}]^+$, as supported by metastable decompositions (B/E scans).

In the spectrum of **6-E**, the ion triplet m/z 269, m/z 270, and m/z 272 can be assigned as $C_{20}H_{29}^+$, $C_{20}H_{28}D^+$, and $C_{20}H_{30}D^+$, respectively. Thus, $C_{20}H_{29}^+$ arises by loss of DMe_2SiOH, and $C_{20}H_{28}D^+$ by loss of $HSiMe_2OH$, from $[M - t\text{-Bu} - H_2O]^+$. $C_{20}H_{30}D^+$ can be formed by $Me_2Si{=}O$ elimination from $[M - t\text{-Bu} - H_2O]^+$ (or, in the latter case, by elimination of a $C_4H_9SiMe_2O$ radical from $[M - H_2O]^{+\cdot}$). Previously, this

Figure 19. Positive EI mass spectra of all four unlabeled (**5-8**) and 5ξ deuterium-labeled (**5-E** to **8-E**) 3ξ-OSiDMTB-17α-hydroxy-17β-methyl-5ξ-androstanes.

Figure 19.

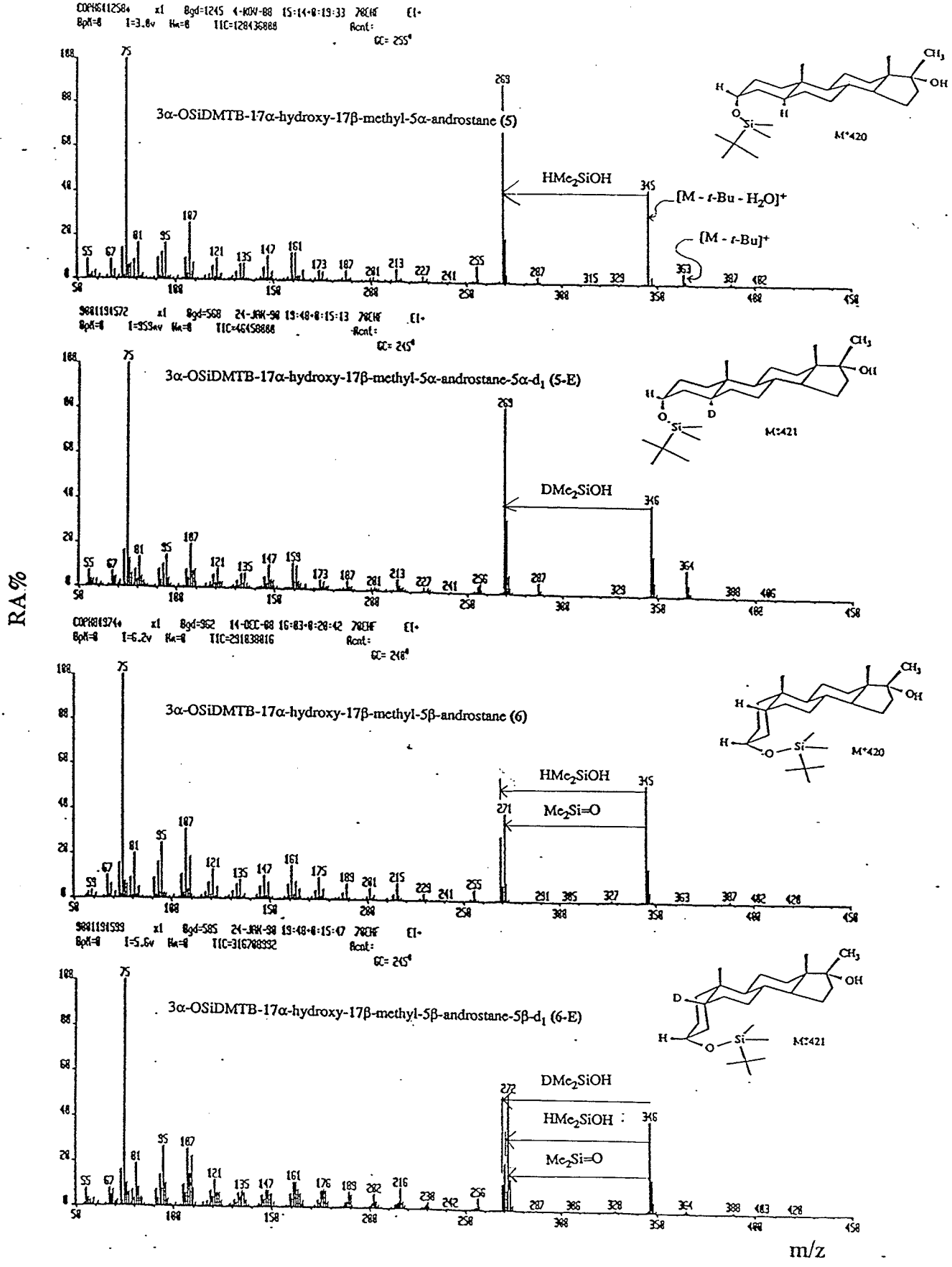

3α-OSiDMTB-17α-hydroxy-17β-methyl-5α-androstane (5)
HMe2SiOH
[M - t-Bu - H2O]+
[M - t-Bu]+
M+420
3α-OSiDMTB-17α-hydroxy-17β-methyl-5α-androstane-5α-d1 (5-E)
DMe2SiOH
M+421
3α-OSiDMTB-17α-hydroxy-17β-methyl-5β-androstane (6)
HMe2SiOH
Me2Si=O
M+420
3α-OSiDMTB-17α-hydroxy-17β-methyl-5β-androstane-5β-d1 (6-E)
DMe2SiOH
HMe2SiOH
Me2Si=O
M+421
RA %
m/z

Figure 19 (continued)

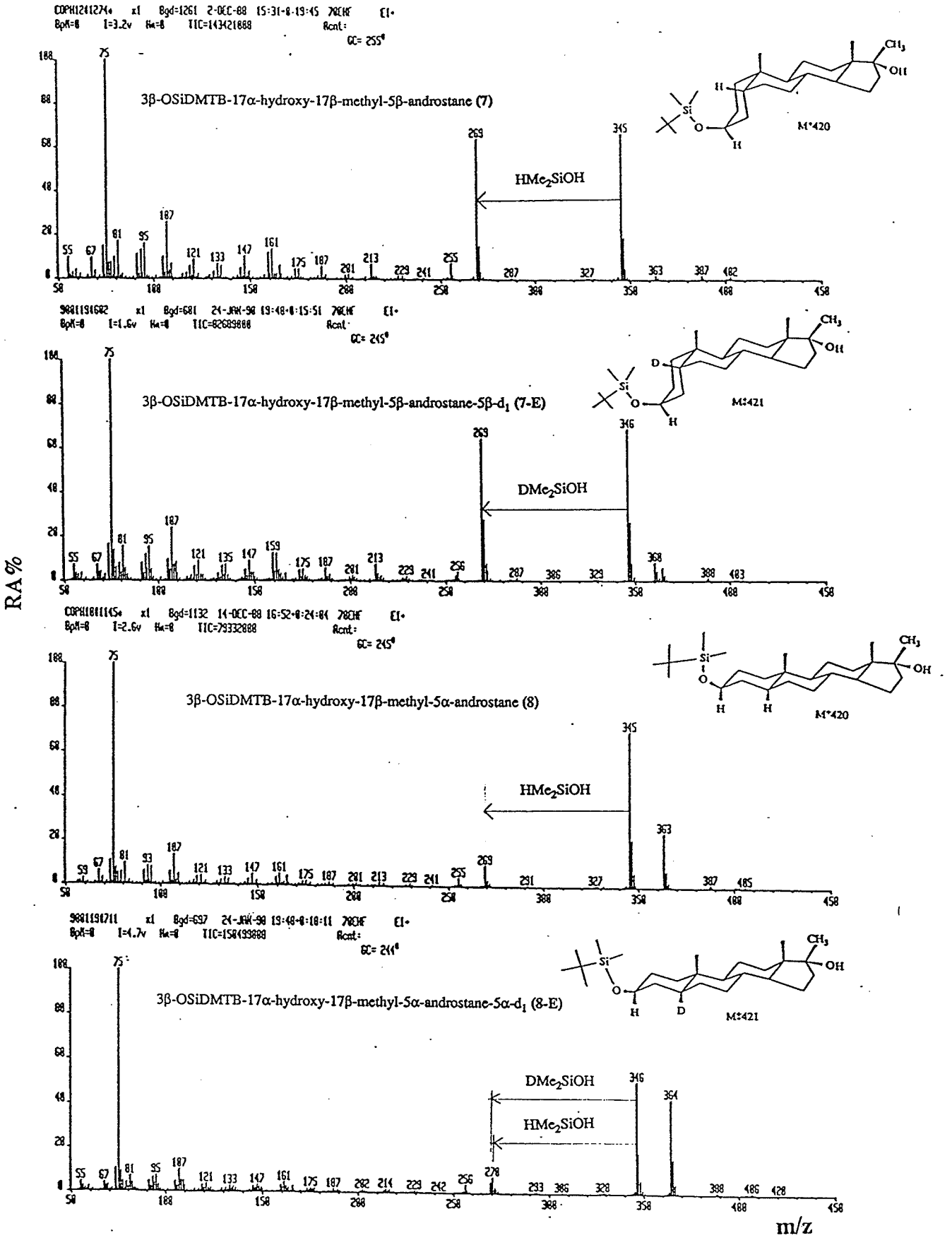


Table 29. Relative abundance (%) of mass spectral peaks of selected ion types of 3ξ-OSiDMTB-17α-hydroxy-17β-methyl-5ξ-androstanes (**5-8**) and their 5ξ deuterium labeled analogs (**5-E** to **8-E**).

synthesized compound	FRAGMENT ION (m/z, %RA)				
	$[F_5]^+$	$[F_4]^+$	$[F_3]^+$	$[F_2]^+$	$[F_1]^+$
	[M - CH_3 -H_2O TBDMSiOH]	[F_3 - - H_2O] or [F_2 - -$HSiMe_2OH$]$^+$	[F_1 - $HSiMe_2OH$]	[F_1 - H_2O]$^+$	[M - *t*-Bu]$^+$
5α-H,3α-OSiDMTB (5)	255/7.3 256/1.5	269/90.0 270/20.1 271/ 3.5* 272/ 0.5*	287/2.7 288/0.6	345/42.6 346/- 347/3.1	363/4.8 364/1.3
5β-H,3α-OSiDMTB (6)	255/4.8 256/1.0	269/28.8 270/ 7.2 271/38.9* 272/ 1.0*	287/< 0.5	345/52.2 346/- 347/ 4.2 348/ 0.7	363/> 0.5
5β-H,3α-OSiDMTB (7)	255/7.1 256/1.6	269/64.2 270/15.2 271/ 3.4* 272/ 0.5*	287/< 0.5 346/18.99	345/66.5 364/ 0.5 347/ 4.9 348/ 0.9	363/2.0
5α-H,3α-OSiDMTB (8)	255/3.6 256/0.9	269/9.2 270/2.4 271/1.4*	287/<0.5	345/70.5 346/21.4 347/ 6.0 348/ 1.0	363/24.4 364/ 6.9 365/ 1.8

cont'd

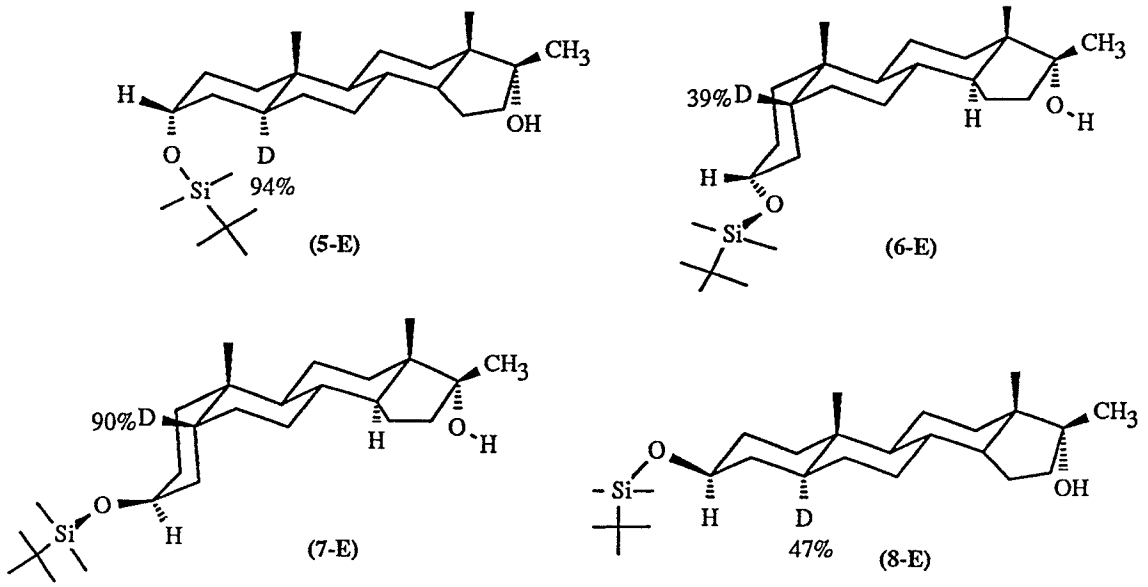
Table 29. (continued)

label in compound	$[F_5]^+$	$[F_4]^+$	$[F_3]^+$	$[F_2]^+$	$[F_1]^+$
5α-D,3α-OSiDMTB (E-5)	255/2.8 256/4.0 257/1.3	269/82.5 270/33.2 271/ 8.0* 272/ 2.5*	287/<0.5 347/18.0 348/ 5.6	345/- 346/40.7 365/ 5.1 366/ 1.6 349/ 1.2	363/- 364/12.0
5β-D,3α-OSiDMTB (E-6)	255/- 256/5.0 257/1.4	269/11.7 270/20.9 271/ 9.1* 272/51.8* 273/13.8* 274/ 2.0*	287/< 0.5	345/- 346/40.7 347/14.5 348/ 4.1	363/- 364/ 1.2
5β-D,3α-OSiDMTB (E-7)	255/2.2 256/3.8 257/1.0	269/64.2 270/27.5 271/ 7.8* 272/ 4.3* 273/ 1.1*	287/< 0.5	345/- 346/68.6 347/26.2 348/ 7.9 349/ 1.5	363/- 364/ 5.7 365/ 2.3 366/- 367/-
5α-H,3α-OSiDMTB (E-8)	255/- 256/3.6 257/1.1	269/4.8 270/7.0 271/2.2* 272/1.6*	287/< 0.5	345/ 1.4 346/50.5 347/18.3 348/ 5.8 349/ 1.3	363/ 1.2 364/42.8 365/15.7 366/ 4.3

* includes contribution from $[M - 149]^+ = [M - t\text{-Bu} - H_2O - Me_2Si{=}O]^+$ or $[M - H_2O - C_4H_9SiMe_2O\cdot]^+$

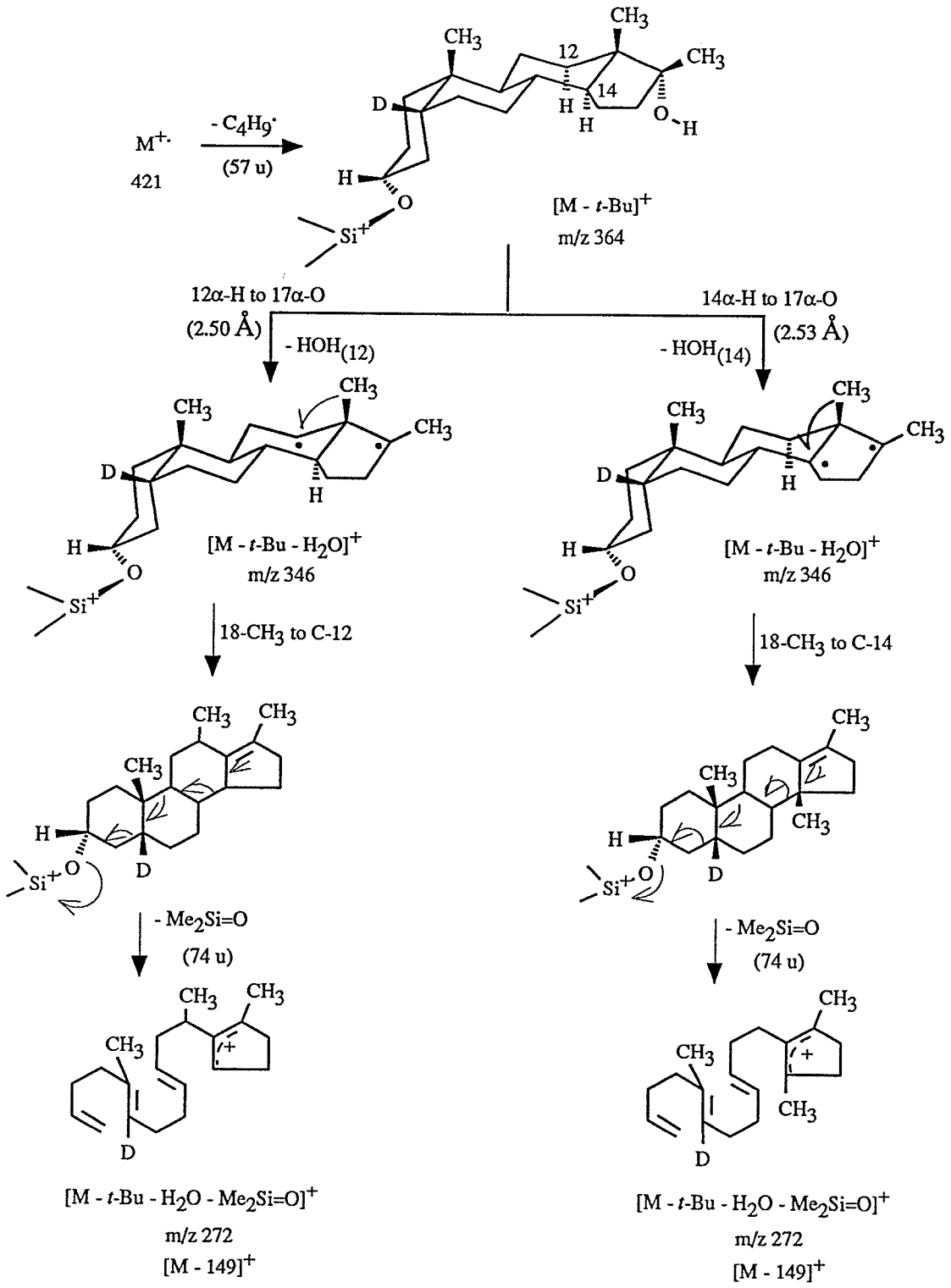
Table 30. Deuterium content (%) of fragment ions from deuterium-labeled 3ξ-OSiDMTB-17α-hydroxy-17β-methyl-5ξ-androstanes (**5-E** to **8-E**).

label in compound		FRAGMENT ION				
		$[F_5]^+$	$[F_4]^+$	$[F_3]^+$	$[F_2]^+$	$[F_1]^+$
		$[M - CH_3 H_2O - TBDMSiOH]$	$[F_3 - H_2O]^+$ or $[F_2 - HSiMe_2OH]^+$	$[F_1 - HSiMe_2OH]$	$[F_1 - H_2O]^+$	$[M - t\text{-Bu}]^+$
		m/z of principal peaks				
	d_n	**255**	**269**	**287**	**345**	**363**
5α-D,3α-OSiDMTB	d_0	41	85	-	-	-
(5-E)	d_1	51	15	-	86	90
	d_2	8		-	12	10
5β-D,3α-OSiDMTB	d_0	-	39	-	-	-
(6-E)	d_1	95	61	-	95	100
	d_2	5	-	-	5	-
5β-D,3β-OSiDMTB	d_0	38	83	-	-	-
(7-E)	d_1	57	17	-	92	92
	d_2	5	-	-	8	8
5α-D,3β-OSiDMTB	d_0	-	45	-	3	2
(8-E)	d_1	92	55	-	90	92
	d_2	8			7	6



Scheme 67. Contribution (%) of hydrogen from the C-5 site, in $HSiMe_2OH$ elimination, for 5ξ-d_1 labeled 3ξ-OSiDMTB-17α-hydroxy-17β-methyl-5ξ-androstanes (**5-E** to **8-E**). (Experimental error ±5%).

ion was observed in the spectra of 3α-OSiDMTB-17β-hydroxy-17α-methyl-5β-androstane (**2**, Fig. 14) but only to a smaller extent, indicating that $Me_2Si{=}O$ (or $C_4H_9SiMe_2O\cdot$) elimination is enhanced when the 17-OH group has the α orientation. Abstraction of the 12α or 14α hydrogens in elimination of H_2O might trigger the "unzipping" mechanisms leading to loss of $Me_2Si{=}O$ (Scheme 68), which could be operative in addition to those depicted previously in Schemes 49 and 50 for **2**. However, confirmation of these proposals is prevented by the non-availability of suitably labeled compounds.



Scheme 68. Proposed "unzipping" mechanism for elimination of $Me_2Si=O$ from the [M - *t*-Bu - H_2O]$^+$ ion of 3α-OSiDMTB-17α-hydroxy-17β-methyl-5β-androstane-5β-d_1 **(6-E)**. (Minimum distances between atoms determined by PCMODEL)

III.C.6 Elimination of H_2O from ring D of the [M - *t*-Bu]$^+$ ion of 3β-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-andostanes (1-4)

Formation of the [M - *t*-Bu - H_2O]$^+$ ion.

Table 31 summarizes losses (%) of HOD from the [M - *t*-Bu]$^+$ ion, for the 16,16-d_2; (**1-J** to **4-J**) or 17α-CD_3 (**1-K** to **4-K**) labeled title compounds. An example of the calculations of deuterium incorporation in the [M - *t*-Bu - H_2O]$^+$ ion, for **1-J** and **1-K**, is given in Appendix I(B).

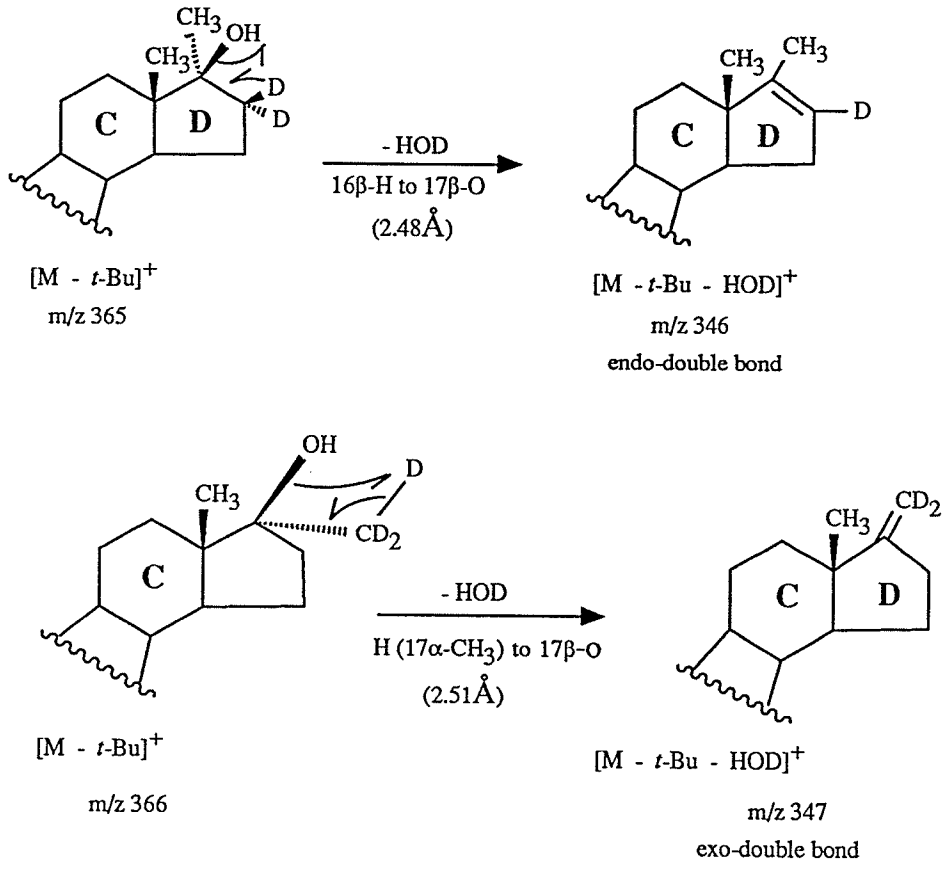
Table 31. Summary of losses (%) of HOD and HOH from the [M - *t*-Bu]$^+$ ion of 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstanes, deuterium labeled at C-16 or 17α-CH_3.

isomer	HOD loss		H_2O loss from all other position(s)
	16,16-d_2	17α-CD_3	
5α-H,3α-OSiDMTB	(1-J) 31	(1-K) 32	37
5β-H,3α-OSiDMTB	(2-J) 28	(2-K) 52	20
5β-H,3β-OSiDMTB	(3-J) 34	(3-K) 30	36
5α-H,3β-OSiDMTB	(4-J) 21	(4-K) 54	25

Incorporation of deuterium from the C-16 site into the eliminated water molecule varies from 21% to 34%. The significance of this observations is not clear. Incorporation of deuterium from the 17α-CD_3 group is significantly lower from compounds **1** and **3**, which have cis-diaxial 5-H and 3-OSiDMTB groups (32% and 30%), than from the other isomers, **2** and **4** (52% and 54%). For the remaining

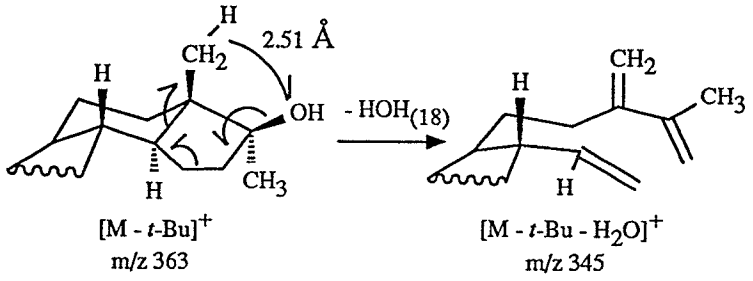
hydrogen transferred the most likely origins are the 18-CH_3 angular group or the C-12 site. The closest distances between 17β-O and 18-methyl hydrogen, 12β-hydrogen (ring C, boat) or 12α-hydrogen (ring C, boat) are 2.51 Å, 3.7 Å, 2.7 Å, respectively.

The major eliminations of water from ring D, through four-membered ring states involving 1,2 elimination, to yield endo- or exo- double bonds[16], are shown in Scheme 69.

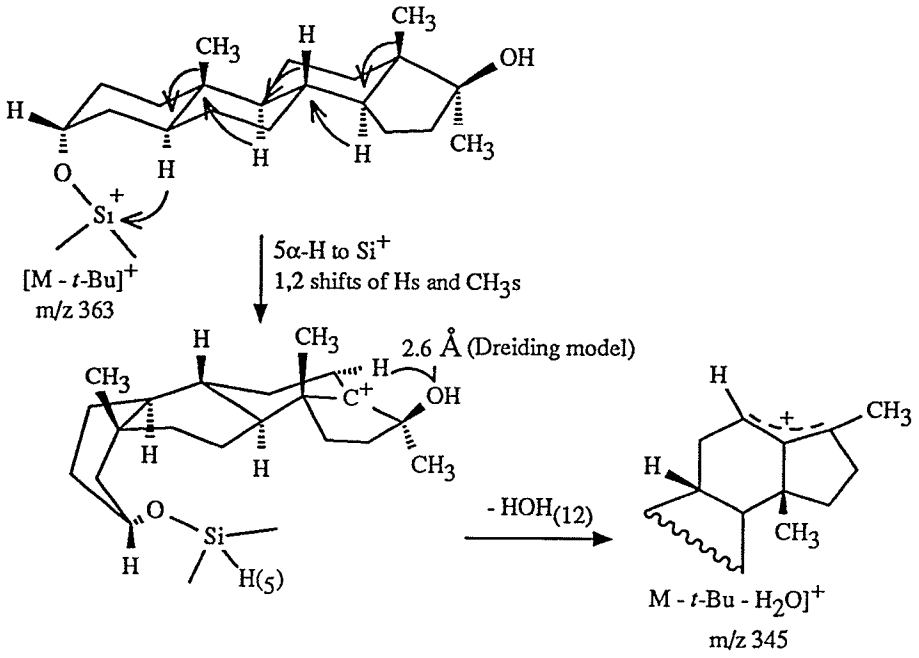


Scheme 69. HOD elimination from ring D, involving H from C-16 or 17α-CH_3. (Distances determined by PCMODEL)

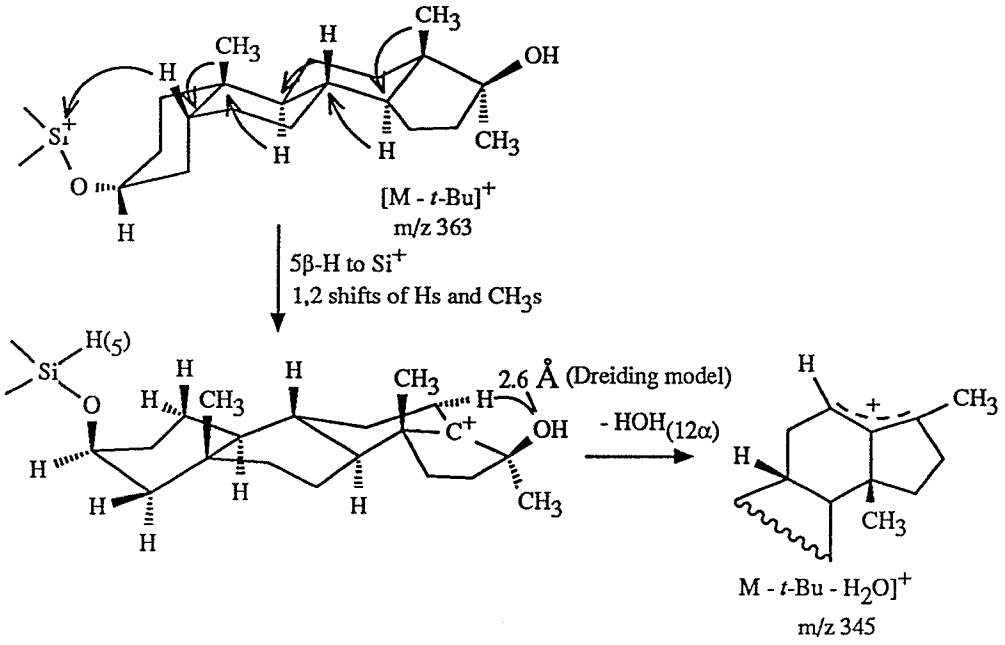
Fragmentation mechanisms for H_2O elimination, involving hydrogen from the 18-methyl angular group or 12α site, are proposed in Schemes 70-73. For the structures presented, transfer of 12α-H to 17β-OH (2.6 Å, Dreiding model) requires positive charge migration from C-5 to C-13 (Schemes 71 and 72) or from C-9 to C-13 (Scheme 73). This can be fullfiled only for isomers in which, after a hydride transfer to Si^+, concerted shifts of Hs and methyls along the steroid backbone can occur.



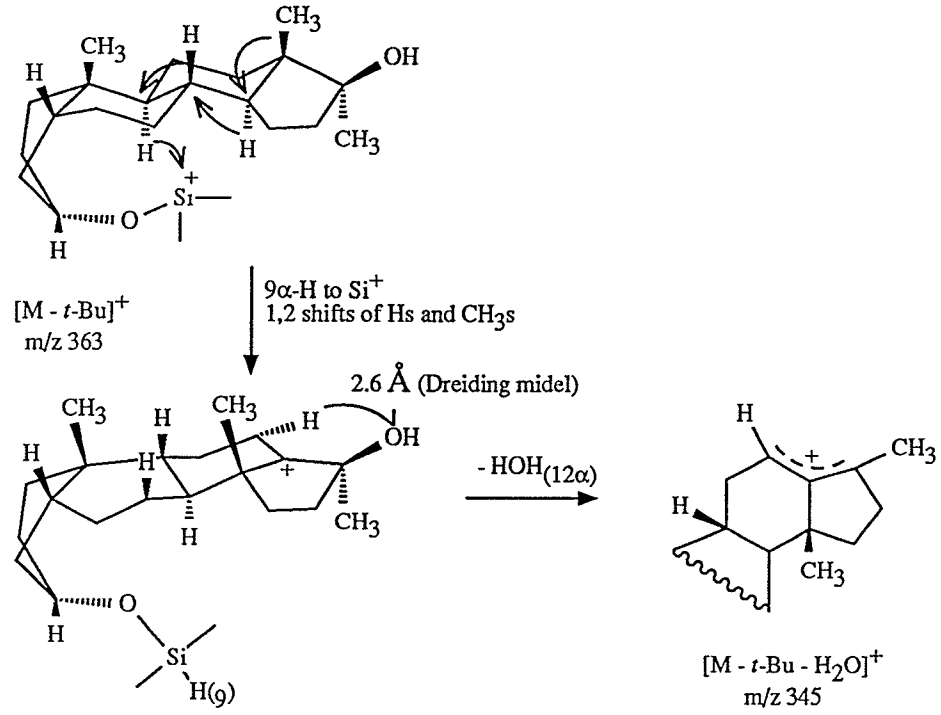
Scheme 70. Proposed H_2O elimination from ring D, involving H from the 18-CH_3 group. (Distances determined by PCMODEL)



Scheme 71. Proposed H_2O elimination from ring D of **1**, involving 12α-H.



Scheme 72. Proposed H_2O elimination from ring D of **3**, involving 12α-H.



Scheme 73. Proposed H_2O elimination from ring D of **2**, involving 12α-H.

III.D. Formation of the $[M - CH_3 - H_2O - C_4H_9SiMe_2OH]^+$ ion for 3ξ-OSiDMTB-17ξ-hydroxy-17ξ-methyl-5ξ-androstanes (1-8).

In this section, formation of the $[M - CH_3 - H_2O - C_4H_9SiMe_2OH]^+$ ion, m/z 255, observed in the spectra of compounds **1-8**, is discussed. Formation mechanisms for this ion are proposed.

III.D.1. 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstanes (1-4).

The ion of m/z 255 in the mass spectra of compounds **1-4** is formed by eliminations of $CH_3^{\cdot}$, H_2O, and *t*-$BuSiMe_2OH$ or ($CH_3^{\cdot}$ + H_2O) and *t*-$BuSiMe_2OH$ from the molecular ion. There is no direct confirmation of the sequence of eliminations, but small peaks corresponding to $[M - 15]^+$ and $[M - 33]^+$ suggest that the sequences are as has been written here.

The deuterium-labeling results in Tables 12, 14, 16 and 18 shed light on these losses. Table 32 shows the extent of deuterium loss for four labeled isomers, based on the m/z 255 to m/z 258 ion peak heights shown in these Tables. The clusters of peaks have similar patterns to those observed in the spectra of 17α-methyl-d_3-5ξ-androstane-3ξ,17β-diol, **13-K**, Fig.8 where a methyl radical and two molecules of water are eliminated from the molecular ion. (For a discussion see section III.B.1.)

The simplest interpretation of the three deuterium losses for the 17α-CD_3 labeled compounds **1-K** to **4-K** is that they show losses of CD_3 ranging from 38% to 53%, i.e. greater than the 33% that would result from statistical elimination of one of the three methyl groups in the molecule. This is not surrprising since loss of the 17α-CD_3 group is an α-cleavage reaction promoted by the ion-radical site on the 17β-$OH^{+\cdot}$ group, as shown in the first step of Scheme 74. After a 1,2 shift of the angular 18-methyl group to C-17[183], with formation of a tertiary carbocation at C-13 (74.3), further fragmentation

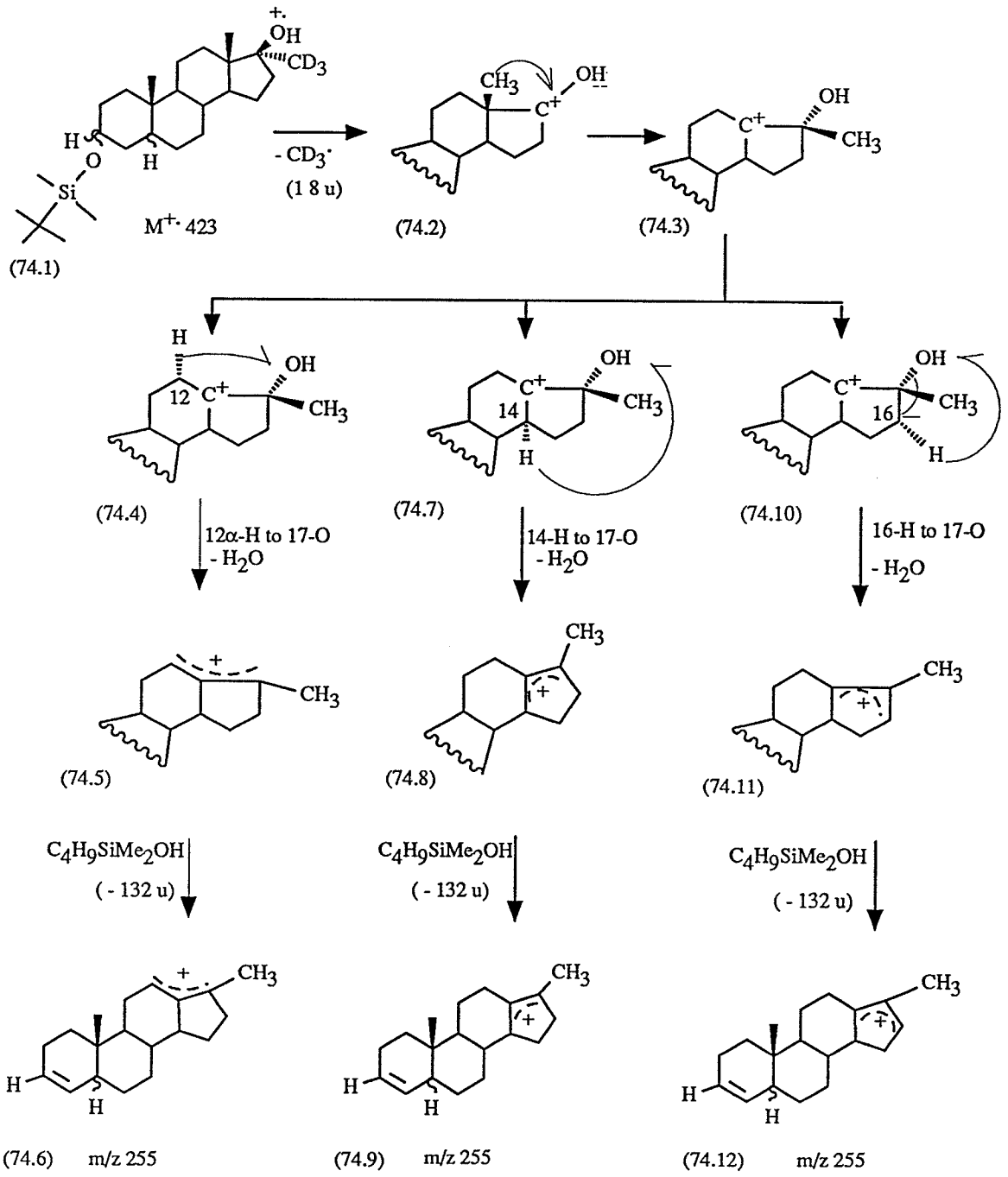
Table 32. Deuterium elimination in formation of the [M - CH_3· - HOH - $C_4H_9SiMe_2OH$]$^+$ ion of labeled 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstanes (**1-4**).

Isomer	labels in compound		% of deuterium loss 3d	2d	1d	0d
5α-H,3α-OSiDMTB	16,16-d_2	(1-J)	-	-	57	43
	17α-CD_3	(1-K)	42	6	18	34
5β-H,3α-OSiDMTB	16,16-d_2	(2-J)	-	-	32	68
	17α-CD_3	(2-K)	38	12	17	33
5β-H,3β-OSiDMTB	16,16-d_2	(3-J)	-	-	56	44
	17α-CD_3	(3-K)	44	6	19	31
5α-H,3α-OSiDMTB	16,16-d_2	(4-J)	-	-	53	47
	17α-CD_3	(4-K)	53	10	24	13

reactions are shown.

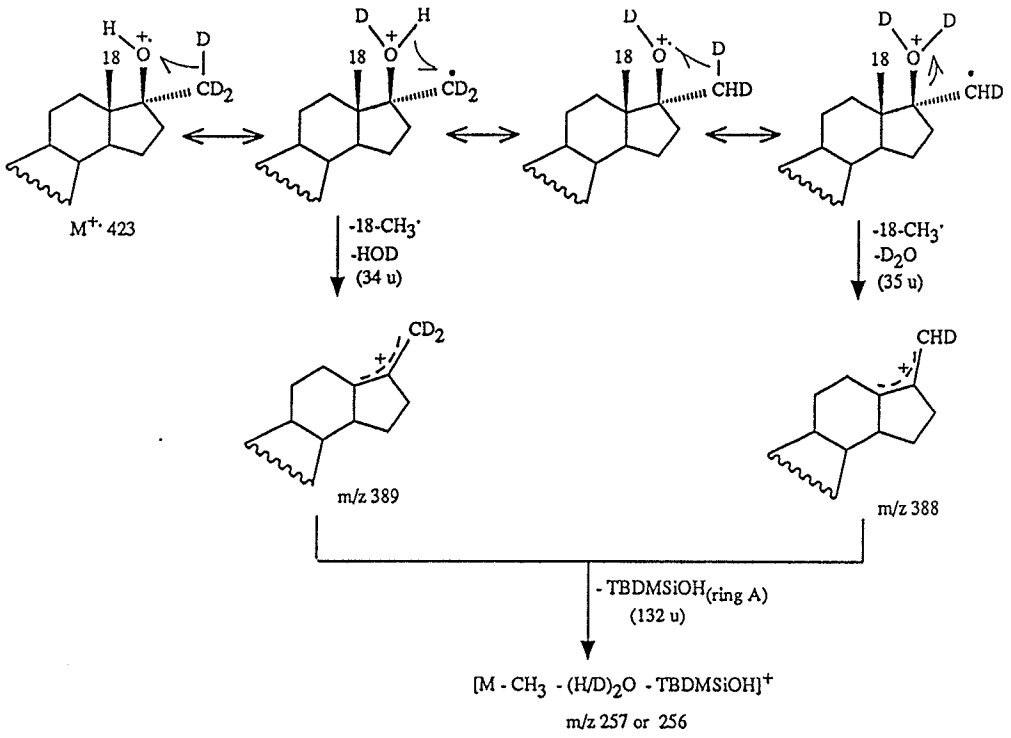
The losses of one or two deuterium atoms are more complex because competing reactions occur. When one deuterium is lost, the C-16 site contributes 32% to 57%, and the 17α-methyl group 17% to 24%, depending upon the isomer. These results are most readily interpreted by losses of HOD. However, since these reactions do not account for all of the transferred hydrogen incorporated in the water molecule eliminated then at least one other reaction must occur (Schemes 74 and 75).

The loss of two deuterium atoms, when a 17α-CD_3 group is present, could, in principle, involve losses of D_2O[184] or CD_2H·, though loss of D_2O is more likely. Thus, in the presence of 17α-CD_3, two deuterium atoms (6% to 12%) can be incorporated into the water molecule lost. One way of explaining this is to propose a rapid reciprocal



Scheme 74. Mechanisms proposed for the successive eliminations of 17α-CD_3, H_2O, and $C_4H_9SiMe_2OH$ from the $M^{+\cdot}$ ions of 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-d_3-5ξ-androstane (**1-K** to **4-K**).

transfer of H/D between the 17α-methyl and 17β-hydroxy groups[184], as shown in Scheme 75 (however, the reciprocal transfer is not rapid enough for equilibration to be achieved).



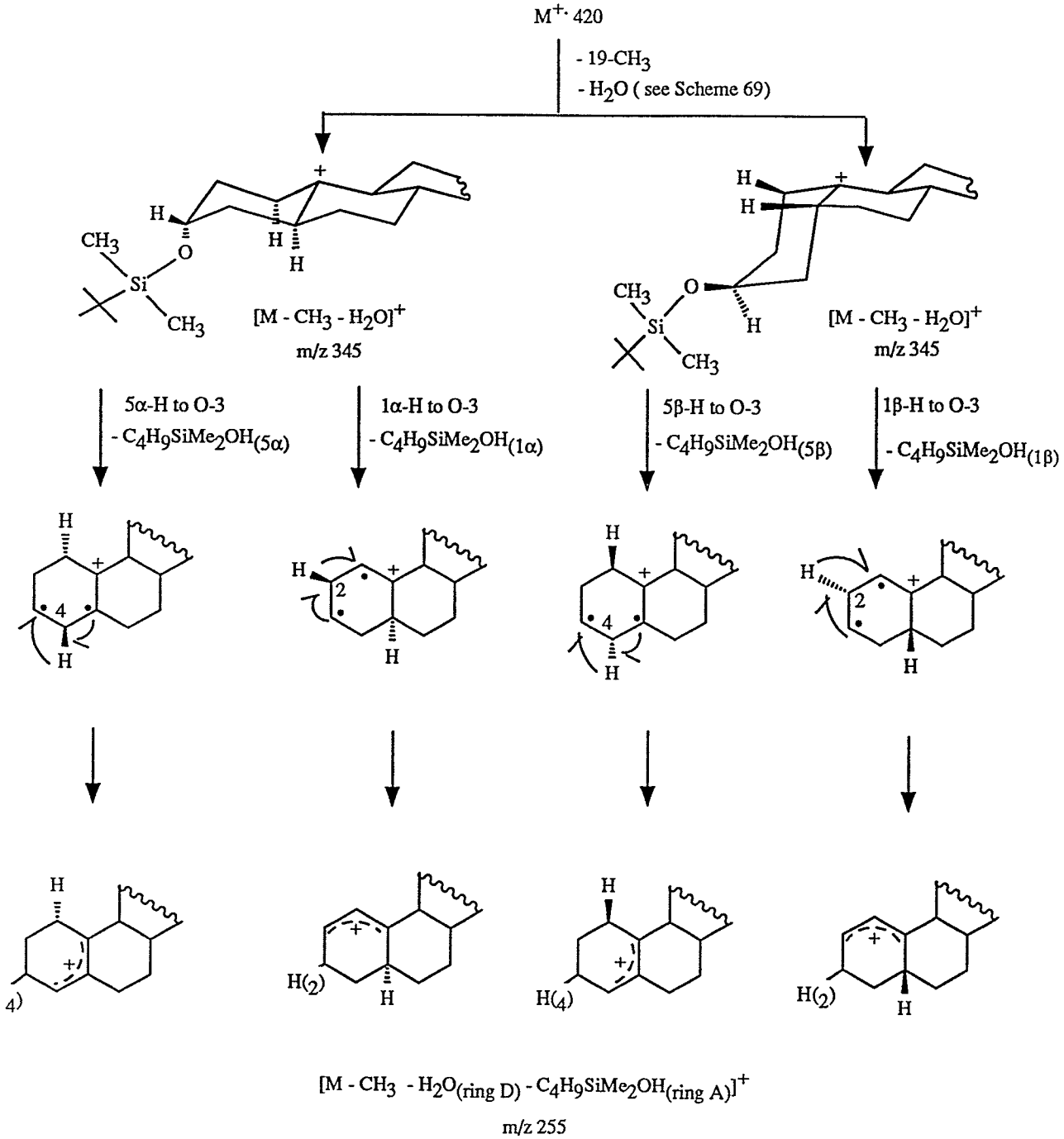
Scheme 75. Losses of one and two deuterium atoms in the formation of the [M- CH_3 - $(H/D)_2O$ - TBDMSiOH]$^+$ ions, m/z 257 or m/z 256, for 3β-OSiDMTB-17β-hydroxy-17α-methyl-d_3-5α-androstane.

III.D.2. 3ξ-OSiDMTB-17α-hydroxy-17β-methyl-5ξ-androstanes (5-8).

Table 33 shows the extent of loss of hydrogen from the C-5 site in the formation of this ion for compounds **5-8**, based on the results in Table 30. We note that the 5β-H of compound **6** and 5α-H of compound **8**, which are both trans to the 3-OSiDMTB substituent, are not eliminated. On the other hand, 5α-H of compound **5** and 5β-H of compound **7**, which are both cis to the 3-OSiDMTB residue, are both eliminated (46% and 42%, respectively). Mechanisms of $C_4H_9SiMe_2OH$ elimination from the $[M - CH_3 - H_2O]^+$ ions of **5** and **7** are proposed in Scheme 76. The reaction follows elimination of the 19-CH_3 radical and expulsion of water from ring D.

Table 33. Transfer (%) of hydrogen from the C-5 site in elimination of $C_4H_9Si(CH_3)_2OH$ from the $[M - CH_3\cdot - H_2O]^+$ ion of 3ξ-OSiDMTB-17α-hydroxy-17β-methyl-5ξ-androstane-5ξ-d_1.

compound		**% loss**
5α-D,3α-OSiDMTB	(**5**-E)	46
5β-D,3α-OSiDMTB	(**6**-E)	0
5β-D,3β-OSiDMTB	(**7**-E)	42
5α-D,3β-OSiDMTB	(**8**-E)	0



Scheme 76. Proposed mechanisms for loss of $C_4H_9SiMe_2OH$ from the $[M - CH_3 - H_2O]^+$ ions of 3α-OSiDMTB-17α-hydroxy-17α-methyl-5α-androstane (**5**) and 3β-OSiDMTB-17α-hydroxy-17α-methyl-5β-androstane (**7**).

IV. CONCLUSION

The gas chromatographic properties of the eight 3ξ-OSiDMTB-17ξ-hydroxy-17ξ-methyl-5ξ-androstane derivatives **(1-8)** on a DB-5 capillary column have been studied. When the 17β-OH (**1-4**) series and 17α-OH (**5-8**) series are chromatographed separately, the four isomers of each series, differing in stereochemistry at C-5 and C-3, were resolved as the individual peaks. The order of elution time for both series was as follows: 5α-H,3α-OR (**1, 5**) < 5β-H,3α-OR (**2, 6**) < 5β-H,3β-OR (**3, 7**) < 5α-H,3β-OR (**4, 8**) (R = TBDMSi). However, distinction of isomers **1, 2**, and **5** as well as **3** and **7** that differ in the stereochemistry of the 17-tertiary hydroxy group is extremely difficult, because their retention times are almost the same.

Positive-ion electron ionization mass spectra (70 eV) of the examined TBDMSi-ether derivatives have been reported. The mass spectra of TBDMSi-ether derivatives were characterized by their marked simplicity in comparison to the underivatized steroids. Ion decompositions were studied by linked-field scanning (B/E or B^2/E constant) and exact mass measurements were made to confirm ion compositions.

Following electron ionization of the molecule, the *t*-butyldimethylsilyl group directs fragmentation by elimination of the *t*-butyl radical, to form $[M - t\text{-Bu}]^+$ fragment ions, which serve as molecular weight indicators. The $[M - t\text{-Bu}]^+$ ions decompose further by elimination of a water molecule (H_2O), dimethylsilanol ($HSiMe_2OH$), or dimethylsilanone ($Me_2Si{=}O$), in various combinations and sequences, to form the $[M - t\text{-Bu} - H_2O]^+$, $[M - t\text{-Bu} - HSiMe_2OH]^+$, $[M - t\text{-Bu} - H_2O - HSiMe_2OH]^+ = [M - t\text{-Bu} - HSiMe_2OH - H_2O]^+$, and/or $[M - t\text{-Bu} - H_2O - Me_2Si{=}O]^+$ ions. The silicon-containing species, $HSiMe_2OH$, is formed by abstraction of two hydrogen atoms from the steroid skeleton by the charged 3ξ-OMe_2Si^+ residue. The m/z 75 ion, $[C_2H_7SiO]^+$, forms the base peak.

The presence of an additional non-derivatized hydroxyl group gives rise to elimination of H_2O from that site, in addition to loss of the *t*-butyl radical from the TBDMSi derivatized site. In some cases, this results in nearly equal peaks for [M - *t*-Bu]$^+$ and [M - *t*-Bu - H_2O]$^+$ ions and in other cases dominant [M - *t*-Bu]$^+$ ion peaks, depending upon the mechanism of the water molecule elimination. In this thesis, I find significant correlations between the [M - *t*-Bu - H_2O]$^+$ fragment ion intensities and the configuration at C-17, in contrast to earlier reports for the eight non-derivatized 17ξ-methyl-5ξ-androstane-3ξ,17ξ-diols. The [M - *t*-Bu - H_2O]$^+$ to [M - *t*-Bu]$^+$ ion intensity ratios distinguish between the 17α-OH and 17β-OH epimers of mono-3ξ-OSiDMTB derivatives, irrespective of C-3 and C-5 configurations, the extent of water elimination being greater for the 17α-OH steroids. For the 17α-OH series, the [M - *t*-Bu - H_2O]$^+$ and [M - *t*-Bu - H_2O - $HSiMe_2OH$]$^+$ ions would be potentially useful for quantitative determination of isomers with the 5α-H,3α-OSiDMTB (**1, 5**), 5β-H,3α-OSiDMTB (**2, 6**), and 5β-H,3β-OSiDMTB (**3, 7**) structure by selected ion monitoring techniques. In the mass spectra of (**1-8**), the [M - *t*-Bu - H_2O - $Me_2Si{=}O$]$^+$ fragment ion, m/z 271, can serve as a diagnostic test for the presence of the 5β-H,3α-OSiDMTB (**2, 6**) isomers in a mixture of all eight isomers. The relative abundances of these ions, which are greater for the 17α-OH structure, differentiate the 17(α, **6**; β-OH, **2**) epimers.

The syntheses of more than forty mono- or poly-deuterated analogues of compounds **1-4** (i.e., 17β-hydroxy-17α-methyl steroids) labeled at positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 16, and 17α-methyl have been synthesized in order to clarify the mechanims of the rearrangements described above. Their mass spectra have been examined. The position of the labels in 17β-hydroxy-17α-methyl-5-androstene-7,7-d_2 was confirmed by ^{2}H-nmr and ^{13}C-nmr spectrometry. The four isomers **5-8**, 3ξ-OSiDMTB-17α-hydroxy-17β-methyl-5ξ-androstanes, were synthesized with a deuterium label only at the C-5 site.

In the mass spectra, the differences between $HSiMe_2OH$ elimination from the [M - *t*-Bu]$^+$ and [M - *t*-Bu - H_2O]$^+$ ions of epimers, are governed ultimately by two factors: an energy factor and a so-called "distance factor". The energy factor comprises the reactivity of the hydrogen atoms (tertiary, secondary, primary) to be abstracted and the stability of the resulting product. The distance factor is a function of the substituent position (C-3), stereochemistry or structure at C-3 and C-5, and ring conformation (chair-twist-boat). The tendency of isomers to eliminate $HSiMe_2OH$, after production of a charged centre at C-5, is enhanced when the 3(α, β)-OSiDMTB substituent and 5(α, β)-hydrogen are *cis*-diaxial with respect to each other. The general mechanism proposed for these eliminations involves rearrangement of a **tertiary hydride** to **positively charged silicon** atom.

For the 5α,3α-OSiDMTB,17β-OH,17α-CH_3 (**1**) epimer, $HSiMe_2OH$ expulsion from the [M - *t*-Bu]$^+$ or [M - *t*-Bu - H_2O]$^+$ fragment ions occurs mostly as a 1,3, 1,4 or 1,5 regiospecific *cis*-diaxial elimination involving, predominantly, the 5α-hydride (100%) together with hydrogen from the 9α (*ca* 46%), C-6 (*ca* 24%) or C-7 (*ca* 36%) sites. Stereospecific rearrangement of the 5α-H indicates the importance of a high positive charge density in the vicinity of the silicon atom. Hydrogen transfers from the distant 9α, 7, and 6 positions are explained by the spatial relationship between the 3α-oxygen and potential hydrogen transfer sites. The proper spatial separation for transfer (H $\rightarrow$ O) is governed by the adopted geometry of the [M - *t*-Bu]$^+$ ion after 5α-hydride rearrangement to Si^+. Thus, sp^2 hybridization at C-5 (planar carbocation) could involve conformational changes of both rings A and B or trigger a 1,2 shift of the 19-CH_3 group from C-10 to C-5 reducing the distance required for transfer of hydrogen.

For the 5α-H,3β-OSiDMTB,17β-OH,17α-CH_3 (**4**) epimer, one tertiary hydrogen atom, i.e. 5α-H (47%), and three secondary hydrogens, i.e. 4-H (14%), 6-H (12%), and

7-H (28%), are involved in competing rearrangements. To allow 5α-H abstraction by the 3β-OMe_2Si^+ group, where the separations are initially too great for hydrogen transfer, the $HSiMe_2OH$ expulsion is preceded by fission of the C-1–C-10 bond. Ring B then isomerizes from a chair to a boat structure to permit abstraction of hydrogen from the C-6 site.

For the 5β-H,3β-OSiDMTB,17β-OH,17α-CH_3 (**3**) epimer, 1,3 *cis*-diaxial elimination of $HSiMe_2OH$ from the $[M - t\text{-Bu}]^+$ ion involves transfer of the 5β-H (100%) to silicon and another hydrogen to oxygen from undetermined positions. On the other hand, elimination of $HSiMe_2OH$ from the $[M - t\text{-Bu} - H_2O]^+$ ion involves 5β-hydride (100%), 1β-H (16%), 7-H (11%) and hydrogen from undetermined sites. The complete retention of labels at C-1α, C-2, C-3, C-4, and C-6 as well as the distances determined between 3β-O and a hydrogen of the 19-CH_3 group (2.60 Å for 19-CH_3 at C-10, or 1.93 Å after a 1,2-shift of 19-CH_3 from C-10 to C-5) provide support for transfer of 19-H as the second hydrogen. The second hydrogen transfer should be confirmed from the mass spectra of 19-CD_3 or 19-CD_2H derivatives of **3.**

For the 5β-H,3α-OSiDMTB,17β-OH,17α-CH_3 (**2**) epimer, elimination of HMe_2SiOH from the $[M - t\text{-Bu}]^+$ or $[M - t\text{-Bu} - H_2O]^+$ ions involves a tertiary hydrogen atom, either 5β-hydride (37%) or 9α-H (63%), a secondary hydrogen atoms (1α-H (6%), 2-H or 4-H (14%), 6-H (7%), 7-H (11%) or an unknown one from ring C or D). Similarly, elimination of HMe_2SiOH from the $[M - t\text{-Bu} - H_2O]^+$ ion involves 5β-H (33%), 9α-H (41%), 1β-H (20%), 2-H or 4-H (14%), 6-H (6%), 7-H (14%) or one from an undetermined site. Since simultaneous abstraction of the 5β-H and 9α-H does not occur the mechanisms were discussed separately.

Transfer of 5β-H to 3α-OMe_2Si^+ should again proceed by skeletal cleavage of the C-1–C-10 bond, because the distances are otherwise large for these trans oriented

groups. Elimination of 5β-H is correlated with loss of 4-H, 6α-H, and 7α-H. Support for this proposal should be proved by deuterium-labeling of the following sites: C-5 and C-4, C-5 and C-6, and C-5 and C-7.

Transfer of 9α-H can occur when ring A is in the inverted boat conformation, which brings the 3α-OMe_2Si^+ group within an appropiate distance. The fact that 1α-H is abstracted indicates that ring A can adopt a **twist** conformation, in which 1α-H is axial. However, to make abstraction of 9α-H and a hydrogen atom from ring C or D possible (12α-H or/and 14α-H), ring A must first adopt the inverted boat configuration. The proposed mechanisms require support from deuterium labeling on rings C and D.

In summary, while substantial progress has been made in understanding the mechanisms of HMe_2SiOH elimination, our knowledge is incomplete. At present there is no strongly supported mechanism for compound **2** and lack of further information about itinerant hydrogen(s) permits only speculation. Further deuterium-labeling studies for compounds **3**, **5-8** are also required.

The major contribution to the $Me_2Si{=}O$ elimination is a "competition" factor, which depends on the type and position of other substituent(s) attached to the steroid skeleton that control the competing $HSiMe_2OH$ elimination. Thus, fragmentation pathways involving elimination of $Me_2Si{=}O$ were found to be specific for 3α-OSiDMTB derivatives of the 17α-OH (**6**) and 17β-OH (**2**) series in isomers with 5β-H structures, and for 3ξ-OSiDMTB-11α-acetoxy-17β-hydroxy-17α-methyl-5ξ-androstanes (**9-12**). Fragmentations triggered by the 17-OH or 11α-CH_3COO groups are strikingly dependent on ring conformation and on the position of the triggering functions (OH, CH_3COO). Mechanisms are proposed for the unexpected $Me_2Si{=}O$ elimination competing with $HSiMe_2OH$ expulsion. Formation of $Me_2Si{=}O$ is rationalized on the basis of the non-availability of one of the two hydrogen atoms required for rearrangement to the 3ξ-OMe_2Si^+ function. In general, various silyloxy steroid

derivatives, having additional polar groups in the steroid skeleton, fragment by elimination of an alkyl radical from the silyl group, a neutral characteristic of the polar group, and $Me_2Si{=}O$. However, the specificity of this mode of fragmentation requires further study.

The elimination of water from $[M - t\text{-Bu}]^+$ fragment ions of deuterium labeled analogues in the conformationally rigid ring D involves hydrogens from the C-16 site, the 17α-CH_3 group, and from undetermined sites. The fragmentation mechanisms proposed require confirmation from the mass spectra of compounds labeled with deuterium at the C-12 site or the 18-methyl group.

The abundance of the $[C_3H_7SiO]^+$ ion, m/z 75, is a function of the energy factor. The labeling results show the extent of a single hydrogen rearrangement from the steroid skeleton to be *ca.* 70% (originating from the C-2 or C-4 sites as well as from other positions) for the saturated TBDMSi-ethers studied. Mechanisms of formation, by transfer of 2-H or 4-H as hydride, are postulated on the basis of metastable decomposition of the m/z 75 ion, as well as by transfer of the remaining 30% of hydrogen from either a *t*-butyl or 19-CH_3 group. A new structure for the m/z 75 ion is proposed, namely, $H(CH_3)Si^+OCH_3$.

Sequential elimination of $CH_3^{\cdot}$, H_2O, and TBDMSiOH from the molecular ion, to form the m/z 255 ion, involves a number of mechanisms. For example, 17α-CD_3 group compounds show elimination of three, two, one, and zero deuterium atoms. Losses of 2D, in elimination of DOD, suggest a reciprocal rearrangement of hydrogen of the 17β-OH group to the 17α-CD_3 group in the odd electron ion. Losses of three or zero deuterium atoms indicates competitive elimination of the 17α-CD_3 substituent or one of the angular methyl groups. To analyse the occurrence of competing reactions at both rings A and D, deuterium-labeled analogues with labels in these rings, as well as derivatives stabilizing the molecular ion, but having similar fragmentation pathways to

the 3ξ-TBDMSi ether derivatives, should be employed.

Understanding of mechanisms of decomposition of homo- and hetero- substituted silyl-ether steroid derivatives under EI would allow us to predict the mass spectrometric fragmentations of steroids.

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VI. APPENDIX I.

Calculation of deuterium content of ions and of deuterium transfer in ion decomposition.

(a) Elimination of $HSiMe_2OH$ from the $[M - t\text{-Bu}]^+$ and $[M - H_2O - HSiMe_2OH]^+$ ions.

The formation of the $[M - t\text{-Bu} - H_2O]^+$, $[M - t\text{-Bu} - HSiMe_2OH]^+$, and $[M - t\text{-Bu} - H_2O - HSiMe_2OH]^+$ ions observed in the mass spectra of all eight 3ξ-OSiDMTB-17ξ-hydroxy-17ξ-methyl-5ξ-androstanes, discussed in sections III.C.1 - III.C.5, involves double or single hydrogen rearrangements. The origins of the transferred hydrogen atoms were investigated by deuterium-labeling at different positions of the steroid skeleton. Complete deuteration of the steroids under study usually does not occur, the extent of label incorporation depending on the synthetic method employed. The mass spectrum of a compound thus contains ions with 0 - n deuteriums. The isotopic purity was determined from the $[M - t\text{-Bu}]^+$ ion for the four 3ξ-OSiDMTB-17β-hydroxy-17α-methyl-5ξ-androstanes (**1-4**) (Tables 13, 15, 18, and 19) and the four 3ξ-OSiDMTB-17α-hydroxy-17β-methyl-5ξ-androstanes(**5-8**) (Table 32).

The isotopic contents of the ions were determined from peak heights calculated with the aid of the computer program, Isotope Cluster Examiner (ICE)[185]. This program calculates relative peak heights for any ion composition, using natural or artificial elemental isotope ratios. The peak height pattern for any group of overlapping groups of peaks with differing deuterium content were then compared with the experimental spectrum. The method is illustrated by the following example.

First, the labeling achieved was determined from the experimental pattern of the $[M - t\text{-Bu}]^+$ ion by simply subtracting the theoretical isotope pattern of each possible d_n ion where n = 0, 1, 2, 3.... in turn, to reduce to zero the intensity of the lowest m/z value

peak. For example, in Table A.1, the group of peaks occurring between m/z 363 and m/z 369 is caused by overlapping of d_0- d_4 ions.

Table A.1 The relative contribution (%) of deuterium-labeled [M - *t*-Bu]$^+$ ions in the mass spectrum of synthesized 3α-OSiDMTB-17β-hydroxy-17α-methyl--5α-androstane-5α,9α,11α-d_3 (**1-I**).

Experimental data			**[(M - *t*-Bu)-d_n]$^+$ peak intensities***					
m/z	RA (%)	normalized (%)	**d_0**	**d_1**	**d_2**	**d_3**	**d_4**	Remaining Intensity
363	2.6	14.6	14.6					
364	4.9	27.5	4.4	23.1				
365	14.7	82.6	1.2	7.0	74.4			
366	17.8	100	0.2	1.9	22.6	75.3		
367	9.1	51.0	---	0.3	6.0	22.9	21.9	
368	2.4	13.5	---	0.05	0.9	6.1	6.7	-0.2
369	0.5	2.8	---	---	0.1	0.9	1.8	-0.05
370	---	---	---	---	---	0.1	0.3	-0.4
371	---	---	---	---	---	---	0.03	-0.03
Relative contributions (%)			**7.0**	**11.0**	**35.5**	**35.9**	**10.5**	

*Based on intensity ratios A = 100%, A + 1 = 30.1%, A + 2 = 8.2%, A + 3 = 1.36%, A + 4 = 0.14%, calculated for natural isotopic abundances of C, H, O, and Si.

The origins of the intinerent hydrogen atoms, in $HSiMe_2OH$ or H_2O elimination, can be determined according to the method described below[184].

The [M - *t*-Bu]$^+$ ion is assumed to retain all deuterium labels and to serve as an indicator of the number of deuterium atoms incorporated into the steroid skeleton. For $HSiMe_2OH$ elimination from [M - *t*-Bu]$^+$ of **1-I** (labeled at 5α,9α, and 11α; Table 12), let us assume that

x - is the fraction of $HSiMe_2OH$ carrying two labeled hydrogens

y - is the fraction carrying one labeled hydrogen

z - is the fraction carrying zero labeled hydrogen

where $x + y + z = 1$.

Now, let us consider decomposition of individual parent ions to product ions:

(a) a d_0 parent can only decompose to a d_0 product, and its contribution to the d_0 product is $7.0(x + y + z)$.

(b) a d_1 parent can decompose to a d_0 or d_1 product; its contribution to the d_0 product is $11.0(x + y)$ and to the d_1 product is $11.0z$

(c) a d_2 parent can decompose to d_0, d_1 or d_2 products; its contribution to the d_0 product is $35.5x$, to the d_1 product is $35.5y$, and to the d_2 product is $35.5z$.

(d) a d_3 parent can decompose to d_1, d_2 or d_3 products; its contribution to the d_1 product is $35.92x$, to the d_2 product is $35.9y$, and to the d_3 product is $35.9z$.

(e) a d_4 parent can decompose to d_2, d_3 or d_4 products; its contribution to the d_2 product is $10.5x$, to the d_3 product is $10.5y$, and to the d_4 product is $10.5z$.

Thus, combining (a) to (e), we can write simultaneous equations to express the relative contributions of deuterated species in the product ion. Using the result for **1-I** (Table 19) we have:

$\mathbf{d_0}$ $\quad 21.3 = 7.0(x + y + z) + 11.0\,(x + y) + 35.5x$

$\mathbf{d_1}$ $\quad 45.9 = 11.0z + 35.5y + 35.9x$

$\mathbf{d_2}$ $\quad 26.2 = 35.5z + 35.9y + 10.5x$

$\mathbf{d_3}$ $\quad 6.7 = 35.9z + 10.5y$

$\mathbf{d_4}$ $\quad 0.4 = 10.5z$

From $\mathbf{d_4}$, we find $z = 0.04$.

From $\mathbf{d_3}$, we find $y = (6.7 - 35.9 \times 0.04)/10.5 = 0.50$

From $x = 1 - z - y$, we find $x = 1 - 0.04 - 0.5 = 0.46$.

Thus, the extent of transfer of two hydrogens is *ca* 46%, and of at least one labeled hydrogen is *ca* 46% + *ca* 50% = *ca* 96%.

(b) Elimination of H_2O from [M - *t*-Bu]$^+$ for **1-J** and **1-K**

The results for **1-J** (16,16-d_2; Table 13) show the deuterium content of [M - *t*-Bu]$^+$ to be d_1= 18% and d_2 = 82%, and for [M - *t*-Bu - H_2O]$^+$ to be d_0 = 4%, d_1 = 39% and d_2 = 57%. These results show that [(33 + 4) -18]/82 = 31% of the hydrogen eliminated in H_2O originates from C-16.

The results for **1-K** (17α-CD_3; Table 13) show d_3 = 100% for [M - *t*-Bu]$^+$ and for [M - *t*-Bu - H_2O]$^+$ d_1 = 4%, d_2 = 33%, d_3 = 63%. Thus, 33% of the eliminated hydrogen originates from 17α-CD_3.

Thus, these two results show that the 17β-hydroxy group is expelled, together with H from C-16 (*ca* 31%) and 17α-CH_3 (*ca* 33%). The remaining eliminated hydrogen (*ca* 35%) comes from undetermined positions.

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APPENDIX II

PCMODEL program.

PCMODEL is a molecular mechanics modelling program[186], which uses most of the features of the MMX program[187]. It also provides graphical displayed molecular models. Molecular mechanics, or so-called force field calculations, have been used to investigate conformations, thermodynamic properties, and vibrational spectra. The expression "molecular mechanics" defines a calculation method designed to give accurate *a prior* structure and energies for three-dimensional molecules. A molecule is considered as a collection of atoms held together by elastic and harmonic forces. These forces may be described by potential energy functions of such structural features as bond length, bond stretching, angle bending, angle torsion, out of plane stretching, and van der Waals interactions. The combination of these potential energy functions is the force field. There are many molecular mechanics programs available[187-191]. They differ in the specific bonded and nonbonded interaction terms included in the potential energy function. The most common is MM2 force field method, developed by Allinger *et al*[187], who have produced force fields that neglect vibrational frequencies but give excellent geometrical results.

The overall molecular potential or steric energy, E_x , of the molecule can be approximated by a sum of energy contributions (the Westheimer equation):

$$E_x = E_s + E_b + E_\omega + E_{nb} + ... \qquad (1)$$

where E_s is the energy of bond deformation (stretching or compression), E_b is the energy of bending, E_ω is the torsional energy, and E_{nb} is the energy of nonbonding interactions. Each of these potential energy functions represents a molecular deformation from an arbitrary reference geometry.

Considering a molecule as a collection of masses held together by springs, and by applying Hook's law, the energy involved in stretching or bending bonds from their natural values is given by:

$$E_s = \sum_{i=1}^{N} 1/2K_i^s (l_i - l_i^o)^2 \quad (2)$$

$$E_b = \sum_{i=1}^{M} 1/2K_{ij}^b (\Theta_{ij} - \Theta_{ij}^o)^2 \quad (3)$$

where N is the total number of bonds in the molecule, and M is the total number of bond angles in the molecule. The K^s and K^b are the stretching and bending force constants selected empirically, the l_i^o and Θ_{ij}^o are the natural bond lengths and angles (Θ_{ij} represents the angle between bonds i and j), and l_i and Θ_{ij} are the deformed values.

The barrier to rotation about bonds (i.e. how the energy of the molecule changes with torsional angle ω) is described by the Fourier series:

$$E_\omega = \Sigma[1/2V_1(1 + \cos\omega) + 1/2V_2(1 - \cos 2\omega) + 1/2V_3(1 + \cos 3\omega) + ...] \quad (4)$$

The fourth term in eqn. (1) is the potential energy term relating to the pairwise nonbonding interactions of atoms as a function of distance between nuclei. As two atoms approach one another, there is the usual attraction due to London dispersion forces and finally a van der Waals repulsion as the atoms get too close. This behaviour is described by the Lenard-Jones and Buckingham potentials:

$$V_{L\text{-}J} = A/r^{12} - B/r^6 \quad (5)$$

$$B_{Buck} = A'\exp B'/r - C/r^6 \quad (6)$$

Equations (5) and (6) describe the attractive part of the curve as r^{-6}-dependent but treat the repulsive part differentially. The Lenard-Jones potential assumes an inverse

12th power dependence, whereas the Buckingham potential assumes the slope increases exponentially. Any other representative functions, such as those which take into account out-of-plane bending, and Coulombic and solvent interactions may also be used.

Molecular mechanics is basically an empirical method and the final molecular model obtained relates to a hypothetical motionless state at absolute zero. To convert steric energy to heat of formation (at say 25°) group enthalpy increments can be included.

The molecular mechanics methods are very well suited for dealing with large molecules, such as a drug or dye molecule, with large ring structures that can exist in many conformations. As Dreiding molecular models can sometimes be misleading, the computational force-field can correctly predict the conformation of various compounds[192].

Determination of the geometry of the structures by the PCMODEL program.

The geometry of the $[M - t\text{-Bu}]^+$ ions, considered in this thesis, was determined by the PCMODEL program as follows.

First, a preliminary structure is drawn in the structure input mode, and then it is transferred to the minimization mode (MIN.).

Second, in the minimization mode, by clicking on the MMX-M option, the structure is transferred into the background minimization screen (BACK. MIN.), where the minimization process is initiated:

(a) first, the total energy of the structure is computed and listed

(b) then, iterative energy minimization is performed, i.e. the atomic movement (in 1.0 - 1.5 Å units) and the energy (in kcal mol^{-1}) is listed every 5 iterations.

Periodically (if the energy starts to increase, or if the energy stabilizes but with large atomic movement), the program pauses to check the effect of each atom's

movement on the total energy. Atoms whose movement causes the energy to rise will become fixed until the next atom movement check. When the energy stabilizes, the minimized structure is redrawn and the energy summary is listed on the screen. As the final energy is displayed, the minimization process is terminated automatically, by transferring the structure and the last energy value into the minimization mode. This option was used to determine the geometry of the structures presented in this thesis.

In some cases, I have noticed discrepancies in energies displayed in the background minimization mode and the minimization mode, after transferring the structure from BACK.MIN to MIN. In this thesis, if such a difference is observed, the energy displayed in the BACK.MIN mode is placed in parentheses.

As the final geometry of the structure, characterized by the lowest energy is determined, distances between two atoms or torsional angles can be measured.