Influence of 11-Trimethylsilyloxy- or 11-Hydroxy-substituents on the Electron-impact-induced Fragmentation of Trimethylsilyl Derivatives of Some Androstanols

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The mass spectra of the trimethylsilyl derivatives of some androstanes containing an 11-hydroxy-substituent exhibit several prominent and characteristic ions containing the trimethylsilyl group. The mechanisms of formation of these ions have been investigated with the aid of high-resolution mass spectrometry and deuterium, ^{18}O, and perdeuteriotrimethylsilyl labelling. Ions of the same mass and composition occurring in the spectra of different compounds do not necessarily have the same structure, but their formation is dependent on the presence of the 11-trimethylsilyloxy-function.

THE use of trimethylsilyl derivatives for the characterization of steroids by g.l.c. was first reported by Horning *et al.*[1] Trimethylsilyl derivatives have since been employed extensively in the separation and identification of steroidal metabolites, especially by combined g.l.c.–mass spectrometry.[2] The presence of the trimethylsilyl substituent in most compounds tends to direct the mass spectrometric fragmentation and often results in the formation of diagnostically useful fragment ions.[3] The mass spectra of steroids containing trimethylsilyloxy-substituents in the 3-position, ring D, or the 17β-side chain have been investigated extensively and several characteristic fragmentations have been noted.[2-4] Comparatively little information, however, is available about the electron-impact-induced fragmentation leading to

[1] T. Luukkainen, W. J. A. VandenHeuvel, E. O. Haahti, and E. C. Horning, *Biochim. Biophys. Acta*, 1961, **52**, 599.

[2] See for example (*a*) E. M. Chambaz, C. J. W. Brooks, M. G. Horning, E. C. Horning, and R. M. Hill, *Compt. rend.*, 1969, **268**, 2817; (*b*) E. C. Horning, C. J. W. Brooks, and W. J. A. VandenHeuvel, *Adv. Lipid Res.*, 1968, **6**, 354; (*c*) H. Eriksson, J.-Å. Gustafsson, and J. Sjövall, *European J. Biochem.*, 1971, **19**. 433; (*d*) C. H. L. Skackleton, J.-Å. Gustafsson, and J. Sjövall, *Steroids*, 1971, **17**, 265, and references cited therein.

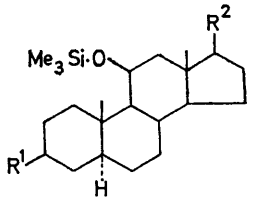
[3] J. Diekman and C. Djerassi, *J. Org. Chem.*, 1967, **32**, 1005.

[4] (*a*) P. Eneroth, K. Hellström, and R. Ryhage, *J. Lipid Res.*, 1964, **5**, 245; (*b*) J. Sjövall and R. Vihko, *Steroids*, 1966, **7**, 447; (*c*) P. Vouros and D. J. Harvey, *Org. Mass Spectrometry*, 1972, **6**, 953.

characteristic ions in the mass spectra of the trimethylsilyl derivatives of 11-hydroxy-steroids. We report a detailed investigation of the mass spectra of the trimethylsilyl derivatives of 5α-androstan-11β-ol and some related polyfunctional steroids.

RESULTS AND DISCUSSION

In addition to the typical $M^{+\cdot}$ (*m/e* 348), $(M - 15)^+$ (*m/e* 333), $(M - 90)^{+\cdot}$ (*m/e* 258), and $(M - 90 - 15)^+$ (*m/e* 243) ions, the mass spectrum of the trimethylsilyl derivative (1) of 5α-androstan-11β-ol exhibits several trimethylsilyl-containing ions, namely *m/e* 103, 117, 143, 156, 169, 184, 197, 251, and 266, whose elemental compositions were determined by high-resolution mass spectrometry (Table 1). These ions were further identified as containing an intact trimethylsilyl group by comparison with the spectrum of the perdeuteriotrimethylsilyl derivative. A similar series of ions was also observed in the mass spectra of the trimethylsilyl derivatives of 5α-pregnan-11β-ol [(2), Table 1], and the 11α-isomers of (1) and (2). The ions at *m/e* 156, 169, 184, and 197 were shifted by 28 mass units in the spectrum of the pregnane derivative (2), indicating incorporation of the 17β-ethyl chain (confirmed by high-resolution mass measurements) and, presumably, parts

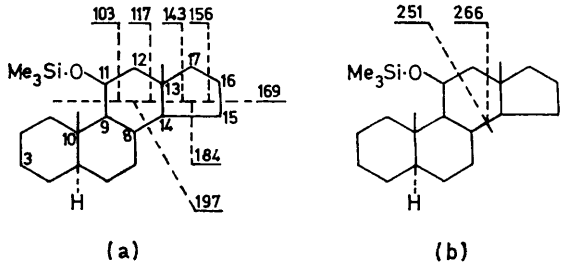


(1) $R^1 = R^2 = H$ (4) $R^1 = O{\cdot}SiMe_3$, $R^2 = H$

(2) $R^1 = H$, $R^2 = Et$ (5) $R^1 = H$, $R^2 = O{\cdot}SiMe_3$

(3) $R^1 = R^2 = O{\cdot}SiMe_3$

of ring D in the respective fragment ions. Formation of the foregoing ions as well as those at *m/e* 103, 117, and 143 may thus be generally depicted as involving the



SCHEME 1 Skeletal fragmentations leading to characteristic ions in the mass spectrum of the trimethylsilyl derivative of 5α-androstan-11β-ol

skeletal fragmentations shown in Scheme 1(a). Hydrogen transfers accompany these skeletal cleavages and the formation of the characteristic ions is discussed later in terms of isotope-labelling results. The ions at *m/e* 251 and 266 were not shifted in the spectrum of the pregnane derivative (2) and may thus be accounted for by the general fragmentation pattern depicted in Scheme 1(b).

These trimethylsilyl-containing ions are also prominent, with minor variations, in the mass spectrum of the trimethylsilyl derivative (3) of 5α-androstane-3β,11β,17β-triol (Table 1). In the case of *m/e* 156 and 251 the ions contain an additional trimethylsilyloxy-group and thus are shifted by 88 a.m.u. to *m/e* 244 and 339, respectively. Other isomeric androstanes do not exhibit such a series of ions. It thus appears that these ions may be characteristic of steroids containing an 11-trimethylsilyloxy-group; consequently it is important to determine their exact mode of formation.

The mechanisms of formation of the foregoing ions were investigated with the aid of deuterium- and ^{18}O-labelled derivatives of (3) as shown in Table 2. This Table shows the elemental compositions of the main trimethylsilyl-containing ions in the mass spectrum of (3) and the mass shifts for these ions in the spectra of the isotopically labelled derivatives. On the basis of this evidence reasonable mechanisms for the formation of the most significant ions are described. The formation of most of the ions is initiated by localization of the charge on the 11-oxygen atom (ion *c*), and subsequent cleavage of the C(9)–C(11) bond as shown in Scheme 2.

m/e 103. The evidence from isotope labelling (Table 2) indicates that *m/e* 103 ($CH_2{=}\overset{+}{O}{\cdot}SiMe_3$ (*a*) contains the 11-trimethylsilyloxy-group, and its formation involves cleavage of the C(9)–C(11) and C(11)–C(12) bonds accompanied by hydrogen abstraction, to a large extent from the 17-position. Molecular models show that cleavage of the 9,11-bond and free rotation about the 12,13-bond brings C-11 into close proximity with the 17α-hydrogen atom. An ion corresponding to the loss of 103 mass units (*m/e* 421) is also observed in the fragmentation of (3). It involves again the 11-trimethylsilyloxy-group, but in this case the hydrogen abstraction occurs from either position 9 or 12.

m/e 129. As previously reported [2,3] this ion has the structure $CH_2{=}CH{-}CH{=}\overset{+}{O}{\cdot}SiMe_3$ (*b*). The data from Table 2 show that in compound (3) it is formed predominantly from ring D with transfer of a 16-hydrogen atom to the steroid nucleus.

m/e 143 (*g*). This ion contains the 11-trimethylsilyloxy-group and a reasonable mechanism for its formation (*c* ⟶ *d* ⟶ *e* ⟶ *f* ⟶ *g*) consistent with the isotope labelling data is shown in Scheme 2. The indicated transfer of the 16-hydrogen atom (*f*) is about 50% specific. A similar fragmentation sequence can be invoked to explain the formation of the ion of mass 143 in the spectra of the monohydroxy-derivatives (1) and (2).

m/e 169 (*i*, *k*). This is a major peak in the spectra of both the monohydroxy- [(1) and (2)] and the trihydroxy-derivatives (3). The isotope-labelling data in Table 2 show the presence of the 17- rather than the 11-trimethylsilyloxy-group in this ion and the complete absence of the 9-, 11-, and 12-hydrogen atoms (3e and *f*). These data together with the observed incorporation of

TABLE 1

Partial mass spectra of compounds (1)—(5); * relative intensities (%) of principal ions

Type of ion or *m/e* value	(1)	(2)	(3)	(4)	(5)
M	348 [a] (54);[b] $C_{22}H_{40}OSi$	376 (53)	524 (33)	436 (25)	436 (44)
M − 15	333 (15); $C_{21}H_{37}OSi$	361 (9)	509 (4)	421 (7)	421 (3)
M − 90	258 (100); $C_{19}H_{30}$	286 (80)	434 (100)	346 (36)	346 (100)
M − 90 − 15	243 (74); $C_{18}H_{27}$	271 (63)	419 (27)	331 (30)	331 (37)
M − 2 × 90			344 (27)	256 (79)	256 (30)
M − 2 × 90 − 15			329 (22)	241 (45)	241 (24)
M − 3 × 90			254 (29)		
M − 3 × 90 − 15			239 (16)		
421			(14)		
347		(12)			
339			(5)		
333					(14)
320				(24)	
318			(23)		
257		(26)			
251	(18); $C_{15}H_{27}OSi$	(28)			(9)
244			(7)		(7)
232	(34); $C_{17}H_{28}$				
230					(54)
228			(21)		
226		(33)			
225		(46)			
215	(8); $C_{16}H_{23}$	(21)	(13)	(24)	(21)
197	(38); $C_{11}H_{21}OSi$	(28)		(59)	
196			(20)		
191			(19)		(20)
184	(33); $C_{10}H_{20}OSi$	(61)		(48)	
182			(36)		(27)
176		(47)			
169	(29); $C_9H_{17}OSi$	(29)	(77)	(56)	(62)
156	(49); $C_8H_{16}OSi$			(100)	
143	(22); $C_7H_{15}OSi$	(100)	(48)	(52)	(40)
129			(47)	(24)	(30)
117	(7); $C_5H_{13}OSi$	(10)	(13)	(19)	(16)
103	(8); $C_4H_{11}OSi$	(9)	(9)	(19)	(9)

[a] Mass of ion indicated. [b] Figures in parentheses are relative intensities.

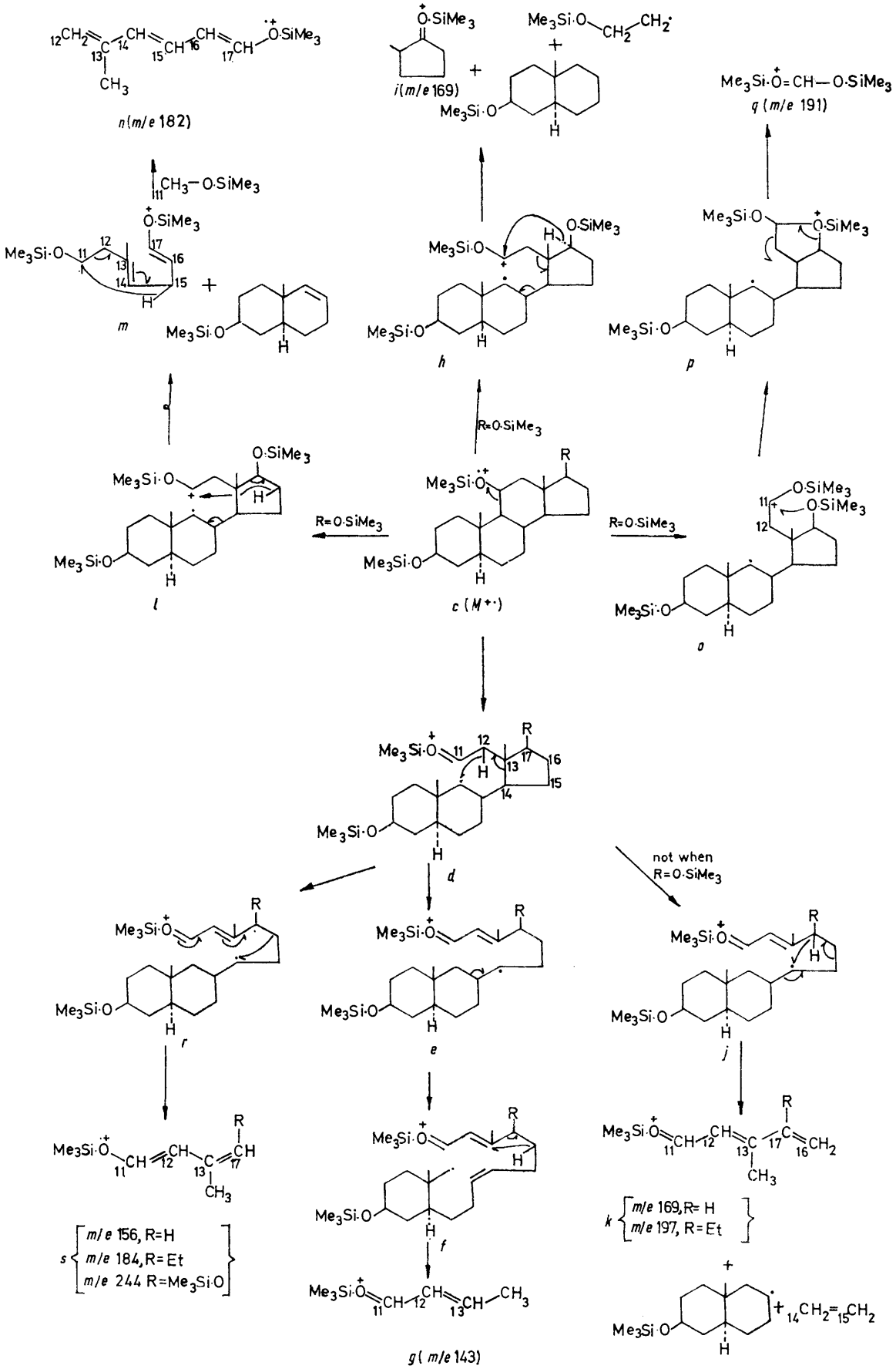
* Complete spectra are available as Supplementary Publication No. SUP 20648 (6 pp.). For details of Supplementary Publications see Notice to Authors No. 7 in *J. Chem. Soc.* (*A*), 1970, Issue No. 20.

TABLE 2

Mass values of the most significant trimethylsilyl-containing ions in the spectra of compound (3) and its isotopically labelled analogues

(3) [a]	Elemental composition	(3a) [b]	(3b) [c]	(3c) [17-^{18}O]	(3d) [17α-^{2}H]	(3e) [11α,17α-$^2H_2$]	(3f) [9,12,12-$^2H_3$]	(3g) [16,16-$^2H_2$]
103	$C_4H_{11}OSi$	112	112	103	103 (6) [d]	104 (3)	103	103
					104 (4)	105 (7)		
117	$C_5H_{13}OSi$	126	126	117	117	*e*	*e*	*e*
129	$C_6H_{13}OSi$	138	129	131	130	130	129	130
143	$C_7H_{15}OSi$	152	152	143	143	144	144	143 (1)
								144 (1)
169	$C_9H_{17}OSi$	178	169	171	169	169	169	171
182	$C_{10}H_{18}OSi$	191	182	184	182 (2)	182 (2)	184	183
					183 (3)	183 (3)		
191	$C_7H_{19}O_2Si_2$	209	200	193	191 (1)	192	191	191
					192 (1)			
196	$C_{11}H_{20}OSi$	205	196	198	196	197	196	197 (4)
								198 (3)
244 (156 + 88)	$C_{11}H_{24}O_2Si_2$	262	253	246	245	246	245	244
303		312	303	303	303 (1)	304 (1)	305 (3)	303
					304 (1)	305 (1)	306 (2)	
318	$C_{20}H_{34}OSi$	327	327	318	318 (3)	319 (3)	321	318
					319 (7)	320 (2)		
339 (251 + 88)	$C_{18}H_{35}O_2Si_2$	357	348	339	339	340	339	339

[a] Unlabelled. [b] 3β,11β,17β-Tris([$^2H_9$]trimethylsilyloxy)-5α-androstane. [c] 3β,17β-Bis(trimethylsilyloxy)-11β-[$^2H_9$]trimethylsilyloxy-5α-androstane. [d] Figures in parentheses refer to the relative ratios of the indicated peaks. [e] No measurement could be made because of other interfering peaks.



SCHEME 2 Fragmentation mechanisms depicting reasonable modes of formation of characteristic trimethylsilyl-containing ions in the spectra of compounds (1)—(5)

both the 16-hydrogen atoms support the structure *i* and a mechanism of formation (*c* ⟶ *h* ⟶ *i*) shown in Scheme 2. Metastable-defocusing data confirmed that *m/e* 169 is formed directly from the molecular ion, and to some extent from the $(M - 90)^{+\cdot}$ ion.

In contrast, the *m/e* 169 ion in the spectrum of the monohydroxy-derivative (1) contains the 11-trimethylsilyloxy-substituent. Its 28 mass unit shift in the mass spectrum of the pregnane derivative (2) supports the fragmentation pattern *c* ⟶ *d* ⟶ *j* ⟶ *k* shown in Scheme 2. The 17-hydrogen atom transfer in *j* and incorporation of C-16 in *m/e* 169 is suggested here because of the presence and well documented mode of formation of the ion of mass 169 having structure *k* (100%; 6·5% Σ_{40}) in the spectrum of the trimethylsilyl derivative of 5α-androstan-16β-ol.[4c]

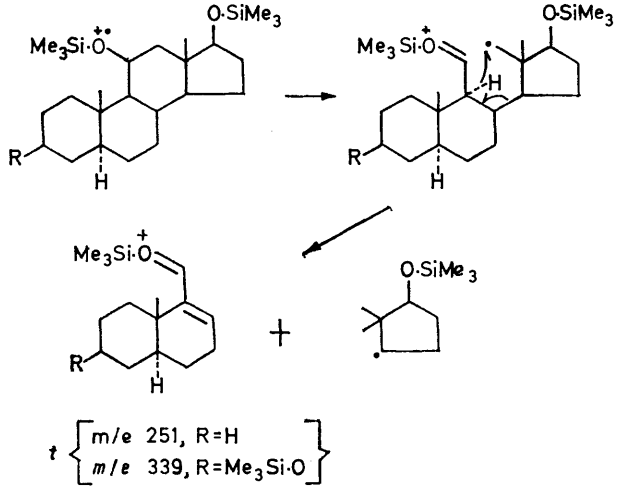
The foregoing data indicate that although *m/e* 169 does not have the same structure in the spectra of the monotrimethylsilyloxy- [(1) and (2)] and tristrimethylsilyloxy- (3) derivatives, its formation appears to be dependent on the presence of the 11-substituent. The fragmentation process leading to its formation is in part activated by the cleavage of the 9,11-bond as shown in Scheme 2. Additional evidence to this effect is demonstrated by the spectrum of 5α-androstane-3β,11β,17β-triol 3β,17β-bis(trimethylsilyl) ether. The peak at *m/e* 169 is again prominent (*ca.* 60%) and contains the 17-trimethylsilyloxy-group, but the 11-hydroxy-substituent undoubtedly plays a major role in initiating the cleavage of the 9,11-bond. Furthermore no *m/e* 169 ion is observed in the spectra of the trimethylsilyl derivatives of 5α-androstane-3β,17β-diol or 5α-androstan-17β-ol where the 11-substituent is absent.

m/e 182 (*n*); *m/e* 244 (*s*). Mechanisms of formation of these ions consistent with the isotope labelling data are shown in Scheme 2 (*c* ⟶ *l* ⟶ *m* ⟶ *n* and *c* ⟶ *d* ⟶ *r* ⟶ *s*, respectively). Ions of type *s* contain the C-17 substituent and therefore appear at *m/e* 156 and 184 in the spectra of (1) and (2) respectively.

m/e 191 (*q*). The ion of mass 191 has been reported in the mass spectra of the trimethylsilyl derivatives of several steroids containing two or more hydroxy-groups in ring D and/or the angular C-13 methyl group, and its mechanism of formation has been studied in detail for these cases.[5] It is also relatively abundant (19%) in the spectrum of the trihydroxy-derivative (3). The labelling data [(3c, d, and e), Table 2] indicate that its formation involves the interaction of the 11- and 17-trimethylsilyloxy-groups following cleavage of the 9,11-bond (*c*), and rotation about the 12,13- and 11,12-bonds (*o*, *p*). Ion *q* (*m/e* 191) is then formed either by bond formation between C-11 and the 17-trimethylsilyloxy-group as shown in Scheme 2, or between C-17 and the 11-trimethylsilyloxy-group as is evident from the spectra of the deuterium labelled compounds (3d and e). The formation of the *m/e* 191 rearrangement ion in the case of the trihydroxy-derivative (3) is not stereochemically dependent because of the prior cleavage of the 9,11-bond, which results in the loss of conformation. This is in contrast to the formation of another common trimethylsilyl rearrangement ion (*m/e* 147), which has been shown to be strongly dependent on stereochemistry.[6]

m/e 196. This ion again contains ring D and the 17-trimethylsilyloxy-group but the lack of the 12-, 11-, and 9-hydrogen atoms suggests a cleavage across the 12,13-, 8,9-, and 6,7-bonds. No further conclusive evidence is available, however, to support a definite mechanism for its formation.

m/e 339 (*t*). An analogue for this ion is observed at *m/e* 251 in the mass spectra of the monohydroxy-derivatives (1) and (2). Unlike the other major ions already discussed, formation of this ion appears to be initiated by the competing α-cleavage of the 11,12- rather than the 9,11-bond. The isotope-labelling data support the mechanism shown in Scheme 3.



SCHEME 3 Mechanism of formation of ion *t* in the spectra of compounds (1)—(5)

The characteristic trimethylsilyl-containing ions observed in the spectrum of 11-trimethylsilyloxy-5α-androstane (1) were also present in that of the 3β,11β-dihydroxy-derivative (4). Preparation of the derivative of the latter compound containing a trimethylsilyl and a perdeuteriotrimethylsilyl group in the 3- and 11-positions, respectively showed that all the foregoing fragment ions contained the 11-trimethylsilyl system. Similarly, the fragmentation of 11β,17β-bistrimethylsilyloxy-5α-androstane (5) parallelled that of the triol (3). It is thus apparent that the presence of the 3-trimethylsilyloxy-substituent has no significant effect on the fragmentation pattern of those compounds. Instead, in all cases the fragmentation is apparently activated by charge localization on the 11-trimethylsilyloxy-substituent. The presence of these characteristic ions may thus be a helpful guide in the identification of steroids containing an 11-hydroxy-function.

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[6] (*a*) R. T. Gray, J. Diekman, G. L. Larson, W. K. Musker, and C. Djerassi, *Org. Mass Spectrometry*, 1970, **3**, 973; (*b*) P. D. Woodgate, R. T. Gray, and C. Djerassi, *ibid.*, 1970, **4**, 257; (*c*) S. Sloan, D. J. Harvey, and P. Vouros, *ibid.*, 1971, **5**, 789; (*d*) S. C. Havlicek, M. R. Brennan, and P. J. Scheuer, *ibid.*, 1971, **5**, 1273.

EXPERIMENTAL

5α-Androstane-3β,11β,17β-triol was commercially available. 5α-Androstan-11β-ol, 5α-pregnan-11β-ol, and 5α-androstane-3β,11β-diol were prepared by Wolff–Kishner reduction [7] of 11β-hydroxy-5α-androstane-3,17-dione, 11β-hydroxy-5α-pregnane-3,20-dione, and 3β,11β-dihydroxy-5α-androstan-17-one, respectively. 5α-Androstane-11β,17β-diol (5) was prepared from 3β-hydroxy-5α-androstane-11,17-dione. Reduction of the *p*-tolylsulphonyl derivative of the latter with lithium aluminium hydride [8] yielded the 11,17-dihydroxy-compound. The assignment of a β-configuration to the 11- and 17-hydroxy-groups in (5) is based on previous data on the hydride reduction of 11- and 17-oxo-steroids.[9]

The purity and elemental composition of all commercially available and synthesised compounds was checked by combined g.l.c.–mass spectrometry and high-resolution mass spectrometry of their trimethylsilyl derivatives. The methods used for the preparation of the trimethylsilyl derivatives of the foregoing compounds have been described before.[10]

The labelled derivatives of 5α-androstane-3β,11β,17β-triol (3) were prepared as follows. 3β,11β,17β-Tris([$^2H_9$]trimethylsilyloxy)-5α-androstane (3a) was prepared by reaction of the trihydroxy-compound with bis[$^2H_9$]trimethylsilylitrifluoroacetamide and chloro[$^2H_9$]trimethylsilane in pyridine.[11] Selective silylation of the trihydroxy-compound (3) and the 3,11-dihydroxy-androstane (4) with trimethylsilyl and [$^2H_9$]trimethylsilyl groups was accomplished [12] on the basis of the variant reactivity of the 11-hydroxy-group as compared to the 17- and/or 3-hydroxy-groups.[13] 5α-Androstane-3β,11β,[^{18}O]17β-triol (3c) was prepared by reduction of 3β,11β-dihydroxy-5α-androstan-17-one with lithium aluminum hydride following exchange of the carbonyl oxygen of the latter compound in 10^{-3}M-HCl in propan-2-ol in the presence of 50% ^{18}O-enriched water.[14] Similarly, 5α-androstane-11β,[^{18}O]17β-diol was prepared by lithium aluminum hydride reduction of 3β-tosyloxy-5α-androstane-11,17-dione, following exchange of the 17-oxygen of 3β-hydroxy-5α-androstane-11,17-dione in the ^{18}O-enriched water–propan-2-ol–HCl solution.

The deuterium-labelled analogues 5α-[17α-^{2}H]androstane-3β,11β,17β-triol (3d) and 5α-[11α,17α-$^2H_2$]androstane-3β,11β,17β-triol (3e) were prepared by the reduction of 3β,11β-dihydroxy-5α-androstan-17-one and 3β-hydroxy-5α-androstane-11,17-dione, respectively, with lithium aluminum deuteride. Finally, 5α-[9,12,12-$^2H_3$]androstane-3β,11β,17β-triol (3f) and 5α-[16,16-$^2H_2$]androstane-3β,11β,17β-triol (3g) were prepared by the reduction of 3β,17β-dihydroxy-5α-androstan-11-one and 3β,11β-dihydroxy-5α-androstan-17-one with lithium aluminum hydride following exchange of the protons α to the carbonyl group in a basic solution of D_2O and dioxan.

The mass spectra were recorded with an LKB-9000 spectrometer. The ionizing voltage was 70 eV, the accelerating voltage was 3·5 kV and the ion source temperature 250°. The samples were introduced *via* the gas chromatographic inlet (6 ft, 1% OV-17 column). The column temperature was programmed over the range 180—240° at a rate of 4° min^{-1}. High-resolution mass spectra were obtained with a CEC 21–110B spectrometer and were photographically recorded.

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