Silyl Derivatives of Steroids. Evidence for Intramolecular Silylation Processes and Electron Impact Induced Reciprocal Exchange of Trimethylsilyl Groups

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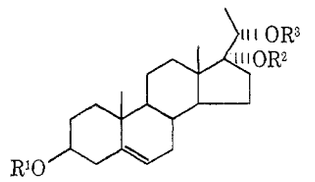
The preparation of mixed trimethylsilyl-d_0 and trimethylsilyl-d_9 derivatives of 17α,20-dihydroxy steroids is described, in which the labeled silyl groups occupy specific positions. The reaction products are analyzed by mass spectrometry and evidence is presented for migration of the 20-trimethylsilyl group to the 17α position during silylation. Electron impact ionization is shown to cause reciprocal exchange of the 17α- and 20α-trimethylsilyl groups in the formation of certain ions in the mass spectrum of the trimethylsilyl derivative of 5-pregnene-3β,17α,20α-triol.

Trimethylsilylation is a well-established technique for the study of numerous types of compounds by gas chromatography–mass spectrometry. Replacement of hydroxylic or other labile hydrogen atoms with trimethylsilyl groups enhances the volatility of most compounds. Consequently the technique has been particularly useful in the identification of hydroxylated steroids as their derived trimethylsilyl ethers.[1] The mass spectra of the trimethylsilyl derivatives of many compounds often exhibit abundant rearrangement ions due to the interaction of the silyl group with other functional groups in the molecule.[2] These ions are often structurally diagnostic and thus recognition of their trimethylsilyl content is of major importance. This has been greatly facilitated by the introduction of perdeuteriotrimethylsilylating reagents.[3]

We have recently reported the preparation of mixed trimethylsilyl (silyl-d_0) and perdeuteriotrimethylsilyl (silyl-d_9), derivatives of hydroxy steroids in which the silyl-d_9 group was selectively introduced in a certain position with a specificity of 90% or more.[4] This selective silylation was accomplished by utilizing the different rates of reaction of sterically hindered and unhindered hydroxyl groups towards various trimethylsilylating reagents.[5] A typical example[4] of the method employed is shown in reaction 1, and it involved (a) the silylation of unhindered hydroxyl groups by treating the sample with a relatively weaker silylating reagent such as bis(trimethylsilyl)trifluoroacetamide (BSTFA) under mild conditions (step I), (b) removal of solvent and reagents, and (c) reaction of the hindered hydroxyl groups under more severe conditions with *N*-trimethylsilyl-d_9-imidazole (TSIM-d_9) (step II). Selectively labeled silyl-d_0 and silyl-d_9 derivatives were helpful in the interpretation of the mass spectra of trimethylsilyl steroidal ethers, since one could identify the parts of the steroid structure involved in the formation of the various trimethylsilyl-containing ions.

In the examples discussed previously the hydroxyl groups selectively silylated with silyl-d_0 and silyl-d_9 groups were widely separated, and this positional identity was retained in the fragment ions produced upon electron impact. We report here on our efforts to introduce selectively silyl-d_0 and silyl-d_9 groups in hydroxyl functions which are attached to adjacent carbon atoms. As examples we have chosen steroids of the pregnane and pregnene series containing both a sterically hindered (17α-) and an unhindered (20α- or 20β-) hydroxyl group. The compounds studied were 5-pregnene-3β,17α,20α-triol (**1**), 5α-pregnane-3β,17α,-20α-triol, and their 20β isomers. Only compound **1** will be discussed in detail here since virtually identical results were obtained with the other compounds as well. The mass spectra of the partial trimethylsilyl ethers (**2** and **3**) of **1** and of its pertrimethylsilyl ether (**4**) are discussed below, and the observed fragmentation patterns are used as a guide to ascertain the mechanism of silylation of the hydroxyl groups.



1, $R^1 = R^2 = R^3 = H$

2, $R^1 = R^3 = Si(CH_3)_3$; $R^2 = H$
2a, $R^1 = R^3 = Si(CD_3)_3$; $R^2 = H$
2b, $R^1 = R^2 = Si(CH_3)_3$; $R^2 = D$
2c, $R^1 = R^2 = Si(CH_3)_3$; $R^2 = H$; 20-^{18}O

3, $R^1 = R^2 = Si(CH_3)_3$; $R^3 = H$
3a, $R^1 = R^2 = Si(CD_3)_3$; $R^3 = H$
3b, $R^1 = R^2 = Si(CH_3)_3$; $R^3 = D$
3c, $R^1 = R^2 = Si(CH_3)_3$; $R^3 = H$; 20-^{18}O

4, $R^1 = R^2 = R^3 = Si(CH_3)_3$
4a, $R^1 = R^2 = R^3 = Si(CD_3)_3$
4b, $R^1 = R^2 = Si(CH_3)_3$; $R^3 = Si(CD_3)_3$
4c, $R^1 = R^2 = Si(CD_3)_3$; $R^3 = Si(CH_3)_3$

Results and Discussion

Structures of Partially Silylated Steroids.—The high efficiency of the selective silylation observed in reaction 1[4] would *a priori* suggest a silylation sequence according to pathway a outlined in reaction 2. Gas chromatography (9-ft, 1% OV-17 column) of the partially silylated steroid from reaction of **1** with BSTFA (step I) revealed in each case a well-resolved doublet

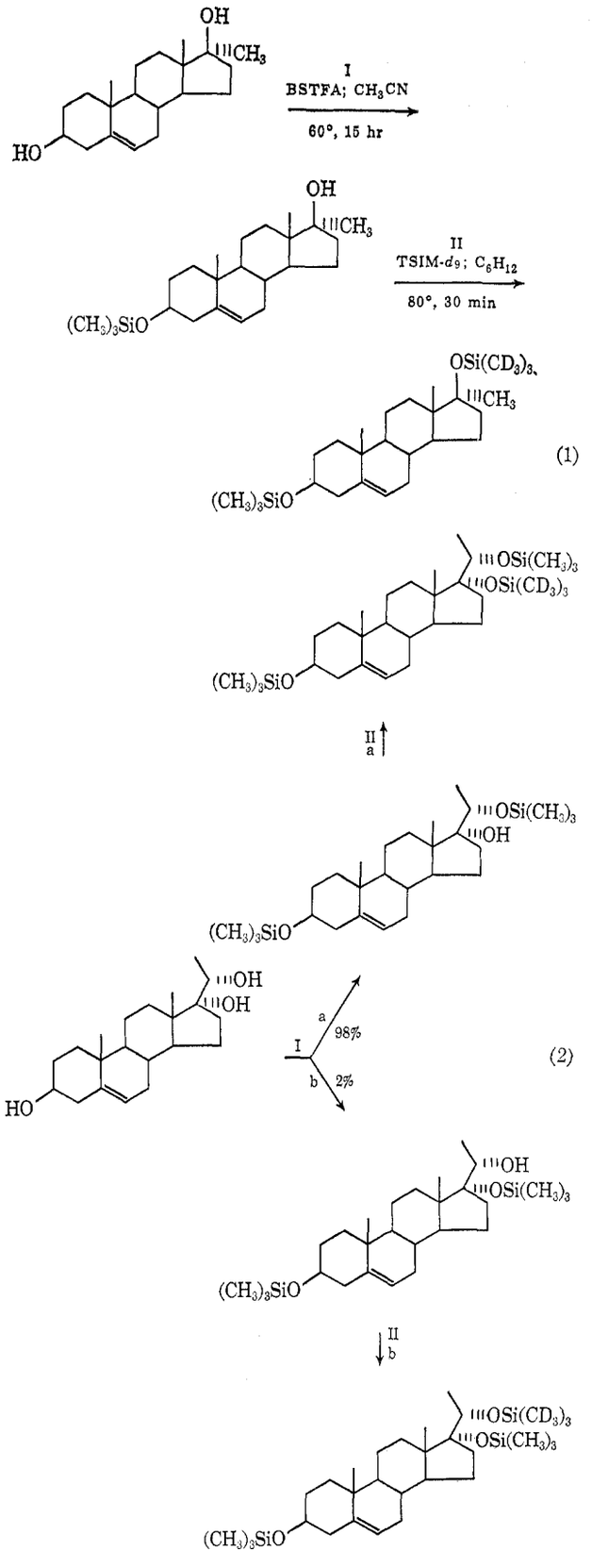
(1) (a) E. C. Horning, C. J. W. Brooks, and W. J. A. VandenHeuvel, *Advan. Lipid Res.*, **6**, 273 (1968); (b) C. J. W. Brooks and B. S. Middleditch, "Modern Methods of Steroid Analysis," E. Heftmann, Ed., Academic Press, New York, N. Y., in press.

(2) (a) J. Diekman, J. B. Thomson, and C. Djerassi, *J. Org. Chem.*, **32**, 3904 (1967); (b) G. H. Draffan, R. N. Stillwell, and J. A. McCloskey, *Org. Mass Spectrom.*, **1**, 669 (1968); (c) E. White, V, and J. A. McCloskey, *J. Org. Chem.*, **35**, 4241 (1970), and references cited therein.

(3) J. A. McCloskey, R. N. Stillwell, and A. M. Lawson, *Anal. Chem.*, **40**, 233 (1968).

(4) P. Vouros and D. J. Harvey, *Anal. Chem.*, **45**, 7 (1973).

(5) (a) E. M. Chambaz and E. C. Horning, *Anal. Lett.*, **1**, 201 (1967); (b) E. M. Chambaz and E. C. Horning, *Anal. Biochem.*, **30**, 7 (1969); (c) E. Sakauchi and E. C. Horning, *Anal. Lett.*, **4**, 41 (1971).



consisting of a major (>98%) and a minor component. The fraction of the latter was observed to increase to ~5% on occasions when step I was carried out at temperatures as high as 68°. This temperature effect suggests that the minor component is formed mainly during silylation rather than as a result of thermal isomerization during gas chromatography. The mass spectra of the principal and minor components following partial silylation of the Δ^5 steroid (**1**) are shown in Figures 1 and 2, respectively.

A major fragmentation process in the mass spectra of 20-trimethylsilyloxy pregnanes or pregnenes involves cleavage of the C-17/20 bond,[6] resulting in the formation of ions of the type $(CH_3)_3\overset{+}{S}iO{=}CHCH_2R$ and/or $[M - (CH_3)_3SiOCHCH_2R]^+$ depending on the disposition of the positive charge. The mass spectrum of the principal product (Figure 1) of partial silylation of 5-pregnene-3β,17α,20α-triol (**1**) exhibits strong and significant peaks at $[M - 117]^+$ (*m/e* 361) and *m/e* 117 corresponding to the presence of a 20α-trimethylsilyloxy function. A metastable peak at *m/e* 359.2 indicates the further decomposition of $[M - 117]^+$ to *m/e* 360 by loss of a hydrogen radical. The intense peak at *m/e* 129[6a,7] confirms the formation of a 3β,20α-bis(trimethylsilyloxy)-5-pregnen-17α-ol (**2**) according to path a of reaction 2. By contrast, the spectrum of the isomeric minor component (Figure 2) exhibits no peak at $[M - 117]^+$, but shows a loss of 45 amu from the molecular ion, providing an ion formed by cleavage of the 17β side chain and elimination of $CH_3\dot{C}HOH$ (*m/e* 433). The intensity of the *m/e* 129 peak is consistent with the presence of a Δ^5-3β-trimethylsilyloxy function as in structure **3**.

The cleavage of the 17β side chain in the mass spectra of the partially silylated products following step I, clearly indicates that silylation step I (reaction 2) proceeds mainly (>98%) *via* reaction path a, and **2** represents the structure of the main component formed. It might be noted here that the aforementioned fragmentations of the 17β side chain were confirmed by deuterium labeling of the C-20 hydrogen and of the free hydroxylic hydrogen, ^{18}O labeling of the C-20 oxygen, and preparation of perdeuteriotrimethylsilyl derivatives (compounds **2a–c**, **3a–c**).

A significant common feature in the mass spectra of both isomers **2** and **3** is the favorable elimination of methane—or the elements thereof—from the respective molecular ions to give the peak at *m/e* 462. The isotope labeling data support the fragmentation mechanism proposed in Scheme I, involving interaction of the 20α and the 17α substituents. This interaction is presumably responsible for the elimination of $C_2H_5O\cdot$ and C_2H_5OH from the molecular ion of the 20-trimethylsilyloxy derivative (**2**) to give the small (<2% R.I.) peaks at *m/e* 432 and 433. Formation of these ions requires reciprocal exchange of the 20α-silyl group and 17α hydrogen and the significance of this observation will become evident in the discussion of the spectra of the fully silylated derivative **4**.

Structures of Selectively Silylated (Silyl-d_0, Silyl-d_9) Steroids. (a) Reaction of 5-Pregnene-3β,17α,20α-triol (1).—As indicated above the initial silylation reaction of **1** (step I) produced mainly a 3β,20α-bis(trimethylsilyloxy) derivative (**2**). Elucidation of the structure of the selectively silylated steroid, following step II, and of the disposition of the silyl-d_0 and silyl-d_9 groups requires

(6) (a) C. J. W. Brooks, E. M. Chambaz, W. L. Gardiner, and E. C. Horning, *Excerpta Med. Found. Int. Congr. Ser.*, **132**, 366 (1966); (b) J. Sjövall and R. Vihko, *Steroids*, **7**, 447 (1966); (c) J.-Å. Gustafsson, C. H. L. Shackleton, and J. Sjövall, *Eur. J. Biochem.*, **10**, 302 (1969).

(7) (a) P. Eneroth, K. Hellström, and R. Ryhage, *J. Lipid Res.*, **245** (1964); (b) J. Diekman and C. Djerassi, *J. Org. Chem.*, **32**, 1005 (1967).

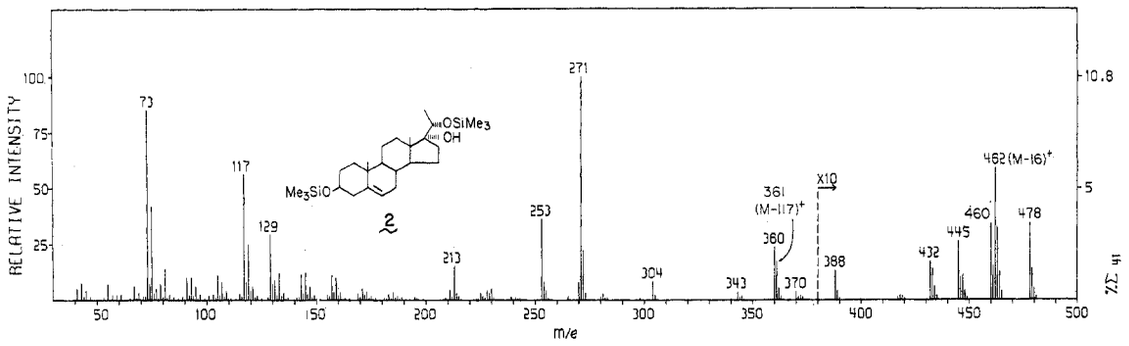


Figure 1.—Mass spectrum of major component following partial silylation of 1 with BSTFA and identified as 3β,20α-bis-(trimethylsilyloxy)-5-pregnen-17α-ol (2).

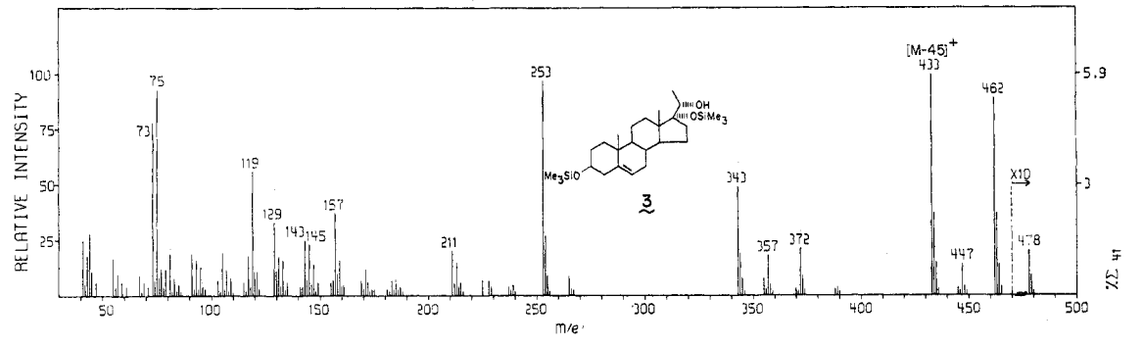
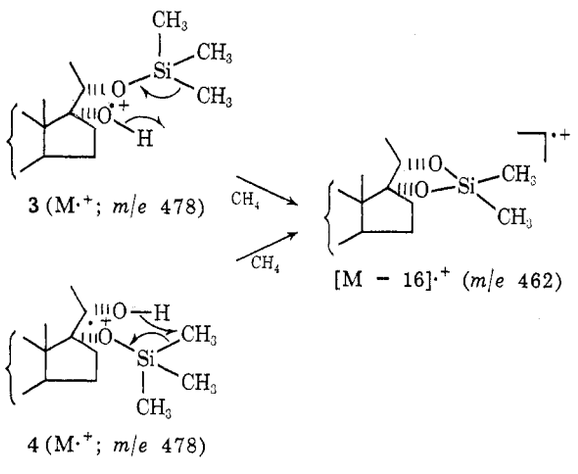


Figure 2.—Mass spectrum of minor component following partial silylation of 1 with BSTFA and identified as 3β,17α-bis-(trimethylsilyloxy)-5-pregnen-20α-ol (3).

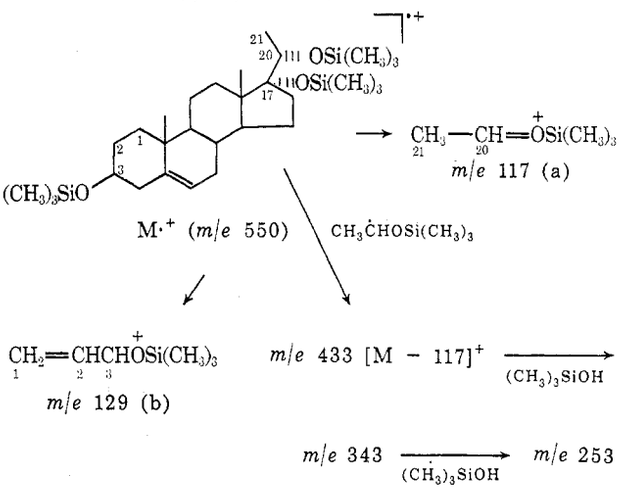
SCHEME I

ELIMINATION OF METHANE (OR $CH_3 + H$) IN THE MASS SPECTRA OF 2 AND 3



SCHEME II

PRINCIPAL FRAGMENTATIONS IN THE MASS SPECTRUM OF 5-PREGNENE-3β,17α,20α-TRIS(TRIMETHYLSILYL) ETHER (4)



comprehension of the major fragmentation pathways of the fully silylated derivative (4). The mass spectrum of 4, prepared by overnight reaction of 1 with *N*-trimethylsilylimidazole (TSIM) at 90°, is shown in Figure 3a and the major fragmentation pathways outlined in Scheme II. The silyl content of the major fragment ions can easily be recognized upon examination of the spectrum of the perdeuteriotrimethylsilyl derivative of 1 (4a, Figure 3b). The mechanism of formation of the ion at *m/e* 129 has been described before.[6b,7] The ion at *m/e* 147 $[(CH_3)_3SiOSi(CH_3)_2{}^+$, c] is a rearrangement ion typical of many compounds containing two or more trimethylsilyloxy groups.[8] Ion c is formed here apparently by interaction of the 17α- and 20α-trimethylsilyloxy groups.[9]

Figure 3c shows the mass spectrum of the mixed silyl-d_0 and silyl-d_9 derivative of 1 prepared as described above by treating initially with BSTFA and

(8) (a) G. H. Draffan, R. N. Stillwell, and J. A. McCloskey, *Org. Mass Spectrom.*, **1**, 669 (1968); (b) J. Dieckman, J. B. Thomson, and C. Djerassi, *J. Org. Chem.*, **33**, 2271 (1968); (c) D. C. DeJongh, T. Radford, J. D. Hribar, S. Hanessian, M. Bieber, G. Dawson, and C. C. Sweeley, *J. Amer. Chem. Soc.*, **91**, 1728 (1969).

(9) S. Sloan, D. J. Harvey, and P. Vouros, *Org. Mass Spectrom.*, **5**, 789 (1971).

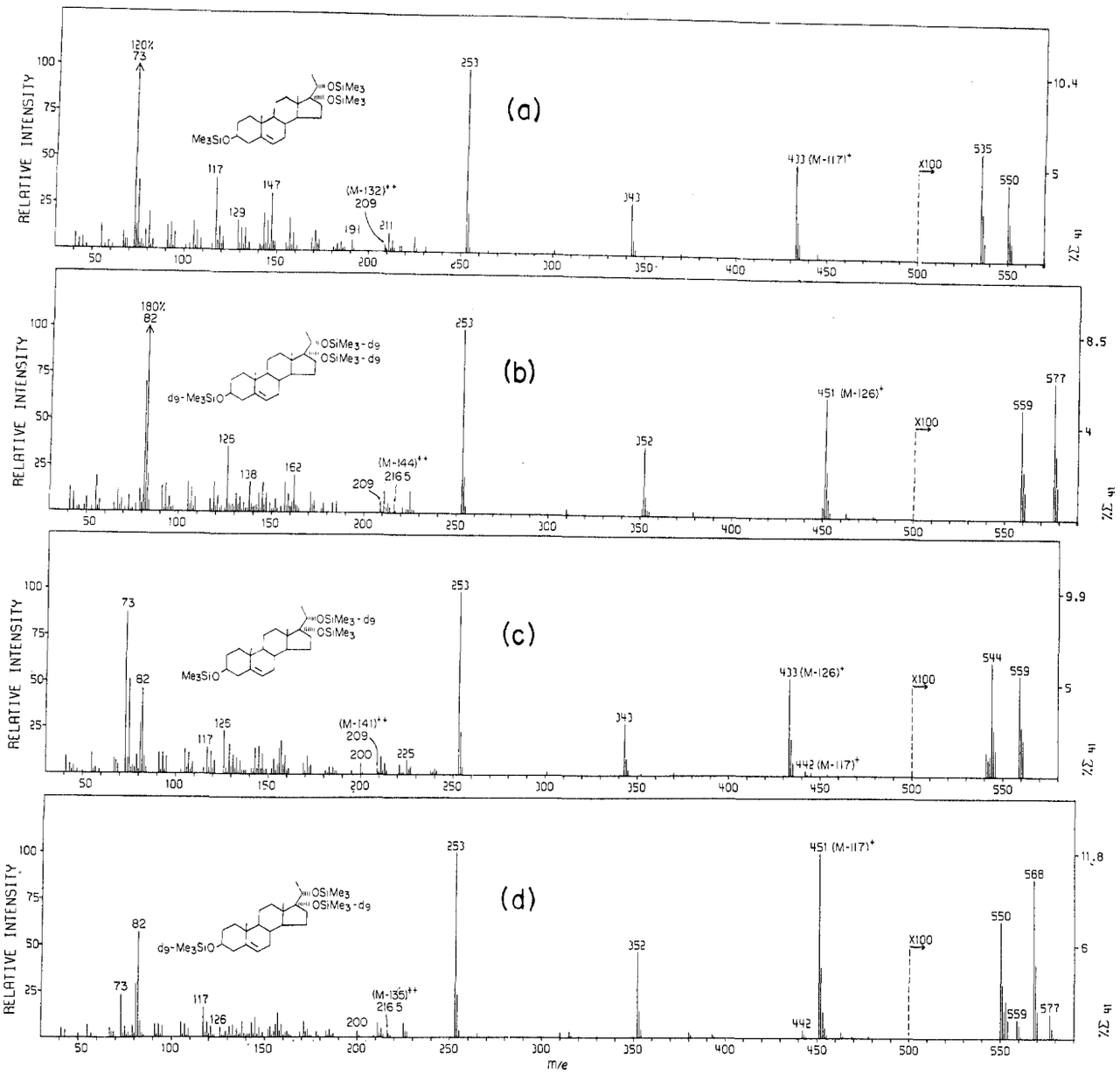
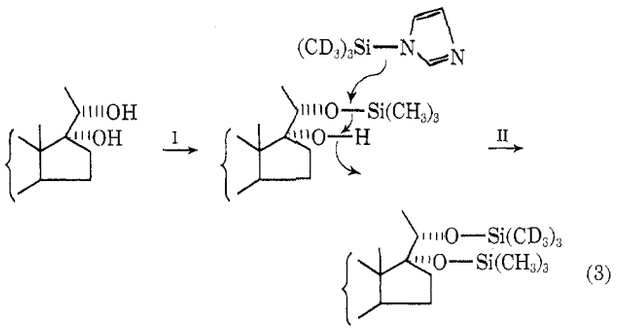


Figure 3.—(a) Mass spectrum of 5-pregnene-3β,17α,20α-tris(trimethylsilyl-d_0) ether (**4**); (b) mass spectrum of 5-pregnene-3β,17α,-20α-tris(trimethylsilyl-d_9) ether (**4a**); (c) mass spectrum of 3β,17α-bis(trimethylsilyloxy-d_0)-5-pregnene-20α-trimethylsilyloxy-d_9 (**4b**); (d) mass spectrum of 3β,17α-bis(trimethylsilyloxy-d_9)-5-pregnene-20α-trimethylsilyloxy-d_0 (**4c**).

then with TSIM-d_9. The shift of the molecular ion from m/e 550 to 559 indicates incorporation of one silyl-d_9 group. If the silylation had proceeded according to the sequence shown in IIa (reaction 2), one would expect loss of 117 amu [$CH_3\dot{C}HOSi(CH_3)_3$] from the molecular ion (m/e 559) to give a peak at m/e 442. Instead of that, however, a predominant loss of 126 amu (95% *vs.* 5%) is observed, corresponding to the elimination of $CH_3\dot{C}HOSi(CD_3)_3$ from $M\cdot^+$. Similarly, an ion of m/e 126 corresponding to $CH_3CH{=}\overset{+}{O}Si(CD_3)_3$ is formed in preference to one of m/e 117 (m/e 126:m/e 117 = 100:38). It thus appears that the final reaction product has a structure corresponding to a 3β,17α-bis(trimethylsilyloxy)-5-pregnene-20α-trimethylsilyloxy-d_9 derivative (**4b**) rather than the anticipated 3β,20α-bis(trimethylsilyloxy)-5-pregenene-17α-trimethylsilyloxy-d_9.

To assess further the validity of the reactions and conclusions drawn above, the selective silylation reaction was performed by reversing the sequence of silyl-d_0 and silyl-d_9 group introduction. A sample of the 5-pregnenetriol (**1**) was first treated under mild conditions with bis(trimethylsilyl)acetamide-d_{18} (BSA-d_{18}), and, after complete incorporation of two silyl-d_9 groups, the solvents and reagent were vaporized. The monohydroxy compound was then treated with TSIM. The spectrum of the resultant bis(silyl-d_9)silyl-d_0-5-pregnene is shown in Figure 3d. The molecular ion has been shifted by 18 amu to m/e 568 indicative of the presence of two silyl-d_9 groups, and the preferential loss of 117 amu rather than 126 amu supports reaction mechanism 3 rather than IIa (reaction 2), and thus the formation of a 3β,17α-bis(trimethylsilyloxy-d_9)-5-pregnene-20α-trimethylsilyloxy-d_0 derivative (**4c**). The peak at m/e 577 in Figure 3d represents the molecular



(3)

ion of the perdeuteriotrimethylsilyl derivative (**4a**) produced during the reaction with BSA-d_{18} and not separated from **4c** during gas chromatography–mass spectrometry. The ion of m/e 559 is formed by the loss of $\cdot CD_3$ from that of m/e 577.

(b) **Mechanism of Selective Silylation.**—Since step Ib in reaction 2 does not appear to occur in any appreciable amount, an intramolecular trimethylsilylation process (reaction 3) would have to be invoked to explain the data in Figures 3a–d. This may be rationalized by the supposition that, following initial silylation of the unhindered 20α-hydroxyl group, the increased steric hindrance inhibits direct attack of the 17α-hydroxyl function by the relatively bulky TSIM-d_9 molecule. The favorable stereochemical disposition of the 20α,β and 17α functions combined with the high mobility of the silyl group provide a suitable pathway for intramolecular completion of the persilylation process. Operation of such an intramolecular silylation mechanism is not surprising in view of previous reports of thermally or otherwise induced intramolecular silyl rearrangements in *N*,*O*-bis(trimethylsilyl)amides,[10] silylacetic acids,[11] tris(organosilyl)hydroxylamines[12] and other systems.[13] The favorable disposition of the 17α and 20α or 20β groups for interaction and intramolecular group transfer is further demonstrated by the fact that pregnane-20α,21-diacetates can be prepared from 17α,21-diacetoxy-20β-tosylpregnanes. Participation of the 17α- or 21-acetoxy groups has been proposed as instrumental in the solvolysis of the 20β-tosyl function and inversion of configuration about C-20.[14] On the other hand, a reverse transfer—from C-20 to C-17—occurs in the Serini reaction,[15] where it was shown that in a 20-acetoxy-17α-hydroxypregnane a hydrogen atom migrates from C-20 to C-17 with inversion at the latter site, resulting in formation of a 20-keto pregnane.[16]

(c) **Electron Impact Induced Exchange of Trimethylsilyl Groups.**—The 100:44 ratio of m/e 117:m/e 126 in the spectrum of **4c** also reflects the presence of a C-20 silyl-d_0 group, but the different ratios of $[M - 117]^+$:$[M - 126]^+$ and m/e 117:m/e 126 in both **4b** and **4c** are mutually inconsistent and indicative of some loss of positional specificity of the trimethylsilyl functions in the process of formation of m/e 117. No change in these ratios was observed when the mass spectra of **4b** and **4c** were determined from sample aliquots taken at different times during step II of the selective silylation reaction. This indicates that the discrepancy in the ratios of m/e 117:m/e 126 and $[M - 117]^+$:$[M - 126]^+$ is not necessarily a reaction related phenomenon, and so we proceeded to investigate the dependence of the relative intensities of those ions on ionizing energy. The results are summarized in Table I. A definite variation of the ratio of m/e 117:m/e 126 with electron beam energy is apparent while the ratio of $[M - 117]^+$:$[M - 126]^+$ remains constant. The variation of m/e 117:m/e 126 with ionizing energy is indicative of scramble of the C-17- and C-20-trimethylsilyl groups upon electron impact. The proximity of the functional groups, coupled with the capability of silicon atom to form additional bonds with its 3d orbitals, and the high energy of formation of the Si–O bond[17] are presumably instrumental in accomplishing this intramolecular scramble (Scheme III).

TABLE I

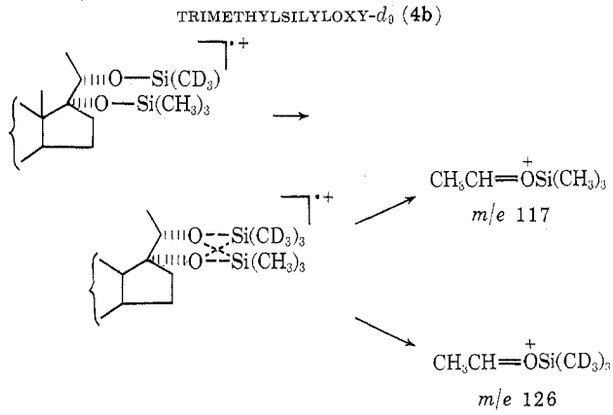
VARIATION OF RELATIVE ABUNDANCE OF THE m/e 117, m/e 126 AND $[M - 117]^+$, $[M - 126]^+$ IONS AS A FUNCTION OF ELECTRON ENERGY IN THE SPECTRA OF **4b** AND **4c**

	4b				4c			
Electron energy, eV	m/e 117	m/e 126	$[M - 117]^+$	$[M - 126]^+$	m/e 117	m/e 126	$[M - 117]^+$	$[M - 126]^+$
100	40[a]	100	5	100				
70	38	100	5	100	100	44	100	5
20	52	100	5	100	100	65	100	5
18	54	100	4	100	100	80	100	4
15	73	100	5	100	95	100	100	5
13.5	75	100	5	100	100	87	100	5
12	100	93	4	100	100	95	100	5

[a] All values have been corrected for contributions from other ions.

SCHEME III

ELECTRON IMPACT INDUCED SCRAMBLE OF 17α- AND 20α-SILYL GROUPS IN THE SPECTRUM OF 3β,17α-BIS(TRIMETHYLSILYLOXY-*d*)-5-PREGNENE-20α-TRIMETHYLSILYLOXY-d_9 (**4b**)



Examples of scrambling of trimethylsilyl hydrogens with other hydrogens during fragmentation[2b,18] as well

(10) (a) J. Pump and E. G. Rochow, *Chem. Ber.*, **97**, 627 (1964); (b) M. Fukui, K. Itoh, and Y. Ishii, *J. Chem. Soc., Perkin Trans. 2*, 1043 (1972).

(11) A. G. Brook, D. G. Anderson, and J. M. Duff, *J. Amer. Chem. Soc.*, **90**, 3876 (1968).

(12) P. Boudjouk and R. West, *J. Amer. Chem. Soc.*, **93**, 5901 (1971).

(13) (a) A. G. Brook, D. M. MacRae, and W. W. Limburg, *J. Amer. Chem. Soc.*, **89**, 5493 (1967); (b) M. S. Biernbaum and H. S. Mosher, *ibid.*, **93**, 6221 (1971); (c) A. G. Brook and J. D. Prascoe, *ibid.*, **93**, 6224 (1971).

(14) D. K. Fukushima, N. S. Leeds, H. L. Bradlow, T. H. Kritchevsky, M. B. Stokem, and T. F. Gallagher, *J. Biol. Chem.*, **212**, 449 (1955).

(15) A. Serini, W. Logemann, and W. Hildebrand, *Chem. Ber.*, **72**, 391 (1939).

(16) T. Goto and L. F. Fieser, *J. Amer. Chem. Soc.*, **83**, 251 (1961).

(17) J. Kleinberg, W. J. Argensinger, Jr., and E. Griswold, "Inorganic Chemistry," D. C. Heath and Co., Boston, Mass., 1960, p 345 ff.

(18) E. White, V. P. M. Krueger, and J. A. McCloskey, *J. Org. Chem.*, **37**, 430 (1972).

as of intermolecular transfer of trimethylsilyl groups[19] have been reported before. This is, however, the first evidence for electron impact induced intramolecular positional exchange of entire silyl functions. It is significant that, at the lowest energies, there is complete randomization (Table I) of the silyl-d_0 and silyl-d_9 groups resulting in essentially 1:1 ratios of m/e 117:126. This is consistent with the observations of Williams and coworkers[20] that an increase in molecular ion lifetime enhances opportunity for interchange. The observed variation of the ratio m/e 117:m/e 126 (Table I) is a reflection of competition between rearrangement and fragmentation. The decreased scramble at higher energies[20] is further evidence that **4b** and **4c** reflect the structures of the products of the selective silylation reaction. The lack of reciprocal exchange of trimethylsilyl groups in the $[M - 117]^+$ ion is surprising in view of the randomization observed in the actual formation of m/e 117. This may be partially due to different charge stabilization and redistribution effects during cleavage of the C-17/20 bond. It is conceivable that charge retention with the 17β side chain requiring charge localization on the oxygen atom at C-20 will enhance bond formation with the 17α-silicon atom, whereas loss of the side chain will result in charge delocalization throughout the remaining steroid structure. The latter hypothesis, together with the high energy of formation of the Si–O bond could in part explain the reason for the occurrence of ions of m/e 117 (~15% R.I.) in the spectrum of the partially silylated derivative **3** but of no ion at $[M - 117]^+$.

Electron impact induced intramolecular exchange of the 17α- and 20α-trimethylsilyl groups was also observed in the mixed silyl-d_0, silyl-d_9 derivatives of 5α-pregnane-3β,17α,20α-triol, the saturated analogs of **4b** and **4c**. It is interesting, however, that no variation was observed in the ratio of m/e 117:m/e 126 with ionizing energy. In all likelihood the Δ^5 double bond in **4b** and **4c** is instrumental in providing sufficient stability or ion lifetime to enhance the intramolecular scramble and complete randomization of the 17α- and 20α-trimethylsilyl groups.

Conclusions

The data presented in the preceding section can be summarized in terms of information pertaining to (a) mechanisms of silylation, and (b) mass spectrometric properties of trimethylsilyl groups. Through the use of selective labeling with silyl-d_0 and silyl-d_9 groups it has been shown that under certain reaction conditions trimethylsilyl groups undergo intramolecular shifts during silylation. Proper stereochemical disposition of the functional groups undergoing silylation appears to be a requirement for the proposed intramolecular migration of trimethylsilyl groups.

Preparation of mixed silyl-d_0 and silyl-d_9 derivatives has shown that in compounds containing vicinal trimethylsilyloxy groups electron impact ionization can cause reciprocal exchange of the entire trimethylsilyl functions. Consequently, although the use of selectively labeled silyl-d_0, silyl-d_9 derivatives can be extremely helpful in mass spectral interpretations, some caution should be exercised in cases involving compounds which contain vicinal trimethylsilyloxy functions. In addition to mutual exchange of trimethylsilyl groups the mass spectra of partially silylated compounds show the occurrence of a hydrogen–trimethylsilyl group interchange upon electron impact. The latter type of reciprocal exchange can occur to a significant extent in certain compounds[21] and thus introduce uncertainties in structural interpretations of mass spectra. Occurrence of such processes further justifies the trend toward the preparation of fully silylated derivatives.[5,22]

Experimental Section

Samples of steroids used in this work were obtained from commercial sources. The methods used for the preparation of their trimethylsilyl derivatives[5] and for selective introduction of silyl-d_0 and silyl-d_9 groups[4] have been described before. The 20-^{18}O labeled analog of **1** was prepared by lithium aluminum hydride reduction of 20-ketopregnene-3β,17α-diol following exchange of the 20-keto oxygen in a mixture of isopropyl alcohol, hydrochloric acid, and 50% ^{18}O-enriched water.[23] Compounds **2b** and **3b** were prepared by exchange of the labile hydroxyl hydrogens of **2** and **3** in the gas chromatographic column attached to the mass spectrometer.[24] The purity of all compounds was checked by combined gas chromatography–mass spectrometry.

Mass spectra were recorded with an LKB-9000 mass spectrometer. The ionizing voltage was 70 eV unless otherwise noted, the accelerating voltage was 3.5 kV, and the ion source temperature was 270°. The samples were introduced to the mass spectrometer *via* the gas chromatographic inlet (6-ft or 9-ft, 1% OV-17 column). The column temperature was programmed over the range of 225–260° at a rate of 3°/min.

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