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GAS CHROMATOGRAPHY—MASS SPECTROMETRY OF EPIMERIC 19-NORANDROSTAN-3-OL-17-ONES AS THE TRIMETHYLSILYL ETHER, METHYLOXIME-TRIMETHYLSILYL ETHER AND TRIMETHYLSILYL-ENOL TRIMETHYLSILYL ETHER DERIVATIVES

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SUMMARY

The capillary gas chromatographic and mass spectrometric properties of trimethylsilyl (TMS), methyloxime-trimethylsilyl (MO-TMS) and trimethylsilyl-enol trimethylsilyl (TMS-enol-TMS) ether derivatives of four stereoisomeric 19-norandrostan-3-ol-17-ones are presented. The best gas chromatographic separation was obtained with the TMS ethers. Their electron-impact mass spectra show identical fragmentation pathways. However, the isomeric steroids can be differentiated by the characteristic relative intensities of their structurally informative ions. The separation of various epimeric pairs is reduced by changing from TMS to MO-TMS and TMS-enol-TMS ether derivatives. The mass spectra of MO-TMS derivatives are dominated by intense $[M - 31]^+$ and $[M - (31 + 90)]^+$ ions, whereas those of the TMS-enol-TMS ethers exhibit prominent ions at M^+ and $[M - 15]^+$. Evidence is presented for the formation of 16-hydroxy-19-norandrosterone TMS derivatives when vigorous conditions were used for the preparation of the TMS-enol-TMS ether derivatives. The mechanisms giving rise to various characteristic fragment ions were investigated with the corresponding $[^2H_9]$TMS derivatives. Gas chromatographic properties and mass spectrometric features are discussed.

INTRODUCTION

The combined technique of gas chromatography—mass spectrometry (GC—MS) has been extensively employed for the analysis of endogenous steroids. Although their major fragmentation pathways are now well defined [1], much attention is still being given to the preparation of specific derivatives suitable for GC—MS analysis of steroids bearing hydroxyl and ketonic functions.

Trimethylsilyl (TMS) ethers have proved to be the most valuable and widely

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used derivatives for GC—MS analysis [2, 3] of hydroxylated steroids. Methyloxime-trimethylsilyl (MO-TMS) ethers, which have also been used extensively, are particularly suitable for quantitative analysis [4]. On the other hand, trimethylsilyl-enol trimethylsilyl (TMS-enol-TMS) ethers [5, 6] have found some applications in the GC—MS profiling of urinary steroids [7, 8], in the analysis of urinary testosterone [9] and corticosteroids [6]. Several other silicon-containing derivatives such as methyloxime-*tert.*-butyldimethylsilyl ether [10], alkoxydialkylsilyl ether [11] and some sterically crowded trialkylsilyl ether derivatives [12] have been used for the GC—MS analysis of endogenous steroids.

It is noteworthy that the capillary column GC and MS properties of isomeric 19-norandrostan-3-ol-17-ones TMS, MO-TMS and TMS-enol-TMS ether derivatives have been little studied. These steroids are of general interest because they are the major urinary metabolites of 19-nortestosterone [13, 14] in humans. This synthetic anabolic steroid is used clinically for the treatment of those conditions in which tissue building is desired, particularly in retarded growth and some endocrine deficiencies. Moreover, 19-nortestosterone is suspected to be widely misused by athletes aiming to increase their athletic performance. It is also used as a growth-promoting agent in farm animals [15] or to increase the physical capabilities of race horses [16].

In order to develop adequate GC—MS methods for the analysis of this group of isomeric steroids in biological fluids, we have prepared their corresponding TMS, MO-TMS and TMS-enol-TMS ether derivatives. This paper describes the GC behaviour and the mass spectral properties of these stereoisomeric derivatives and those of their perdeuterated TMS analogues.

EXPERIMENTAL

Reagents and steroids

Trimethylchlorosilane (TMCS), N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) and methoxyamine hydrochloride were obtained from Pierce (Rockford, IL, U.S.A.). Perdeuterated bis(trimethylsilyl)acetamide (BSA) was purchased from Merck Sharp and Dohme of Canada (Montreal, Canada). All solvents and liquid reagents were glass-distilled prior to use.

Pure reference steroids: 3α-hydroxy-5α-estran-17-one (19-norandrosterone), 3α-hydroxy-5β-estran-17-one (19-noretiocholanolone), 3β-hydroxy-5β-estran-17-one (19-norepietiocholanolone), 3β-hydroxy-5α-estran-17-one (19-norepiandrosterone) were kindly supplied by Schering (Berlin, F.R.G.); 5α-androstane, 5α-cholestane, 3α,16α-dihydroxy-5α-androstan-17-one (16α-hydroxyandrosterone) and 3α,16β-dihydroxy-5α-androstan-17-one (16β-hydroxyandrosterone) were obtained from Steraloids (Wilton, NH, U.S.A.).

Preparation of the derivatives

TMS and [$^2H_9$] TMS derivatives. The TMS derivatives of the isomeric 19-norsteroids were prepared as follows: 5 μg of the steroid were dissolved in 85 μl of dry pyridine and 15 μl of MSTFA, and 1 μl of TMCS was added. This solution was heated at 60°C for 15 min. One microlitre was injected into the chromatograph. Perdeuterated TMS derivatives were prepared by heating the

sample (5 μg) at 60°C for 30 min in a mixture of pyridine—perdeuterated BSA (85:15, v/v).

MO-TMS and MO-[$^2H_9$] TMS derivatives. The steroid (5 μg) was dissolved in a 10% (w/v) solution of methoxyamine hydrochloride in dry pyridine and the solution was heated at 60°C for 30 min. Excess pyridine was evaporated under a nitrogen stream at 60°C and 100 μl of pyridine—MSTFA (85:15, v/v) were added. The mixture was heated at 60°C for 15 min and 1 μl was injected for GC and GC—MS analysis. The perdeuterated derivatives were prepared using perdeuterated BSA instead of MSTFA as the silylation reagent.

TMS-enol-TMS ether and [$^2H_9$] TMS-enol-[$^2H_9$] TMS ether derivatives. The derivatives were prepared according to the method described by Chambaz et al. [6]. An alternative method was also used: the sample (5 μg) was dissolved in 100 μl of MSTFA, and 1 μl of trimethyliodosilane (TMIS) was added. The resulting mixture was heated at 60°C for 30 min. For the preparation of the perdeuterated TMS-enol-TMS ethers, the steroid (5 μg) was dissolved in 50 μl of dry pyridine and 50 μl of perdeuterated BSA were added. The mixture was heated at 75°C for 1 h in the presence of anhydrous sodium acetate.

Gas chromatography

Gas chromatography was performed on a Perkin-Elmer Sigma 2 gas chromatograph equipped with a flame-ionization detector. The injector and the detector temperatures were maintained at 250°C and 300°C, respectively. The chromatograph was fitted with a 30 mm × 0.25 mm fused-silica SE-30-coated capillary column. The oven temperature was programmed from 100°C (5 min hold) to 180°C at 8°C/min and then to 280°C at 3°C/min. Helium was the carrier gas with a flow-rate of 2.0 ml/min. One microlitre of the derivatization mixture was injected into the gas chromatograph in the splitless mode.

Gas chromatography—mass spectrometry

Low-resolution mass spectra were recorded in the electron-impact mode at 70eV with a Kratos MS-25 mass spectrometer interfaced to a Data General Nova 3 (DS-55) data system and to a Perkin-Elmer Sigma 3 gas chromatograph. The capillary column was directly introduced into the mass spectrometer ion source. Chromatographic separations were performed under the above-mentioned conditions. Other conditions were: ion source temperature, 250°C; accelerating voltage, 2.5 kV; trap current 100 mA; scan speed, 1 sec/decade.

RESULTS AND DISCUSSION

GC characteristics

Table I presents the GC relative retention times and steroid numbers [17] for the four isomeric 19-norandrostanolones *1—4* as their TMS, MO-TMS and TMS-enol-TMS ether derivatives. The first interesting aspect illustrated by these data is that the epimeric hydroxyl group at position C-3 has an important effect upon the GC mobility and separation of the steroidal derivatives. This influence is much more pronounced for the steroids having a *trans* A—B ring junction (5α-steroids). As shown in Fig. 1, 19-norandrosterone (*1*) and 19-norepiandrosterone (*4*) TMS derivatives are easily resolved on the SE-30

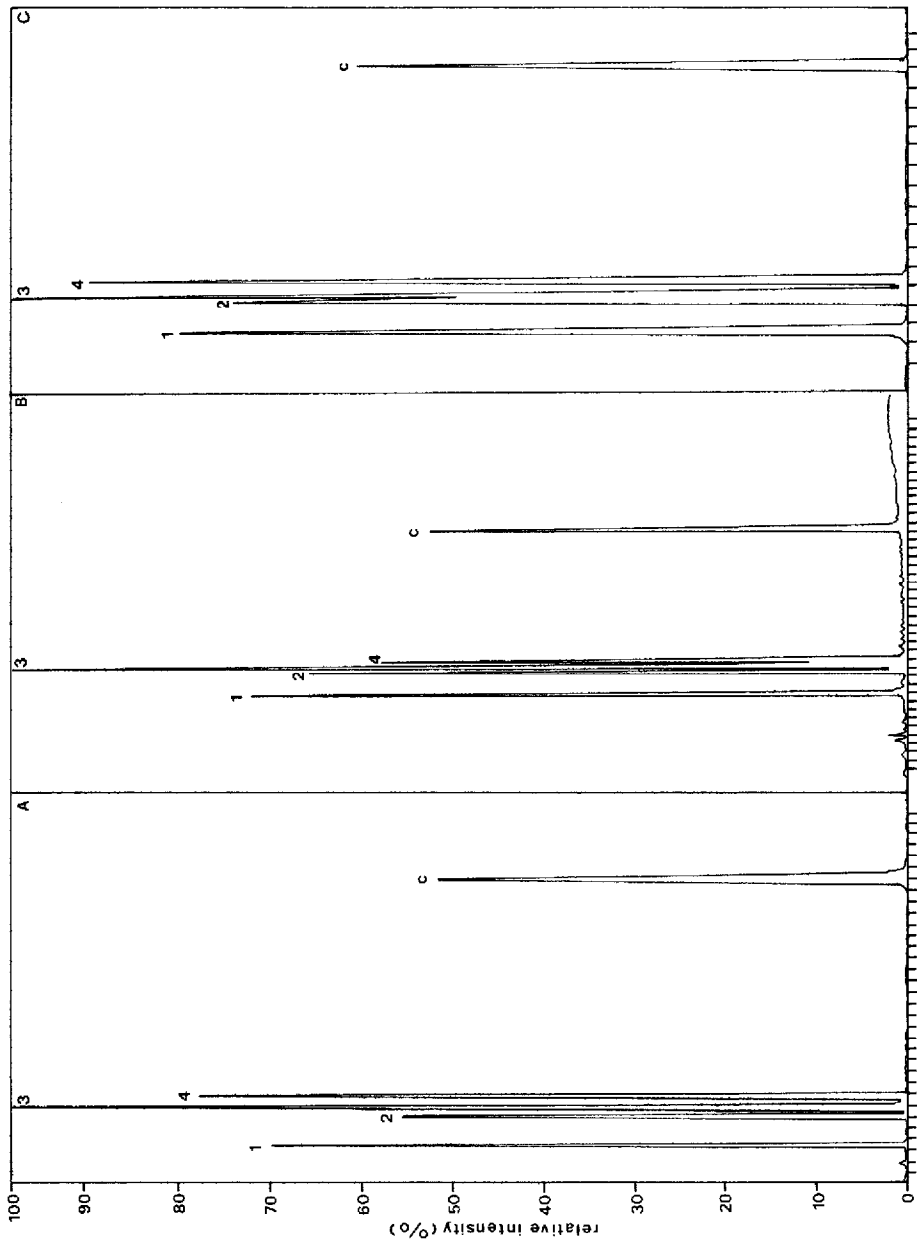
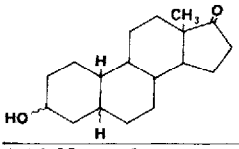


Fig. 1. Total ion chromatograms obtained by GC—MS analysis of a mixture of four 19-norandrostanolones (50 ng each) as (A) TMS, (B) MO-TMS and (C) TMS-enol-TMS ether derivatives. Numbers on the peaks refer to compounds listed in Table I. Peak c is 5α-cholestane (50 ng).

TABLE I

GAS CHROMATOGRAPHIC DATA FOR 19-NORSTEROID DERIVATIVES ON SE-30 CAPILLARY COLUMN

For gas chromatographic conditions, see the experimental part.



	TMS		MO-TMS		Enol-TMS	
	RRT*	SN**	RRT	SN	RRT	SN
1 19-Norandrosterone: 3α,5α	1.39	20.40	1.47	21.54	1.59	23.28
2 19-Norepietiocholanolone: 3β,5β	1.44	21.16	1.52	22.30	1.63	23.94
3 19-Noretiocholanolone: 3α,5β	1.46	21.33	1.54	22.56	1.64	24.08
4 19-Norepieandrosterone: 3β,5α	1.48	21.67	1.55	22.71	1.66	24.40

*Retention time relative to that of 5α-androstane: 22.83 min.

**Steroid numbers calculated from relative retention times; 5α-androstane and 5α-cholestane were used as reference standards [17].

capillary column. On the other hand, the GC resolution of 19-norepietiocholanolone (*2*) and 19-noretiocholanolone (*3*) is achieved only when derivatized as TMS and MO-TMS derivatives. Their corresponding TMS-enol-TMS ether derivatives are only partially resolved.

Another interesting aspect of these data is the effect of derivatization of the 17-oxo group upon the chromatographic resolution of the epimeric steroids. One can observe that the change from TMS to MO-TMS or TMS-enol derivatives does not improve the chromatographic resolution of the steroids as illustrated in Fig. 1. On the contrary, the introduction of MO or TMS-enol substituents at C-17 has a negative effect upon the GC resolution of the steroids, particularly upon that of the MO-TMS derivatives of 19-noretiocholanolone (*3*) and 19-norepiandrosterone (*4*) and the TMS-enol-TMS ethers of the 5β-steroidal epimers *2* and *3*. Thus, derivatization of the C-17 oxo group slightly decreased the GC resolution of the steroids *2*, *3* and *4* under the experimental conditions used.

Formation of OTMS derivatives

Trimethylsilylation of hydroxylated oxo steroids can yield secondary products when the oxo group is unprotected. These products arise mainly from the formation of enol-TMS ethers. However, when oxo steroids are treated under vigorous silylation conditions, other products than the TMS-enol-TMS ether can be formed. For example, when 19-norandrosterone (*1*) was heated at 100°C for 16 h with a mixture of MSTFA—pyridine (1:1, v/v) in the presence of anhydrous sodium acetate, three silylation products were formed. In addition to 19-norandrosterone TMS (peak A) and TMS-enol-TMS derivatives (peak B) a third compound (peak C) was detected by GC—MS analysis (Fig. 2). Its mass spectrum shows a molecular ion at *m/z* 436 and a prominent ion at *m/z* 202 $[M - 90 - 144]^+$ (Fig. 3). Other diagnostically important ions were observed at *m/z* 421 $[M - 15]^+$, 331 $[M - 15 - 90]^+$, 292 $[M - 144]^+$ (ring D cleavage), 290 $[M - 144 - 2H]^+$, 187 $[M - 15 - 90 - 144]^+$ and 117 $[CH_3{-}CH{=}\overset{+}{O}TMS]$.

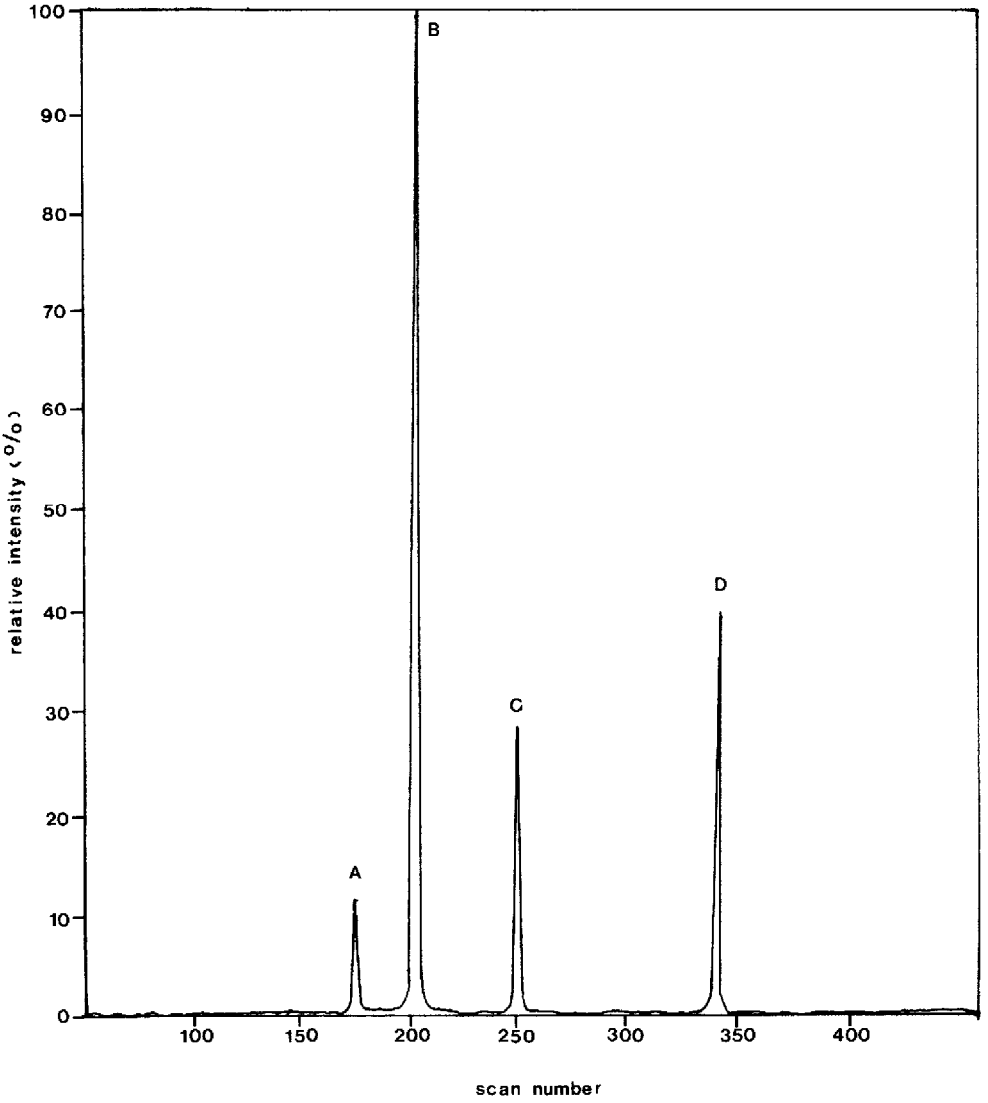


Fig. 2. Computer-reconstructed total-ion chromatogram after reaction of 19-norandrosterone (5 μg) with MSTFA—sodium acetate in dry pyridine at 100°C for 10 h: (A) TMS derivative, (B) TMS-enol-TMS ether derivative, (C) oxysilylation by-product, and (D) 5α-cholestane. Temperature programmed at 3°C/min from 200°C (2 min hold) to 280°C.

To further assess the reproducibility of the reaction and the identity of peak C, the reaction was performed under the same conditions using the 19-norsteroids *2*, *3* and *4* as substrates. The GC—MS analysis of the resulting reaction mixtures gave total ion chromatographic profiles identical to the one obtained for 19-norandrosterone (*1*) (Fig. 2). The mass spectra of the corresponding oxysilylation secondary products were identical to that of peak C. It thus appears that peak C and its isomeric analogues have a structure corresponding to 3,16-bis(trimethylsilyloxy)estrane-17-one. Comparison of this mass

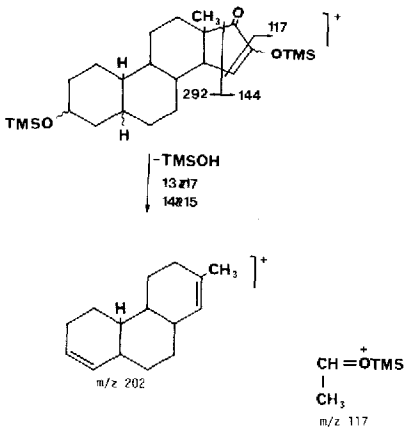


Fig. 3. Origin of ions at *m/z* 117, 144, 202, 292 in the mass spectrum of 19-norandrosterone oxysilylation product.

spectrum with literature spectra for the corresponding steroid TMS ethers in the androstane series [18—20] indicated that peak C was an isomer of 3α,16-dihydroxyestran-17-one. Further comparison with the mass spectrum of the TMS derivative of 3α,16-dihydroxyestran-17-one, a metabolite of 19-nortestosterone in the horse [21], confirmed that peak C was an isomer of 3α,16-dihydroxyestran-17-one. These products are apparently formed by the addition of one trimethylsilyloxy group at C-16 to a TMS-enol ether double bond, with subsequent elimination of the TMS-enol group. The formation of similar products have been reported by Chambaz et al. [5] following the trimethylsilylation of testosterone with a mixture of BSA—TMCS (10:1, v/v) under vigorous conditions.

Mass spectral properties

TMS ether derivatives. The mass spectra of the TMS ethers of the four 19-norsteroids *1—4* are summarized in Table II. The mass spectrum of the TMS derivative of 19-noretiocholanolone (*3*) given in Fig. 4 is representative of those of its corresponding epimers. Although all derivatives exhibit similar fragmentation patterns, the isomeric steroids can be easily differentiated from each other by comparison of the relative intensities of their diagnostically important ions at *m/z* 348 $[M]^+$, 333 $[M - 15]^+$, 258 $[M - 90]^+$, 257 $[M - 90 - H]^+$ and 216 $[M - 90 - 42]^+$ (Table II). Interpretation of the spectra was facilitated by comparison with those of their corresponding $[^2H_9]$TMS derivatives (Table III). TMS-containing fragments were easily identified in this manner. The fragment ion at *m/z* 257 was found to be characteristic of the TMS ethers of 19-norandrosterone (*1a*) and 19-norepiandrosterone (*4a*). The values of the ratio *m/z* 258/*m/z* 257 were 1.30 and 2.11, respectively. This ion was also recorded in the mass spectra of the 5β-steroid TMS derivatives *2a* and *3a*, but the values of the ratios of the relative intensities of the ions at *m/z* 258/*m/z* 257 were much more prominent (3.22 and 8.70, respectively) than those recorded in the mass spectra of their 5α-epimers. As can be seen from Table II, the $[M - 15]^+$ ions are much more abundant in the 5α-steroids, whereas the $[M - 90]^+$ ions are more prominent in the 5β-steroids *2a* and *3a*.

TABLE II

PARTIAL MASS SPECTRA OF THE UNLABELLED TMS, MO-TMS and TMS-ENOL-TMS ETHER DERIVATIVES OF 19-NORANDROSTANOLONES *1—4*

	M⁺	Principal ions in mass spectrum													
19-Norandrosterone (1)															
TMS	348*	333	292	258	257	243	230	216	202	201	155	145	129	75	73
	(42)**	(95)	(3)	(53)	(41)	(10)	(20)	(4)	(18)	(15)	(25)	(12)	(52)	(100)	(58)
MO-TMS	377	362	346	330	286	272	256	201	199	129	75	73			
	(3)	(8)	(100)	(5)	(8)	(4)	(91)	(10)	(12)	(8)	(38)	(13)			
TMS-enol	420	405	315	225	182	169	129	75	73						
	(100)	(78)	(25)	(6)	(8)	(22)	(7)	(8)	(70)						
19-Norepietiocholanolone (2)															
TMS	348	333	292	258	257	243	230	216	202	201	155	145	129	75	73
	(2)	(10)	(2)	(100)	(31)	(7)	(28)	(10)	(11)	(15)	(14)	(10)	(33)	(63)	(32)
MO-TMS	377	362	346	330	286	272	256	201	199	129	75	73			
	(2)	(3)	(52)	(2)	(6)	(3)	(100)	(7)	(9)	(6)	(44)	(19)			
TMS-enol	420	405	315	225	182	169	129	75	73						
	(72)	(56)	(31)	(14)	(11)	(24)	(8)	(49)	(100)						
19-Noretiocholanolone (3)															
TMS	348	333	292	258	257	243	241	230	216	202	201	155	145	129	75	73
	(8)	(43)	(2)	(87)	(10)	(4)	(14)	(28)	(60)	(16)	(20)	(20)	(15)	(47)	(100)	(59)
MO-TMS	377	362	346	286	272	256	201	199	129	75	73					
	(4)	(12)	(91)	(9)	(2)	(100)	(9)	(12)	(10)	(77)	(41)					
TMS-enol	420	405	315	225	182	169	129	75	73							
	(78)	(64)	(28)	(8)	(6)	(21)	(7)	(14)	(100)							
19-Norepiandrosterone (4)															
TMS	348	333	292	258	257	243	230	216	155	145	129	75	73		
	(20)	(62)	(7)	(57)	(27)	(3)	(10)	(5)	(22)	(28)	(29)	(100)	(33)		
MO-TMS	377	362	346	286	272	256	201	199	129	75	73				
	(2)	(7)	(68)	(5)	(3)	(100)	(6)	(11)	(4)	(56)	(25)				
TMS-enol	420	405	315	225	182	169	129	75	73						
	(89)	(96)	(28)	(8)	(9)	(23)	(8)	(7)	(100)						

*m/z value.

**In parentheses, relative intensity as percentage of base peak.

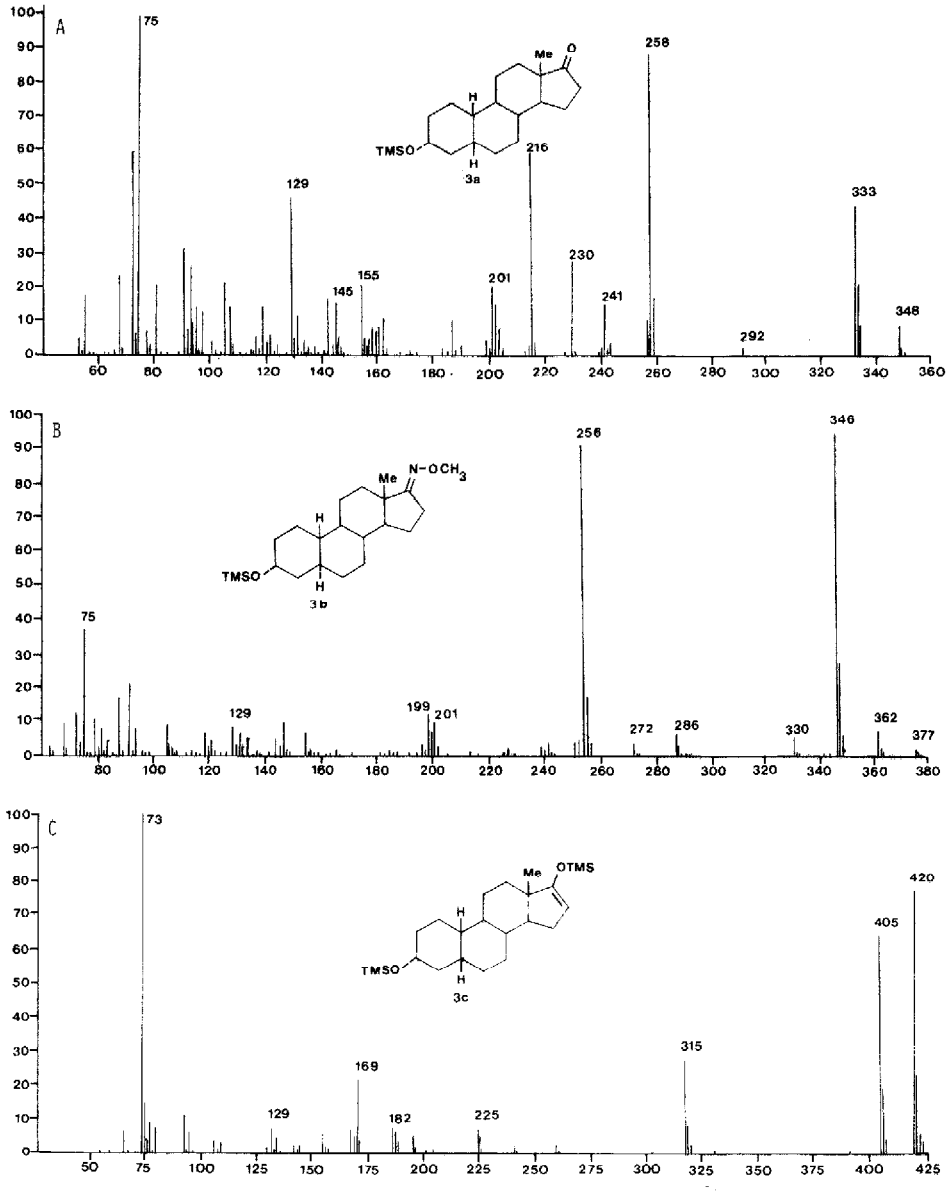


Fig. 4. Electron-impact mass spectra at 70 eV of 19-noretiocholanolone (*3*) as the TMS (A), MO-TMS (B), and TMS-enol-TMS ether derivatives (C).

These data indicate that their respective mechanism of formation is influenced by stereochemical factors. Indeed, several authors [22—28] have studied the effect of the configuration of the 3-hydroxyl group and that of ring A on the fragmentation pathways of underivatized steroidal alcohols in the androstane series. We may assume that the mechanisms of elimination of trimethylsilanol (TMSOH) in the TMS derivatives of the epimeric 19-norandrostanolones *1—4* are similar to the mechanisms proposed for the loss of water in the corresponding alcohols. The $[M - CH_3]^+$ ions arise mainly from

TABLE III

PARTIAL MASS SPECTRA OF THE [$^2H_9$]TMS, MO-[$^2H_9$]TMS and [$^2H_9$]TMS-enol [$^2H_9$]TMS ETHER DERIVATIVES OF 19-NORANDROSTANOLONES *1–4*

	M$^+$	Principal ions in mass spectrum																	
19-Norandrosterone (1)																			
[$^2H_9$]TMS	357*	342	339	301	258	243	241	240	239	230	216	207	202	201	199	187	164	138	82	81
	(35)**	(2)	(59)	(2)	(44)	(3)	(9)	(5)	(7)	(13)	(3)	(13)	(13)	(10)	(7)	(8)	(18)	(30)	(60)	(100)
MO-[$^2H_9$]TMS	386	371	355	336	286	272	256	241	201	200	199	138	82	81						
	(2)	(3)	(100)	(4)	(4)	(2)	(85)	(5)	(10)	(6)	(10)	(8)	(34)	(50)						
[$^2H_9$]TMS-enol	438	423	420	339	324	225	191	178	145	138	82	81								
	(91)	(100)	(4)	(13)	(19)	(8)	(10)	(16)	(10)	(19)	(98)	(89)								
19-Norepietiocholanolone (2)																			
[$^2H_9$]TMS	357	342	339	301	258	243	241	240	239	230	216	207	202	201	199	187	164	138	82	81
	(3)	(1)	(9)	(1)	(100)	(7)	(5)	(4)	(4)	(26)	(11)	(17)	(10)	(15)	(6)	(5)	(16)	(5)	(53)	(68)
MO-[$^2H_9$]TMS	386	371	355	286	272	256	241	201	200	199	138	82	81							
	(3)	(2)	(63)	(3)	(2)	(100)	(3)	(7)	(3)	(10)	(7)	(38)	(42)							
[$^2H_9$]TMS-enol	438	423	420	339	324	225	191	178	145	138	82	81								
	(100)	(43)	(3)	(3)	(24)	(7)	(7)	(14)	(4)	(3)	(70)	(46)								
19-Noretiocholanolone (3)																			
[$^2H_9$]TMS	357	342	339	301	258	243	241	240	239	230	216	207	202	201	199	187	164	138	82	81
	(13)	(1)	(31)	(3)	(84)	(11)	(13)	(8)	(4)	(26)	(60)	(15)	(14)	(19)	(8)	(9)	(19)	(33)	(68)	(100)
MO-[$^2H_9$]TMS	386	371	355	286	272	256	241	201	200	199	138	82	81							
	(2)	(4)	(80)	(–)***	(–)***	(100)	(4)	(5)	(11)	(9)	(12)	(49)	(54)							
[$^2H_9$]TMS-enol	438	423	420	339	324	225	191	178	145	138	82	81								
	(100)	(43)	(3)	(3)	(24)	(6)	(9)	(19)	(5)	(4)	(84)	(45)								
19-Norepiandrosterone (4)																			
[$^2H_9$]TMS	357	342	339	301	258	243	241	240	239	230	216	207	202	201	199	187	164	138	82	81
	(11)	(1)	(34)	(4)	(40)	(3)	(5)	(3)	(3)	(11)	(3)	(26)	(7)	(3)	(3)	(5)	(16)	(19)	(61)	(100)
MO-[$^2H_9$]TMS	386	371	355	336	286	272	256	241	201	200	199	138	82	81						
	(2)	(2)	(100)	(2)	(4)	(2)	(82)	(2)	(6)	(5)	(13)	(7)	(47)	(60)						
[$^2H_9$]TMS-enol	438	423	420	339	324	225	191	178	145	138	82	81								
	(100)	(70)	(4)	(4)	(14)	(5)	(6)	(14)	(4)	(3)	(59)	(37)								

*m/z value.

**Relative intensity as percentage of base peak.

***Relative intensity less than 1% of base peak.

loss of $CH_3^{\bullet}$ from the TMS group and also from the C-18 methyl group as shown by the spectra of the [$^2H_9$]TMS derivative (Table III). Other diagnostically important ions resulting from the loss of TMSOH and/or steroid skeleton cleavage are observed at *m/z* 243, 230, 216, 202—199 and 129 [25, 29—32].

MO-TMS derivatives. The mass spectra of the MO-TMS ethers (Table II and Fig. 4) of the isomeric 19-norsteroids are less complex than those of their TMS ether derivatives. They are dominated by two prominent ions at *m/z* 346 $[M - 31]^+$ and *m/z* 256 $[M - 31 - 90]^+$. The latter ions are the result of a fragmentation sequence which is initiated by the loss of MeO radical from the molecular ion with subsequent loss of one molecule of trimethylsilanol [33]. Direct loss of TMSOH from $[M]^+$ is negligible and occurs with concomitant elimination of one hydrogen atom to yield a low intensity ion at *m/z* 286 $[M - 90 - H]^+$. This fragmentation was ascertained by comparison with the mass spectrum of the corresponding MO-[$^2H_9$]TMS derivatives (Table III). In addition to the major $[M - 31]^+$ and $[M - 31 - 90]^+$ ions, few characteristic low-intensity ions are observed in the mass spectra of the four isomeric steroids. One would expect that the ion of *m/z* 362 $[M - 15]^+$ arises from the elimination of a methyl radical from the 3-OTMS group. Surprisingly the mass spectra of the corresponding MO-[$^2H_9$]TMS derivatives (Table III) show an ion of low intensity at *m/z* 371 $[M - CH_3]^+$, instead of the one expected at *m/z* 368 which would normally arise from the elimination of one [$^2H_9$]methyl group $[M - 18]^+$ from the perdeuterated TMS group.

The mass spectral data (Tables II and III) indicate that the $[M - CH_3]^+$ ion at *m/z* 362 arises from the elimination of a methyl radical from the methyloxime moiety and/or the C-18 methyl group and not from the TMS group as shown by the absence of $[M - C^2H_3]^+$ ions at *m/z* 368 (Table III).

This loss of CH_3 radical can be rationalized by charge localization and stabilization at the nitrogen and oxygen atoms of the methyloxime group. Another fragmentation giving rise to an ion at *m/z* 330 is characteristic to the 5α-steroid derivatives *1b* and *4b*. This ion arises from the consecutive losses of one molecule of methanol and a methyl radical from the TMS group $[M - 15 - 32]^+$ as indicated by TMS deuterium labelling (Table III).

TMS-enol-TMS ether derivatives. As observed previously for the MO-TMS derivatives, the mass spectra of all four isomeric TMS-enol-TMS ether derivatives *1c—4c* showed less fragmentation than those of the TMS ethers (Table II and Fig. 4). Molecular ions and $[M - 15]^+$ ions were of very high abundance. Mass spectral data obtained from the corresponding [$^2H_9$]TMS derivatives (Table III) indicate that, in contrast to the TMS derivatives, initial loss of methyl radical originates almost entirely from the C-18 position. Only 3—4% of the ejected methyl group arose from the TMS groups (Table III).

This mass spectrometric behaviour can be attributed to the formation of an allylic ion stabilized by the TMS-enol group. Although the elimination of TMSOH from the molecular ions is not favoured, a moderately abundant ion at *m/z* 315 $[M - 15 - 90]^+$ is observed in the mass spectra of the 19-norsteroid TMS-enol derivatives. A complementary ion at *m/z* 225 arises from the expulsion of a second molecule of trimethylsilanol from the ion at *m/z* 315. Additional evidence to this stepwise elimination of methyl radical and TMSOH giving rise to the peaks at *m/z* 405, 315 and 225 is ascertained by the mass

spectral data from the corresponding [$^2H_9$]TMS-enol ethers. Fragmentation of the steroidal skeleton gives rise to two characteristic ions at *m/z* 169 and 182 (Fig. 4) containing the TMS group (Table III).

CONCLUSIONS

In conclusion, the gas chromatographic and mass spectrometric data and interpretation outlined herein provide a firmer basis for the use of combined GC—MS in the characterization of isomeric 19-norandrostanolones *1—4*. GC separation of all four steroids is obtained only with the TMS derivatives, whereas the peaks corresponding to the MO-TMS derivatives of 19-noretiocholanolone (*3*) and 19-norepiandrosterone (*4*) and to the TMS-enol-TMS ethers of 19-norepietiocholanolone (*2*) and 19-noretiocholanolone (*3*) are only partially resolved. The mass spectra of the TMS derivatives permit the identification and the differentiation of all four isomeric 19-norsteroids.

On the other hand, the mass spectra of the corresponding MO-TMS and TMS-enol-TMS ether derivatives are so similar that differentiation between the epimeric steroids on the basis of their respective mass spectral features is practically impossible. Consequently, definitive identification of each of the epimeric 19-norsteroids as the MO-TMS and TMS-enol-TMS ether derivatives must be performed by comparison of their GC and MS properties with those of authentic samples.

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