Influence of the 6-Trimethylsilyl Group on the Fragmentation of the Trimethylsilyl Derivatives of some 6-Hydroxy- and 3,6-Dihydroxy-Steroids and Related Compounds

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The 25 eV mass spectra of the trimethylsilyl derivatives of a number of 6-hydroxy and 3,6-dihydroxy steroids together with deuterium and ^{18}O-labeled analogs have been examined to determine the influence of the 6-OTMS group on fragmentation patterns. Ions in the cholestane series at *m/z* 321 and 403 were the most characteristic ions derived from the 6-OTMS function; their relative abundances, although low in the spectra of 6-OTMS steroids themselves, were considerably elevated when a 3-OTMS or 3-oxo group was present. Similar ions were present in the spectra of androstane and pregnane derivatives. No correlation was found between the abundance of these ions and the stereochemistry at C_3, C_5, or C_6. Fragmentation mechanisms and gas chromatographic data are discussed.

It is generally recognized that analysis of hydroxy steroids by gas chromatography mass spectrometry (GCMS can best be accomplished using trimethylsilyl (TMS) ether derivatives.[1] It has been known for many years[2] that trimethylsilyl substituents tend to direct the fragmentation of the steroid nucleus, and are thus instrumental in the formation of structurally diagnositic ions. For example, an ion of *m/z* 129 is characteristic of the TMS derivatives of both Δ^5-3-hydroxy[3,4] and 17-hydroxy C-19 steroids,[5] while a rearrangement ion of *m/z* 147 has been shown to reflect the steric disposition of trimethylsilyloxy groups on the steroid nucleus.[6] Similarly, we have shown that the spectra of the TMS derivatives of 11- and 16-hydroxy steroids exhibit TMS-containing fragment ions which are characteristic of the position of TMSO substitution on the steroid nucleus.[7,8] In an analogous manner we have now examined the mass spectra of the TMS derivatives of representative 6-hydroxy steroids in order to establish some criteria for recognizing this position of substitution on the basis of TMS-containing fragment ions. 6-Hydroxy steroids are of considerable biological interest as it has been shown that one of the pathways for the elimination of steroids in both the adult and the newborn infant is their excretion as free substances following 6β-hydroxylation.[9]

In evaluating the mode of fragmentation of steroids containing a 6-OTMS group we first examined the mass spectra of 6-monohydroxy cholestane derivatives epimeric about C-6. As naturally occurring 6-hydroxy steroids also have hydroxylation at C-3 we further considered the mass spectra of several 3,6-dihydroxy steroid derivatives epimeric about C-3, C-5 and C-6, or various combinations thereof. Finally, the effect of Δ^4-unsaturation on the fragmentation of the 3,6-diols was evaluated. Specifically, the compounds examined in this study were the O-TMS derivatives of the following steroids: 5α-cholestane-6β-ol (**1**), 5α-cholestane-6α-ol (**2**), 5α-cholest-2-ene-6β-ol (**3**), 5α-cholest-2-ene-6α-ol (**4**), 5β-cholestan-3-one-6β-ol (**5**), 5α-cholestan-3-one-6β-ol (**6**), 5β-androstane-3α,6α-diol-17-one (**7**), 5β-pregnane-3α,6α-diol (**8**), 5β-pregnane-3α,6α-diol-20-one (**9**), 5α-cholestane-3α,6β-diol (**10**), 5α-cholestane-3β,6α-diol (**11**), 5α-cholestane-3α,6β-diol (**12**), 5β-cholestane-3α,6β-diol (**13**), 5β-cholestane-3β,6β-diol (**14**), cholest-4-ene-3β,6β-diol (**15**), cholest-4-ene-3β,6α-diol (**16**), cholest-4-ene-3α,6β-diol (**17**) and pregn-4-ene-3β,6β-diol-20-one (**18**).

The 'structures' and possible mechanisms of formation of the most significant TMS-containing ions in the spectra of their trimethylsilyl derivatives were determined with the aid of high resolution mass measurements and isotopic labeling. In addition to perdeuteriotrimethylsilyl derivatives,[10] isotopically labeled compounds were prepared by replacement of selected skeletal hydrogens with deuterium. To distinguish between ions containing the 3- vs the 6-OTMS group, selectively labeled TMS/[$^2H_9$]TMS derivatives[11] and mono^{18}O-derivatives[12] were prepared.

EXPERIMENTAL

Steroids

The following compounds were purchased from commercial suppliers: 5α-cholest-2-en-6-one (Steraloids), 5α-cholestan-3β-ol-6-one (Aldrich), 5β-androstan-3α,6α-diol-17-one (**7**, Ikapharm), 5β-pregnane-3α,6α-diol (**8**, Ikapharm), 5β-pregnane-3α,6α-diol-20-one (**9**, Steraloids) and pregn-4-ene-3β,6β-diol-20-one (**18**, Ikapharm).

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5α-Cholest-2-ene-6β-ol (3) and 5α-cholest-2-ene-6α-ol (4)

These were prepared by reduction of 5α-cholest-2-ene-6-one with lithium aluminum hydride. The major product (91%) was the 6β-alcohol[13] and the two isomers were well separated as their TMS derivatives on 3% SE-30 (Table 1).

5α-Cholestan-6β-ol (1) and 5α-cholestan-6α-ol (2)

These were prepared either by catalytic hydrogenation of **3** and **4** (10% Pd/C, EtOH) or by hydrogenation of 5α-cholest-2-ene-6-one under similar conditions. Again the 6β-isomer predominated.

Cholest-4-ene-3β,6β-diol (15)

This was obtained from cholesterol by hydroxylation of the double bond followed by dehydration.[14,15]

5β-Cholestane-3β,6β-diol (14)

This was prepared from **15** by catalytic hydrogenation.[15]

The following oxo-steroids were also prepared as described by Harvey and Reid:[15] 5β-cholestan-6β-ol-3-one, 5α-cholestane-3,6-dione, 5β-cholestan-3,6-dione, cholest-4-ene-3,6-dione[16], 5α-cholestan-6β-ol-3-one, and cholest-4-ene-6β-ol-3-one.[17] These were reduced with lithium aluminum hydride or deuteride to the corresponding diols and deuterium substituted diols respectively.

α-Deuteriated diols were prepared by reduction of the corresponding ketones after exchange of the α-hydrogen for deuterium (NaO^2H, ^2H_2O, dioxane). Oxygen-18 labeled sterols were prepared by lithium aluminum hydride reduction of the corresponding ketones, following exchange of the carbonyl oxygen with 50% isotopic purity $H_2\,^{18}O$ in a 0.001 M solution of HCl is isopropanol.

Derivative preparation

TMS and [$^2H_9$]TMS derivatives were prepared by standard methods.[18] Hindered 6β-hydroxyl groups were silylated by the addition of trimethylsilyl imidazole. Selective TMS and [$^2H_9$]TMS derivatives were prepared by utilizing the reactivity differences of the 3- and 6-OH groups as described.[11]

Gas chromatography

Methylene unit values were recorded with a Varian 2400 gas chromatograph fitted with a flame ionization detector and a 2 m × 2 mm (i.d.) glass column packed with 3% SE-30 on 100/120 mesh Gas Chrom Q (Applied Science Laboratories, Inc., State College, Pennsylvania, USA). The injector and detector block temperatures were 300 °C and the nitrogen flow rate was 30 ml min^{-1}.

Mass spectrometry

Mass spectra were recorded with a VG Micromass 70-70 F mass spectrometer interfaced via a glass jet separator to a chromatographic system similar to that described above, and a Nuclide 12-90-G mass spectrometer interfaced to a Varian 2700 gas chromatograph via a direct transfer line. No major differences in the spectral patterns between the two instruments were noted. The ion source temperature was 250 °C, electron energy 25 eV and accelerating voltage source temperature was 250 °C, electron energy 25 eV and accelerating voltage 4 kV (4.5 kV in the Nuclide 12-90-G). Spectra obtained with the VG Micromass 70-70 F instrument were recorded with a VG 2040 Data System at 3 s per decade. High resolution ($M/\Delta M$ 5000) spectra were also recorded with the VG 70-70 F–2040 GCMS data system.

RESULTS AND DISCUSSION

Gas chromatography

All of the alcohols, diols and hydroxy-ketones chromatographed well as TMS derivatives on 3% SE-30; the diketones, on the other hand, gave very asymmetrical peaks. Methylene unit values are given in Table 1. Reduction of the oxo-steroids with lithium aluminum hydride produced both isomeric alcohols in unequal amounts (Table 2) and in general these isomeric pairs were well separated. A notable exception to this was the isomeric pair produced by reduction of 6β-hydroxy-5β-cholestan-3-one (Table 1). Determination of the

Table 1. Methylene unit values recorded on 3% SE-30 for the steroid ketones and TMS derivatives of hydroxycholestanes

(a) Saturated steroids

Steroid 5	3	6	No.	Methylene unit
α	—	β-OH	**1**	29.72
α	—	α-OH	**2**	30.25
α	=O	β-OH	**6**	33.54
α	β-OH	=O		33.66
α	=O	=O		32.93
β	=O	β-OH	**5**	31.77
β	=O	=O		32.91
α	β-OH	α-OH	**11**	32.80
α	α-OH	β-OH	**12**	31.43
α	β-OH	β-OH	**10**	32.30
β	β-OH	α-OH		32.29
β	α-OH	β-OH	**13**	31.52
β	β-OH	β-OH	**14**	31.43

(b) Unsaturated steroids

Position of unsaturation	3	6	No.	Methylene unit
2	—	β	**3**	29.70
2	—	α	**4**	30.22
4	=O	β-OH		32.31
4	=O	=O		33.02
4	α-OH	α-OH		31.41
4	β-OH	α-OH	**16**	32.46
4	α-OH	β-OH	**17**	31.18
4	β-OH	β-OH	**15**	31.78

Table 2. Products formed by $LiAlH_4$ reduction of the steroid ketones

	Steroid reduced		Product %			
	3	6	3α, 6α	3β, 6β	3α, 6β	3β, 6α
5α	=O	β-OH	—	86	14	—
5α	β-OH	=O	—	89	—	11
5α	=O	=O	—	68.5	13.5	18
5β	=O	β-OH	—	[a]	[a]	—
5β	=O	=O	minor	intermediate	major	intermediate[a,b]
Δ^4	=O	β-OH	—	96	4	—
Δ^4	=O	=O	[c]	[c]	[c]	[c]

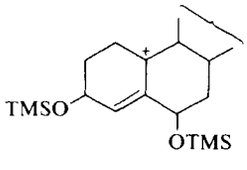
[a] 3β, 6β and 3α, 6β did not separate on GC.
[b] Major, minor and intermediate peaks indicated.
[c] Peaks not homogeneous.

stereochemistry of the hydroxyl groups was based on the studies of Barton,[19] who proposed that unhindered ketones are reduced primarily to equatorial alcohols whereas the reverse is true for hindered ketones. In addition, the reduction of cholest-4-en-3,6-dione has been shown to give mainly cholest-4-en-3β,6α-diol (**16**),[20] whereas the reduction of 5α-cholestane-3,6-dione[21] and 5α-cholestan-3-one-6β-ol[13] both give the 3β,6β-diols as the major product.

Mass spectrometry

Metastable ions were observed for all the fragmentations involving loss of trimethylsilanol or $CH_3\cdot$ and for the formation of the ions at m/z 403 and 321 from $[M]^{+\cdot}$ in the spectra of the cholestane derivatives. High resolution mass measurements were made on all ions in the spectra of the TMS derivatives of **14** and **15** and supported the structures of the ions discussed below. The spectra of the compounds are summarized in Table 3. Tables 4 and 5 summarize the labeling data for compounds **10** and **15** respectively.

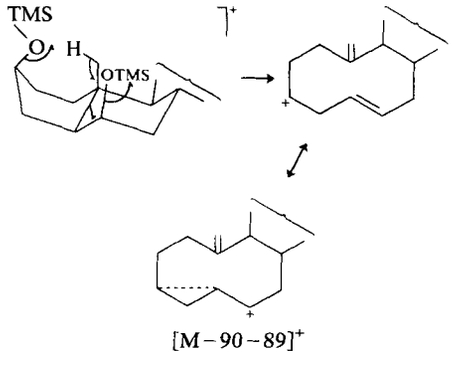
Ions arising from loss of $CH_3\cdot$ and TMSOH. In common with the spectra of other steroid TMS derivatives, the spectra of the TMS ethers of 6-hydroxy steroids contained abundant ions produced by successive losses of methyl radicals and TMSOH molecules. The $[M-CH_3]^+$ ions in the spectra of the saturated and Δ^2-steroids (**1–14**) all arose from loss of $CH_3\cdot$ from a TMS group as shown by the spectra of the $[^2H_9]$TMS derivatives. In the case of the TMS derivatives of 5α-cholestane-3β,6β-bis-OTMS where selective TMS and $[^2H_9]$TMS labeling was possible, about 60% of the $CH_3\cdot$ loss originated from the 6-OTMS group. Loss of the methyl radical from the Δ^4-steroids (**15–18**) arose almost entirely (for example, 92% in the spectrum of cholest-4-ene-3β,6β-bis-OTMS, **15**) from the steroid nucleus. This variation can be attributed to a stabilized ion such as *a* which can be formed by loss of the 19-angular methyl group.



a

The elimination of trimethylsilanol from $[M]^{+\cdot}$ led to abundant ions, often the base peaks, in most of the spectra. Where selective TMS/$[^2H_9]$TMS labeling was possible (6β-OTMS group), this loss was shown to be almost entirely (over (90%) from the 6-position. This was further concurred from the spectrum of the 6-^{18}O derivative (**10i**, Table 4). In general, the spectra of the steroids containing axial TMSO groups exhibited more abundant $[M-TMSOH]^{+\cdot}$ ions than those containing equatorial groups; this was almost certainly a reflection of hydrogen availability. For example, it has been known[22,23] that 1,3-diaxial or related interactions in the molecular ion in which the TMSO groups and a hydrogen can come to within bonding distance (less than 3.2 Å) usually lead to abundant losses of trimethylsilanol in steroids. Therefore, it could be surmised that the elimination of the 6β-OTMS group from 5α-cholestane-3β,6β-bis-OTMS (**10**) involves abstraction of the 4β, 8β or 19-angular methyl hydrogens. Indeed, deuterium labeling of the 2, 3α, 4, 5α, 6α and 7 positions (Table 4) showed that an insignificant amount of hydrogen abstraction occurred from these positions, thus strongly implicating the tertiary 8 hydrogen or the hydrogens of the 19-methyl group. Moreover, in the case of cholest-4-ene-3β,6β-bis-OTMS, where again the $[M-TMSOH]^{+\cdot}$ ion was derived from loss of the 6-OTMS group, the bulk of the hydrogen abstraction also appeared to be from these positions (Table 5). However, in this case, about 20% of the hydrogen loss involved the 3α-hydrogen. This can best be rationalized by cleavage of the C-2—C-3 bond prior to hydrogen abstraction. This cleavage is facilitated by the Δ^4-double bond.

Elimination of a second molecule of TMSOH (from the $[M-TMSOH]^{+\cdot}$ ion) again reflected the availability of hydrogen atoms. For example, in the steroid containing diaxial OTMS substituents (5α-cholestan-3α,6β-bis-OTMS, **12**), this ion was the base peak (Table 3). In the spectra of the diols containing a 3β-OTMS group, however, the loss of the second TMSO group was not accompanied by hydrogen abstraction although the resulting ion (m/z 369) was abundant (69%). In this compound, with ring A in the chair conformation, the 3β-OTMS group cannot come to within bonding distance of a hydrogen atom. However, with ring A in the boat conformation, interaction between the 3β-oxygen and the 19-angular methyl hydrogens can occur, resulting in the concerted loss of TMSOH and TMSO shown in Scheme 1.



Scheme 1

Table 3. Mass spectrometric data for trimethylsilyl derivatives of selected 6-hydroxy sterols

Compound	Base peak	Molecular ion	Principal ions in mass spectrum															
5α-Cholestane-6β-ol (**1**)	370[a](0)[b]	460(9) (8%)[c]	445(6) (18%)	403(9) (3%)	355(0) (22%)	321(9) (14%)	260(0) (9%)	257(0) (13%)	231(0) (17%)	230(0) (36%)	216(0) (45%)	215(0) (58%)	197(18) (8%)	148(0) (36%)	147(0) (46%)	129(9) (10%)		
5α-Cholestane-6α-ol (**2**)	370(0)	460(9) (9%)	445(6) (60%)	403(9) (<1%)	355(0) (33%)	321(9) (13%)	305(9) (15%)	257(0) (23%)	230(0) (34%)	216(0) (50%)	215(0) (83%)	197(9) (20%)	129(9) (14%)					
5α-Cholestane-2-ene-6β-ol (**3**)	368(0)	458(9) (1%)	443(6) (7%)	404(9) (1%)	403(9) (1%)	389(9) (9%)	353(0) (17%)	314(0) (18%)	255(0) (35%)	250(0) (15%)	213(0) (20%)	201(0) (16%)	129(9) (12%)	106(0) (85%)	105(0) (35%)			
5β-Cholestane-2-ene-6α-ol (**4**)	368(0)	458(9) (1%)	443(6) (17%)	404(9) (5%)	403(9) (3%)	389(9) (14%)	353(0) (22%)	314(0) (31%)	255(0) (32%)	213(0) (33%)	201(0) (18%)	106(0) (50%)	105(0) (34%)					
5β-Cholestan-3-one-6β-ol (**5**)	459(6)	474(9) (11%)	403(9) (17%)	384(0) (20%)	351(0) (14%)	321(9) (95%)	229(0) (18%)	211(0) (16%)	173(0) (15%)	159(0) (13%)	129(9) (18%)							
5α-Cholestan-3-one-6β-ol (**6**)	384(0)	474(9) (11%)	459(6) (27%)	369(0) (15%)	321(9) (31%)	271(0) (14%)	244(0) (52%)	231(0) (16%)	230(0) (64%)	229(0) (56%)	211(0) (18%)	147(0) (22%)	124(0) (23%)					
5β-Androstane-3α,6α-diol-17-one (**7**)	270(0)	450(18) (13%)	435(15) (2%)	360(9) (23%)	345(6, 9) (14%)	332(9) (4%)	305(9) (22%)	271(0) (56%)	253(18) (21%)	231(0) (19%)	223(9) (30%)	215(0) (19%)	191(18) (9%)	155(9) (19%)	147(0, 15) (40%)	143(9) (18%)	129(9) (23%)	
5β-Pregnane-3α,6α-diol (**8**)	284(0)	468(18) (9%)	374(9) (21%)	359(6) (15%)	319(9) (20%)	278(9) (9%)	269(0) (32%)	245(0) (18%)	239(0) (63%)	229(0) (20%)	191(18) (14%)	161(0) (37%)	147(15) (45%)	129(9) (35%)				
5β-Pregnane-3α,6α-diol 20-one (**9**)	298(0)	478(18) (15%)	388(9) (31%)	375(9) (6%)	373(6, 9) (18%)	333(9) (27%)	318(9) (6%)	283(0) (28%)	255(0) (24%)	251(9) (50%)	243(0) (27%)	213(0) (28%)	204(18) (18%)	191(18) (18%)	161(0) (47%)	147(15) (57%)	143(9) (22%)	129(9) (37%)
5α-Cholestane-3β,6β-diol (**10**)	458(9)	548(18) (11%)	533(15) (11%)	443(9) (9%)	417(9) (5%)	403(9) (4%)	369(0) (64%)	368(0) (67%)	353(0) (19%)	329(0) (16%)	321(9) (20%)	318(9) (20%)	305(9) (20%)	304(9) (31%)	285(18) (5%)	228(0) (40%)	204(18) (11%)	194(9) (16%)
5α-Cholestane-3β,6α-diol (**11**)	204(18)	548(18) (28%)	533(15) (38%)	458(9) (72%)	445(9) (15%)	443(6, 9) (25%)	417(9) (3%)	403(9) (20%)	369(0) (94%)	368(0) (78%)	353(0) (24%)	329(0) (65%)	321(9) (18%)	285(18) (15%)	195(9) (21%)	191(9) (70%)	161(0) (42%)	129(9) (45%)
5α-Cholestane-3α,6β-diol (**12**)	368(0)	548(18) (3%)	533(15) (3%)	458(9) (66%)	443(6, 9) (17%)	403(9) (37%)	353(0) (21%)	329(0) (12%)	321(9) (24%)	318(9) (7%)	305(9) (9%)	285(18) (4%)	228(0) (37%)	215(0) (18%)	213(0) (21%)	196(9) (12%)	161(0) (14%)	129(9) (16%)
5β-Cholestane-3α,6β-diol (**13**)	458(9)	548(18) (2%)	533(15) (2%)	443(9) (15%)	403(9) (84%)	368(0) (73%)	353(0) (23%)	329(0) (7%)	321(9) (49%)	314(0) (24%)	313(0) (17%)	285(18) (3%)	255(0) (25%)	228(0) (27%)	213(0) (26%)	161(0) (13%)	129(9) (31%)	
5β-Cholestane-3β,6β-diol (**14**)	458(9)	548(18) (7%)	533(15) (6%)	443(6, 9) (22%)	417(9) (3%)	403(9) (17%)	368(0) (96%)	353(0) (41%)	329(0) (32%)	321(9) (59%)	318(9) (15%)	304(9) (9%)	285(18) (4%)	228(0) (17%)	213(0) (39%)	169(9) (13%)	143(9) (24%)	129(9) (52%)
Cholest-4-ene-3β,6β-diol (**15**)	403(9)	546(18) (19%)	531(15, 18) (22%)	517(18) (7%)	456(9) (78%)	441(9) (35%)	389(9) (6%)	366(0) (8%)	321(9) (5%)	314(0) (6%)	283(18) (9%)	195(9) (12%)	194(9) (19%)	168(9) (7%)	154(9) (7%)	143(9) (21%)	142(9) (19%)	129(9) (7%)
Cholest-4-ene-3β,6α-diol (**16**)	456(9)	546(18) (19%)	531(18) (9%)	517(18) (3%)	441(9) (43%)	428(9) (6%)	403(9) (54%)	389(9) (6%)	366(0) (7%)	326(0) (7%)	321(9) (4%)	314(0) (5%)	283(18) (5%)	194(9) (20%)	168(9) (8%)	154(9) (7%)	143(9) (17%)	142(9) (14%)
Cholest-4-ene-3α,6β-diol (**17**)	456(9)	546(18) (22%)	531(15, 18) (32%)	517(18) (9%)	441(9) (72%)	428(9) (8%)	403(9) (85%)	389(9) (8%)	384(0) (15%)	366(0) (14%)	321(9) (6%)	314(0) (6%)	283(18) (9%)	195(9) (17%)	194(9) (19%)	143(9) (36%)	142(9) (34%)	129(9) (12%)
Pregn-4-ene-3β,6β-diol-20-one (**18**)	333(9)	476(18) (21%)	461(18) (23%)	447(18) (11%)	386(9) (96%)	371(9) (54%)	319(9) (7%)	283(18) (12%)	251(9) (7%)	195(9) (22%)	194(9) (27%)	181(9) (21%)	147(15) (26%)	143(9) (31%)	142(9) (28%)	129(9) (16%)		

[a] Indicates *m/z* value.
[b] Value refers to *amu* shift in spectrum of [$^2H_9$]TMS derivative.
[c] Relative intensity given in terms of % base peak. Ion abundances have not been corrected for ^{13}C or ^{29}Si isotopic contribution.

Table 4. Partial mass spectra of the TMS derivatives of 5α-cholestane-3β,6β-diol (10) and its isotopically labeled analogs (10a–10i). *M/z* values of most significant ions

Type of ion	10	3,6-bis[$^2H_9$]TMS (10a)	3-TMS,6-[$^2H_9$]TMS (10b)	3α-^{2}H (10c)	6α-^{2}H (10d)	3α,6α-$^2H_2$ (10e)	2,2,4,4-$^2H_4$ (10f)	2,2,4,4, 5α,7,7-$^2H_7$ (10g)	5α,7,7-$^2H_3$ (10h)	6β-^{18}O (10i)
$[M]^{+\cdot}$	548	566	557	549	549	550	552	555	551	550
$[M-CH_3]^+$	533	548	542(3)[a] 539(4)[a]	534	534	535	537	540	536	535
$[M-90]^+$	458	467	467(1) 458(9)	459	459	460	462	464	461	458
$[M-90-15]^+$	443	452(8) 449(1)	452(3) 449(1) 443(6)	444	444	445	447	449	446	445(1) 443(1)
$[M-131)^+$	417	426	426	417	418	418	419	420	419	419
	403	412	412	403	404	404	403	404[c] 405[c]	403[c] 404[c]	405
$[M-90-89]^+$	369	369	369	370	370	371	373	375	372	369
$[M-2\times90]^{+\cdot}$	368	368	368	369	369	370	372	374	371	368
$[M-2\times90-15]^+$	353	353	353	354	354	355	357	359	356	353
	329	329	329	329	330	330	332	[b]	332	329
	321	330	330	321	322	322	321	[b]	322	323
	318	327	318	319	319	320	322	[b]	321	318
	305	314	314	306	305	306	[b]	[b]	308	307
	304	313	304	305	305	306	[b]	[b]	307	304
	285	303	294	286	286	287	289	289	286	287
	204	222	213	205	205	206	204	[b]	204	206

[a] Values in parentheses refer to relative ratios of indicated peaks.
[b] Not possible to ascertain *m/z* value due to interfering peaks.
[c] Correct ratios not determined because of interfering peaks.

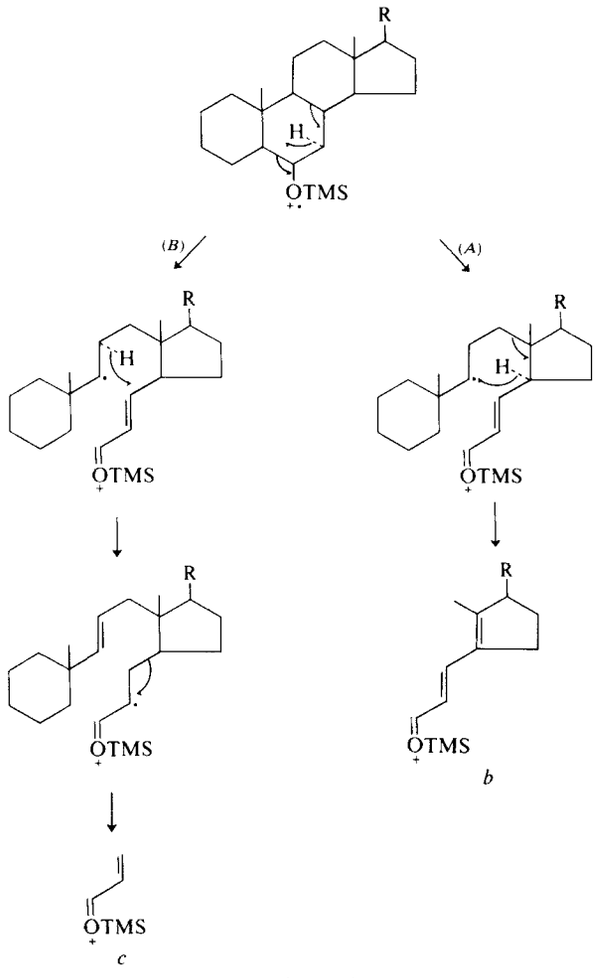
Table 5. Partial mass spectra of the TMS derivatives of cholest-4-ene-3β,6β-diol (15) and its isotopically labeled analogs (15a–15d).[a] *M/z* values of most significant ions.

Type of ion	(15)	3β,6β-bis[$^2H_9$]TMS (15a)	3β-TMS, 6β-[$^2H_9$]TMS (15b)	3α-^{2}H (15c)	3α,6α-$^2H_2$ (15d)
$[M]^{+\cdot}$	546	564	555	547	548
$[M-15]^+$	531	549(9)[a] 546(1)[a]	540	532	532
$[M-29]^+$	517	535	526	518(2) 517(1)	519(5) 518(1)
$[M-90]^{+\cdot}$	456	465	465(1) 456(9)	457(4) 456(1)	458
$[M-90-15]^+$	441	450	450(3) 441(2)	442(20) 441(1)	443(10) 442(1)
$[M-90-28]^{+\cdot}$	428	438		429(3) 438(1)	430(5) 429(1)
	403	412	412	404(1) 403(4)	404(1) 403(1)
	389	398	398	390(1) 389(1)	390
$[M-2\times90]^{+\cdot}$	366	366	366	367(6) 366(1)	367
	337	355	346	338	338
	321	330	330	321	321
	283	301	292	284(4) 283(3)	285(1) 284(1)
	217	235	226	217	217
	194	203	194	195	196
	180–183	189–192	180–183	181–184	181–184
	168, 169	177, 178	168, 169	169–170	169–170
	142–143	151, 152	142, 143	143, 144	143, 144
	131	140	131	132	[b]
	129	138	138	129	130

[a] Values in parentheses refer to ratios of indicated peaks.
[b] Not possible to ascertain *m/z* value due to interfering peaks.

While the loss of a 6β-OTMS group was the major mechanism producing the $[M-TMSOH]^{+\cdot}$ ions, the ion produced by elimination of TMSOH and $CH_3\cdot$ ($[M-105]^{+}$), at least in the cases of the labeled analogs (**10** and **15**), appeared to involve a substantial loss of the 3-OTMS group and a methyl radical, probably C-19, from the steroid nucleus. This was particularly evident by the retention of about 50% of the ^{18}O-label in the spectrum of **10i** (Table 4) and the low abundance of species containing 6-deuterium atoms in the spectrum of the selectively labeled TMS/[$^2H_9$]TMS derivative (**10b**).

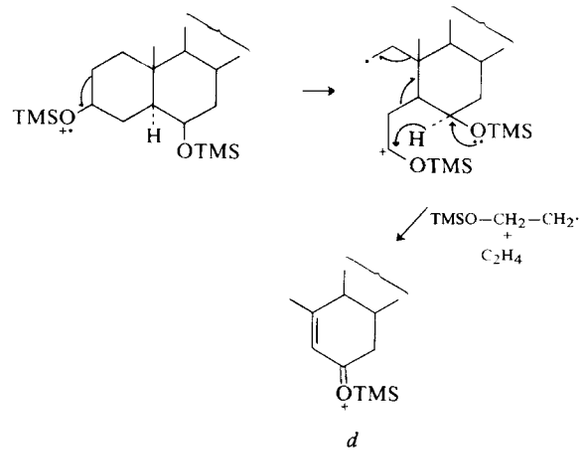
Ions characteristic of 6-substitution. Two ions (*b*, Scheme 2 and *d*, Scheme 3) were observed in the spectra of all the steroids and appeared to be diagnostic of 6-OTMS substitution. As both contained the D-ring, their masses varied with the composition of the 17-substituents. In the cholestane series ions *b* and *d* were observed at *m/z* 321 and 403 respectively. The abundances of these ions were low in the spectra of the monosubstituted derivatives – but increased considerably in the presence of 3-substitution (Table 3).



Scheme 2

Ion *b* has been observed before in the spectra of 6-OTMS cholestanes, and a mechanism for its formation has been proposed by Brooks *et al.*[24] This mechanism (Scheme 2, route A) is fully consistent with the labeling data summarized in Tables 4 and 5. Ions of this type are not exclusive to TMS derivatives: they have been observed also in the spectra of 6-methoxy[25] and 6-ethylene acetal derivatives.[26] The abundance of ion *b* appeared to be greater in compounds with 5β stereochemistry but much lower in the steroids containing unsaturation in ring A. Substitution in the 3-position, particularly by a keto- group, tended to increase its abundance. No correlation could be found between its abundance and the stereochemistry at C-6.

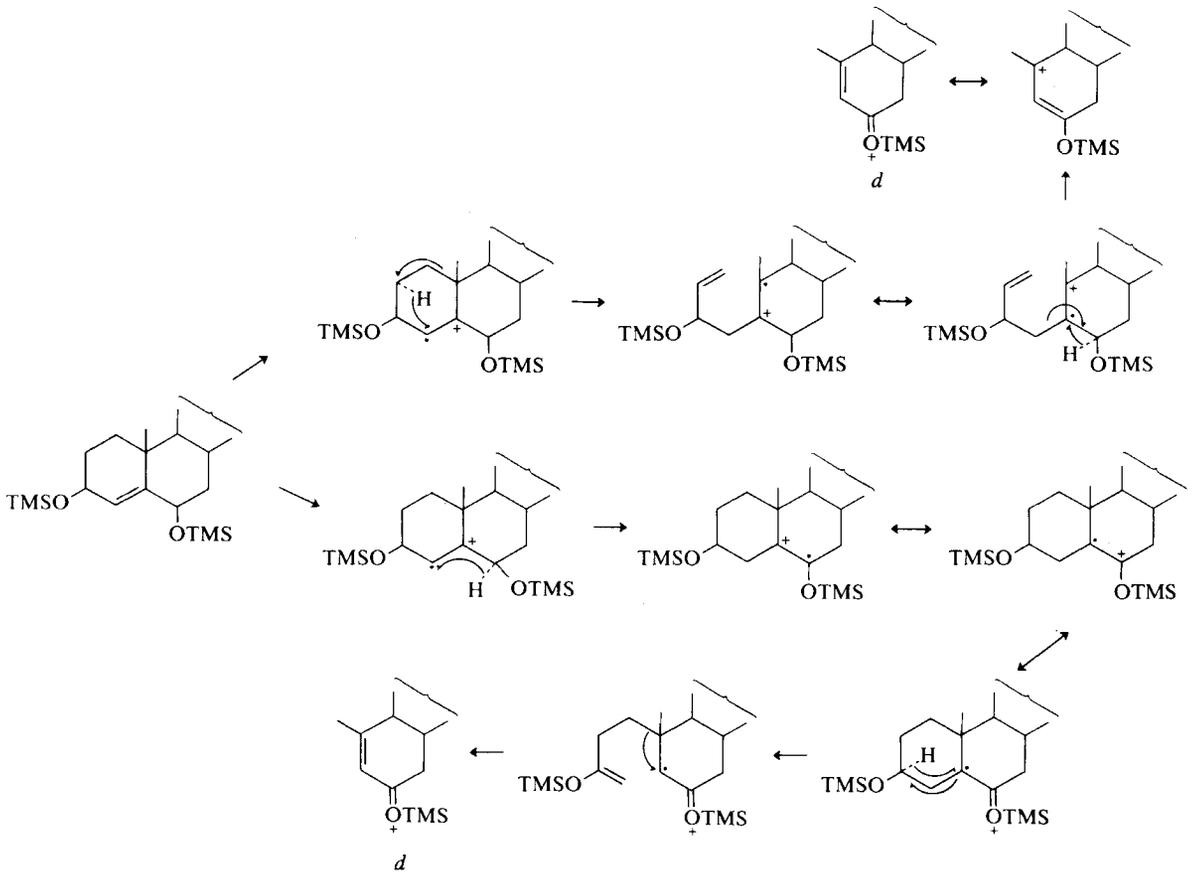
In a manner analogous to that of ion *b*, ion *d* was also of low abundance in the spectra of the monosubstituted steroids, but became much more prominent in the spectra of steroids containing 3-substitution. It was particularly abundant in the steroids containing a 3β-OTMS-Δ^4 group and formed the base peak in the spectrum of cholest-4-ene-3β,6β-bis-TMS. Again this ion is not exclusive to TMS derivatives; some underivatized steroids, particularly 3α,6β,20β-trihydroxy-5β-pregnane, fragment in a similar manner.[27] The mechanism proposed by Grupe and Spiteller[27] for the formation of this ion from the underivatized steroid involves elimination of water from the 3-OH group and the 6α-hydrogen with rings A and B in the boat conformation. This is followed by loss of the C_4H_7 radical, the latter comprising carbons C_1–C_4. This mechanism probably explains the high abundance of ion *d* in the spectrum of the bis-TMS derivatives of the related cholestane (**13**), but cannot account for its presence in the spectra of the other isomers, where abstraction of the 6α-hydrogen is not possible on steric grounds. Neither does it explain the metastable evidence suggesting formation of ion *d* from $[M]^{+\cdot}$ or the apparent hydrogen scramble evident in Table 4. In these instances a mechanism such as that shown in Scheme 3 is proposed.



Scheme 3

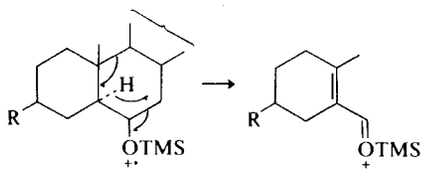
In the case of the 3,6-bis-OTMS-Δ^4 steroids, the labeling data in Table 5 suggest the participation of several mechanisms. Some 20% of ion *d* is formed by migration of the 3-hydrogen to the charged fragment and loss of, presumably, the 6-hydrogen, whereas the bulk of the ions of type *d* retain the 6-hydrogen. The formation of ion *d* can thus best be rationalized in terms of ionization of the double bond followed by hydrogen migrations as shown in Scheme 4.

A third TMS-containing ion (*e*, R=H) was observed at *m/z* 197 in the spectra of the monosubstituted



Scheme 4

compounds and at low abundance at m/z 285 in the spectra of the diols where it also contained the 3-OTMS group. A mechanism for its formation, consistent with the labeling data, is shown in Scheme 5. This ion has also been observed by Sjövall *et al.* in the spectrum of

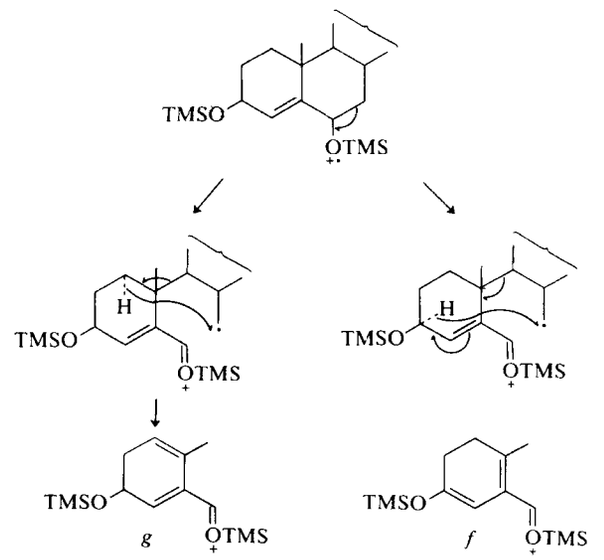


R = H, m/z 197
R = OTMS, m/z 285

Scheme 5

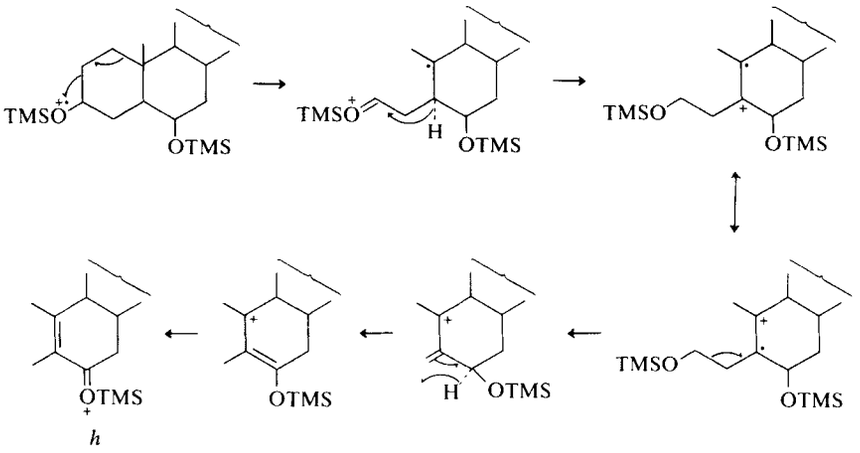
3α,6α(or β),7β-tris-OTMS-cholanate,[28] and related ions have been reported in the spectra of ethylene acetal derivatives[26] and of underivatized 5α-pregnane-3β,6α,20β-triol.[27]

In the spectra of the unsaturated diols (**15–17**), a related ion was observed in low abundance at m/z 283. The labeling data show hydrogen abstraction partly from C-3 and partly from another site (Table 5). The spectrum of the 2,2-[$^2H_2$] analog, not shown in the table as the exchange reaction did not go to completion, provided sufficient evidence to show that the C-2 hydrogens were not involved. Formation of these ions (f, g) can thus be rationalized by the two mechanisms shown in Scheme 6. In both cases, rotation about the C-9–C-10 bond brings the radical site at C-7 to within bonding distance of the indicated hydrogens.



Scheme 6

A rather weak TMS-containing ion was observed at $[M-131]^+$ (m/z 417, h) in the spectra of the cholestane



Scheme 7

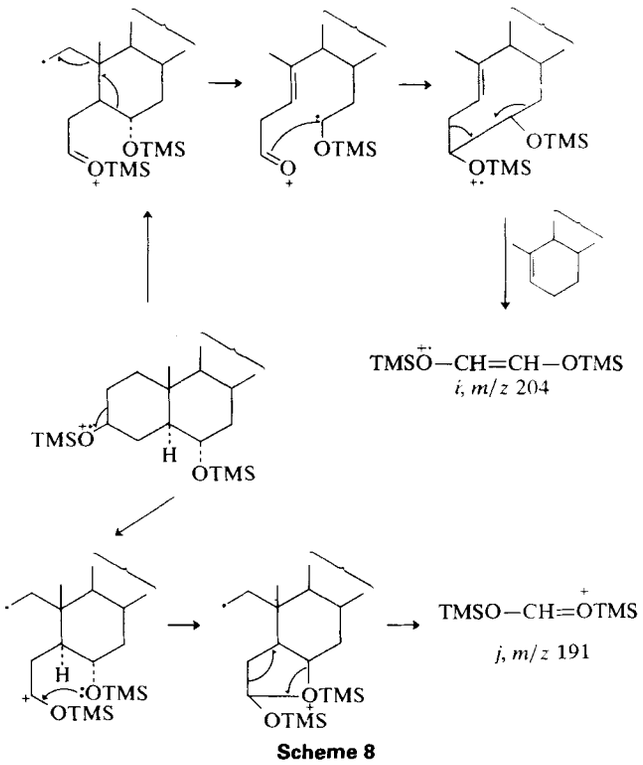
diol derivatives. From the isotope labeling data, this ion contains the hydrogens at C-6 and C-7 and two of the four hydrogens at C-2 and C-4 in addition to the 6-OTMS function. On this evidence its mechanism of formation can be rationalized as shown in Scheme 7. A complementary ion at m/z 131 was also observed.

Another ion containing the 6-OTMS group was present at m/z129 (*c*, Scheme 2, pathway B). The labeling data suggest that its mechanism of formation is similar to that proposed by Ahmad and Ansari[26] for the equivalent ion in the spectra of the corresponding ethylene acetals. This is triggered by the same cleavages that were involved in the formation of ion *b*, but the paths diverge after rupture of the C-8–C-9 bond; migration of the C-14 hydrogen leads to *b*, whereas transfer of the C-11 hydrogen to the double bond gives ion *c*. The presence of similar ions in the spectra of Δ^5-3-OTMS and 17-OTMS steroids precludes its use as an ion diagnostic of 6-substitution.

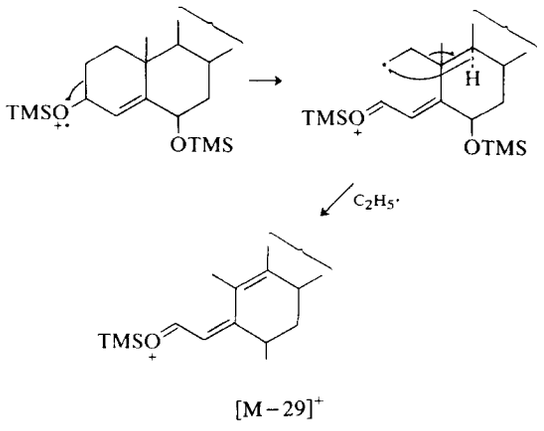
Other silicon-containing ions. The spectrum of the diequatorial compound, 5α-cholestane-3β,6α-bis-OTMS (**11**) was interesting in that its base peak was at m/z 204 (*i*) and an abundant ion was present at m/z 191 (*j*). These ions were of only minor importance in the spectra of the other compounds. Both ions contained two TMS groups and the 3-hydrogen, and thus probably also contained the 3-carbon. In addition, the ion at m/z 204 contained the 6-hydrogen. These ions are probably related, and possible mechanisms for their formation are suggested in Scheme 8. The compositions of both ions were verified by high resolution measurements. Ion *j* is frequently encountered in the spectra of steroids containing vicinal OTMS groups.[29] The high abundance of ions *i* and *j* is probably a reflection of the diequatorial OTMS groups which are not readily eliminated as TMSOH. In addition, the axial and somewhat shielded 6β-hydrogen is not so readily abstracted to form ions such as *d* (Scheme 3) which involve abstraction of the 6α-hydrogen. Moderately abundant ions at m/z 191 were also observed in the spectra of the other dihydroxy steroids containing a 6α-OTMS group (**7–9**).

The ion at $[M-29]^+$ in the spectrum of the Δ^4-cholestene diols (**15–17**), although containing the 6-OTMS-group, appears to be characteristic of the 3-OTMS-Δ^4 group, as it is also prominent in the spectra of 3-OTMS-Δ^4-steroids themselves. High resolution data are indicative of elimination of $C_2H_5\cdot$ from $[M]^{+\cdot}$, and although some hydrogen scrambling occurs, the labeling data indicate retention of the C-3 and C-6 hydrogens. Assuming that fragmentation is initiated by cleavage of the C-2–C-3 bond, as observed for several of the other fragmentations, a hydrogen transfer from C-9 to the radical site (Scheme 9) seems the most likely mechanism by inspection of molecular models.

Several other TMS-containing ions were present at low mass in the spectra of the TMS derivatives of most of the diols, particularly those containing Δ^4-unsaturation. The most abundant of these were at m/z 142, 143, 154, 155, 168, 169, 180–183 and 194–196. Selective TMS/[^{2}H$_9$]TMS labeling showed that these contained the 3-OTMS group and that they were not characteristic of 6-OTMS substitution. Indeed, similar ions were observed in the spectra of 3-OTMS steroids. Insufficient



Scheme 8



Scheme 9

labeling data are available to define the origin of these ions, and, as they are not characteristic of 6-OTMS substitution, they will not be discussed further. Most other ions were hydrocarbon fragments typical of steroids in general.[30,31]

In conclusion, the ions most characteristic of the presence of a 6-OTMS group appear to be ions *b*, *d* and *e*. Although of very low abundance in the spectra of the 6-monohydroxy steroids, these ions became very prominent when an additional 3-substituent was present. No general conclusions were reached which could relate ion abundances to stereochemistry, although ion *b* generally seemed to be more abundant in the steroids carrying a 6β-OTMS group. Ion *d* was particularly abundant in the spectra of the Δ^4-compounds. It is of interest that the characteristic ions produced by the 3-OTMS groups were still present in the spectra of the 3,6-diols, although they were of low abundance. Indeed, many of the ions containing the 6-OTMS group appeared to be produced by initial ionization around the 3 position, particularly the C-2–C-3 bond, thus explaining their greater prominence in the spectra of the compounds containing 3-substitution. Charge localization at the 6-OTMS function often resulted in elimination of this group as trimethylsilanol, if suitable hydrogen atoms were present. The most significant difference between the spectra of stereoisomeric alcohols appeared to be related to their axial or equatorial configuration. The presence of axial OTMS groups gave rise to molecular ions of low abundance, and prominent ions produced by loss of TMSOH; this was probably the result of greater hydrogen availability in these isomers.

Acknowledgments

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