the *n*-butyl group), a triplet at 2.9 (benzylic hydrogens of *n*-butyl group), and singlet at 8.38 (proton on C-1 of isoquinoline nucleus).

Registry No.—1-*n*-Propylisoquinoline, 7661-37-2; 1-*n*-butylisoquinoline, 7661-38-3; 2-*n*-propylquinoline, 1613-32-7; 2-*n*-butylquinoline, 7661-39-4; 1-isobutylisoquinoline, 7661-40-7; 3-butyrylisoquinoline, 7661-41-8; 3-*n*-butylisoquinoline, 7661-42-9; 2-phenyl-3-heptanol, 7661-43-0; 2-phenyl-3-heptanone, 7661-44-1; 2-phenyl-3-aminoheptane, 7634-70-0; 3-*n*-butyl-4-methylisoquinoline, 7661-45-2; 1-(3,3,3-d_3-*n*-propyl)isoquinoline, 7661-46-3; 1-(3,3-d_2-*n*-butyl)isoquinoline, 7634-71-1; 7-methylquinoline, 612-60-2; 3-methylquinoline, 612-58-8; 2,4-dimethylquinoline, 1198-37-4; 2-ethylquinoline, 1613-34-9; 7-ethylquinoline, 7661-47-4; 2-(3,3-dimethyl-*n*-butyl)quinoline, 7661-48-5; 2-*n*-propyloxyquinoline, 945-83-5; 1-*n*-propylcarbostyril, 944-70-7; 4-*n*-propylpyridine, 1122-81-2; 4-isobutylquinoline, 7661-51-0; 6-*n*-butylquinoline, 7634-74-4; 7-*n*-butylquinoline, 7661-52-1; 8-*n*-propylquinoline, 7661-53-2; 6-*n*-pentyl-3,8-di-*n*-propylquinoline, 7661-54-3; 8-*n*-pentyl-3,6-di-*n*-propylquinoline, 7634-75-5.

Acknowledgment.—Dr. Wai H. Hui (on leave from the University of Hong Kong) synthesized 2-neopentyl-,[24] 2-isobutyl-,[24] and 2-ethylquinoline,[24] and Mr. Roger Kornberg (undergraduate summer research assistant) synthesized 1-ethylisoquinoline.[24] Dr. Z. Velenta[25] (University of New Brunswick, Fredericton, New Brunswick, Canada) provided 6-*n*-pentyl-3,8-di-*n*-propyl- and 8-*n*-pentyl-3,6-di-*n*-propylquinoline. Dr. Yasuo Makisumi[26] (Shionogi and Co., Osaka, Japan) provided 1-*n*-propylcarbostyril and 2-*n*-propyloxyquinoline, while Dr. K. Schofield (University of Exeter, Exeter, England) donated 6-*n*-propyl-, and *n*-butylquinoline as well as 7-ethyl-, 7-*n*-propyl-, and 7-*n*-butylquinoline.

(24) W. Bradley and S. Jeffrey, *J. Chem. Soc.*, 2770 (1954).
(25) Z. Valenta, H. Rashid, R. Wightman, and J. Wilson, *Tetrahedron Letters*, No. 23, 1559 (1963).
(26) Y. Makisumi, *ibid.*, **39**, 2833 (1964).

Mass Spectrometry in Structural and Stereochemical Problems. CXXV.[1] Mass Spectrometry of Some Steroid Trimethylsilyl Ethers[2]

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Using deuterium and substituent labeling as well as high-resolution mass measurements, plausible assignments could be made for the diagnostically most significant peaks in the mass spectra of some steroid trimethylsilyl ethers which have been used in the past for structural purposes. The variations in these spectra, which are shown to be strongly dependent upon experimental conditions, are commented upon as are other features of the mass spectra. Charge retention in the silicon-containing moiety does not compete very effectively with the more favored hydrocarbon carbonium ions produced when methyl groups are substituted at C-4.

In recent years, trimethylsilyl ether derivatives have been used extensively in the purification and identification of nonvolatile materials, especially in connection with a technique combining gas–liquid partition chromatography and mass spectrometry.[4] This method has found application in a variety of fields[5-8] but has been particularly relevant to steroid metabolites.[5,9,10] Aside from the fact that trimethylsilyl ether derivatives greatly facilitate gas–liquid partition chromatographic separation in comparison with the parent steroids,[11] they also appear to direct mass spectrometric fragmentation in a manner characteristic of particular groups of steroids. In order to acquire information other than empirical formula determinations from a mass spectrum, it is most important that some insight be gained into such electron-impact-induced fragmentation patterns. Ryhage[4] and his collaborators have pointed out that there is a particularly characteristic fragmentation sequence for trimethylsilyl ethers of Δ^5-3-hydroxy steroids, and, in fact, the existence of an intense peak at m/e 129 has been used by them as practically conclusive evidence for the identification of the trimethylsilyl derivative of this type of sterol. Sjövall and Vihko[5] have found this same diagnostically useful peak, but with reduced intensity, in the mass spectrum of the trimethylsilyl ethers of 3-hydroxy 5α-steroids. Ryhage[4] has attributed the composition of this molecular fragment to the trimethylsiloxy group, carbon atoms 2, 3, and 4 of ring A, and a loss of a hydrogen atom from the charge-retaining species (see wavy line in I), and Sjövall and Vihko[5] state that the exact composition of this frag-

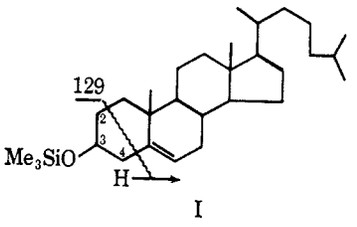
(1) Paper CXXIV: S. D. Sample, D. A. Lightner, O. Buchardt, and C. Djerassi, *J. Org. Chem.*, **32**, 997 (1967).
(2) Financial assistance by the National Institutes of Health (Grants No. CA 07195 and AM 04257) is gratefully acknowledged. The purchase of the Atlas CH-4 mass spectrometer was made possible by NASA Grant Gs 81-60.
(3) National Science Foundation Predoctorate Fellow, 1966–1967.
(4) P. Eneroth, K. Hellström, and R. Ryhage, *J. Lipid Res.*, **5**, 245 (1964).
(5) J. Sjövall and R. Vihko, *Steroids*, **7**, 447 (1966).
(6) J. B. Castillo, C. J. W. Brooks, and M. M. Campbell, *Tetrahedron Letters*, 3731 (1966).
(7) K. Karlsson, *Acta Chem. Scand.*, **19**, 2425 (1965); R. C. Gaver and C. C. Sweeley, *J. Am. Chem. Soc.*, **88**, 3643 (1966).
(8) B. T. Golding, R. W. Rickards, W. E. Meyer, J. B. Patrick, and M. Barber, *Tetrahedron Letters*, 3551 (1966); G. Gaudiano, P. Bravo, A. Quilico, B. T. Golding, and R. W. Rickards, *ibid.*, 3567 (1966).
(9) E. C. Horning, W. L. Gardiner, and C. J. W. Brooks, Abstracts, Second International Congress of Hormonal Steroids, Milan, Italy, 1966, p 19.
(10) P. Eneroth, K. Hellström, and R. Ryhage, *Steroids*, **6**, 707 (1965); C. C. Sweeley, W. H. Elliott, I. Fries, and R. Ryhage, *Anal. Chem.*, **38**, 1549 (1966); H. Adlercreutz, T. Luukkainen, and W. Taylor, *Europ. J. Steroids*, **1**, 117 (1966).
(11) T. Luukainen, W. J. A. VandenHeuvel, E. O. A. Haahti, and E. C. Horning, *Biochim. Biophys. Acta*, **52**, 599 (1961); E. C. Horning, W. J. A. VandenHeuvel, and B. G. Creech, *Methods Biochem. Anal.*, **11**, 69 (1963); P. P. Nair, C. Bucana, S. de Leon, and D. A. Turner, *Anal. Chem.*, **37**, 631 (1965); A. Rozanski, *ibid.*, **38**, 36 (1966); M. Kirschner and M. B. Lipsett, *J. Clin. Endocrinol. Metab.*, **23**, 255 (1963); Abstracts, Second International Congress of Hormonal Steroids, Milan, Italy, 1966: R. R. Burtner, E. A. Brown, and R. A. Mikulec, p 50; C. J. W. Brooks, E. M. Chambaz, W. L. Gardiner, and E. C. Horning, p 32; S. Hara, T. Watabe, and Y. Ike, *Chem. Pharm. Bull.* (Tokyo), **14**, 1311 (1966).

TABLE I

VARIATIONS IN THE MASS SPECTRA (70 EV) OF CHOLESTEROL TRIMETHYLSILYL ETHER (II) DEPENDING UPON MEASURING CONDITIONS

Method of prepn	Mass spectrometer	Inlet system[a]	*m/e* 129 % Σ_{40}	*m/e* 129 relative abundance, %	M^+ % Σ_{40}	M^+ relative abundance, %
HMDS[b]	Atlas CH-4	Direct	2.86	30	9.54	100
HMDS	CEC 21-103C	Heated	3.67	82	0.22	5
HMDS	AEI MS-9	Direct	6.18	100	2.78	45
AcTMS[c]	Atlas CH-4	Direct	2.97	40	7.43	100
AcTMS	CEC 21-103C	Heated	0.60	13	0.20	4
AcTMS	AEI MS-9	Direct	5.69	100	2.22	39

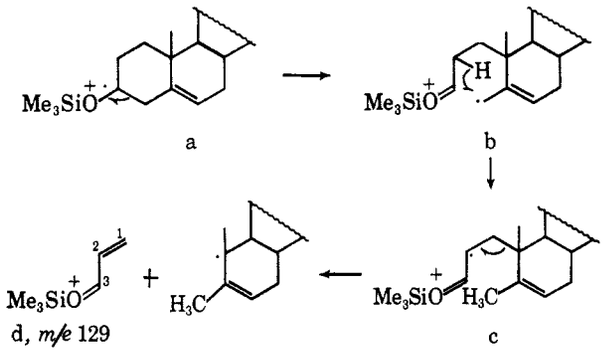
[a] For further details, see footnote 29. [b] HMDS = hexamethyldisilazane. [c] AcTMS = N-(trimethylsilyl)acetamide.



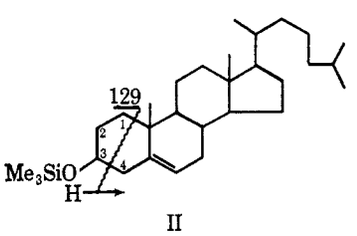
I

ment is not known but probably involves the trimethylsiloxy group and three carbon atoms of ring A.

In light of the extensive work on the mass spectral fragmentation behavior of ethylene ketals of steroids[12] and especially those with a Δ^5 double bond, such a formulation seems unlikely since it would involve the unfavorable fission of a vinylic bond between carbon atoms 4 and 5. It seems more plausible to propose a trimethylsilyl ether directed fragmentation sequence (a → d) which is analogous to the well-substantiated[12] ethylene ketal directed fragmentation path.



In this scheme, cleavage of both the 3,4 and 1,10 bonds is favored by allylic activation, hydrogen transfer from C-2 occurs through a six-membered transition state, and the resulting ion of mass 129 is a conjugated even-electron ion. This process (II) differs from Ryhage's[4] rationalization in that it encompasses carbon atoms 1, 2, and 3 (see II) rather than carbon atoms 2, 3, and 4 (see I).



II

If such importance is going to be placed upon the existence of an *m/e* 129 peak for identification purposes, it seems essential to elucidate the exact origin of this species. For instance, a substituent at C-4 (frequently encountered in naturally occurring sterols) would have no effect upon the *m/e* 129 peak according to sequence II; while, according to the alternate sequence (I), this peak would be shifted by the mass corresponding to that substituent. It was therefore desired to determine the exact composition of this fragment of mass 129 utilizing deuterium labeling and substitution of methyl groups at C-4; as will be shown below, such experiments verified the proposed fragmentation sequence (a → d).

Preparation of Deuterium-Labeled Compounds

Deuterium-labeling experiments were undertaken with the intention of placing deuterium atoms at positions 2, 3, and 4 of cholesteryl trimethylsilyl ether. Work done by Ringold and Malhotra[13,14] on conjugate anion formation in α,β-unsaturated keto steroids involves an attractive method of deconjugating the readily available Δ^4-cholesten-3-one (III) to Δ^5-cholesten-3-one (VI), which can be subsequently reduced and converted to the trimethylsilyl ether (II) of cholesterol. The particular advantage in this procedure centers on the possibility of achieving the desired deuterium labeling during the deconjugative process. Synthesis of the following three labeled compounds was therefore undertaken: 2,2,4,4-d_4-Δ^5-cholesten-3β-ol (IX), 3α-d_1-Δ^5-cholesten-3β-ol (X), and 2,2,4-d_3-Δ^5-cholesten-3β-ol (XI).

Ringold[14] achieved deconjugation by treating Δ^4-cholesten-3-one (III) with potassium *t*-butoxide in *t*-butyl alcohol, followed by acetic acid protonation of the enolate anion (IV). It was decided to perform this reaction in *t*-butyl alcohol-O-d_1,[15] whereby deuterium was incorporated at C-2 and C-4, but not at C-6 of the enolate anion (V). Deuteration of the latter with acetic acid-O-d_1 then yielded 2,2,4,4-d_4-Δ^5-cholesten-3-one (VII); deuteration at the C-4 position with acetic acid-O-d_1 rather than at C-6 is in accord with Ringold's conclusions regarding the position of protonation of enolate anions of this type.[16] Reduction of the keto group with lithium aluminum tri-*t*-butoxyhydride[17] gave the labeled sterol, 2,2,4,4-d_4-Δ^5-cholesten-3β-ol (IX). Formation of 3α-d_1-Δ^5-cholesten-3β-ol (X) was

(12) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 2, Holden-Day, Inc., San Francisco, Calif., 1964, Chapter 18.

(13) H. J. Ringold and S. K. Malhotra, *Tetrahedron Letters*, 669 (1962).

(14) H. J. Ringold and S. K. Malhotra, *J. Am. Chem. Soc.*, **84**, 3402 (1962).

(15) The authors wish to express their appreciation to Dr. H. J. Ringold of the Worcester Foundation for Experimental Biology, for discussion and unpublished information regarding this procedure.

(16) S. K. Malhotra and H. J. Ringold, *J. Am. Chem. Soc.*, **87**, 3228 (1965).

(17) O. H. Wheeler and J. L. Mateos, *Can. J. Chem.*, **36**, 1431 (1958).

accomplished by reduction of unlabeled Δ^5-cholesten-3-one (VI) with lithium aluminum tri-*t*-butoxy deuteride.

The availability of an alternate procedure for converting Δ^4-cholesten-3-one (III) to cholesterol (VIII) offered a method of synthesizing the third required species, 2,2,4-d_3-Δ^5-cholesten-3β-ol (XI). Instead of deuterating the labeled enolate anion (V) with acetic acid-O-d_1, 2,2,4-d_3-$\Delta^{3,5}$-cholestadien-3β-yl acetate (XII) was formed by addition of acetic anhydride. Hydrolysis and reduction of the enol acetate with sodium borohydride[18] gave 2,2,4-d_3-Δ^5-cholesten-3β-ol (XI). All sterols were converted to their trimethylsilyl derivative by reaction with hexamethyldisilazane[19] (eq 1) or with N-(trimethylsilyl)acetamide (eq 2).[20] A disadvantage of the N-(trimethylsilyl) acetamide procedure is that on a small scale it is difficult to remove traces of acetamide.

III

IV, $R_1 = R_2 = H$
V, $R_1 = R_2 = D$

VI, $R_1 = R_2 = H$
VII, $R_1 = R_2 = D$

VIII, $R_{1-5} = H$
IX, $R_{1,2,4,5} = D$; $R_3 = H$
X, $R_{1,2,4,5} = H$; $R_3 = D$
XI, $R_{1,2,4} = D$; $R_{3,5} = H$

XII

XIV

$$2ROH + (CH_3)_3SiNHSi(CH_3)_3 \longrightarrow 2ROSi(CH_3)_3 + NH_3 \quad (1)$$

$$ROH + CH_3\overset{\overset{O}{\|}}{C}NHSi(CH_3)_3 \longrightarrow ROSi(CH_3)_3 + CH_3\overset{\overset{O}{\|}}{C}NH_2 \quad (2)$$

Discussion and Results

Before discussing the nature of the individual peaks, one hitherto unreported general feature must be noted, namely the fact that the mass spectrum of cholesterol trimethylsilyl ether (II) exhibited striking variations depending upon (1) the mass spectrometer used to record the spectrum, (2) the inlet system and ion source temperature of the mass spectrometer, and (3) the method of synthesizing the silyl derivative. Table I indicates extensive variations in both the relative abundance and the percent total ionization of the *m/e* 129 peak; similar variations occur in the molecular ion (*m/e* 458). Ryhage[4] utilized the hexamethyldisilazane method of synthesis (eq 1) and an Atlas CH-4 mass spectrometer with an ionizing energy of 20 ev coupled directly to a gas chromatography apparatus; he found the *m/e* 129 peak to be the most intense one. When the mass spectrum was recorded in our laboratory on an isolated, crystalline sample using an Atlas CH-4 mass spectrometer, at 20 ev instead of the customary 70 ev, the *m/e* 129 peak was only 2% of the base peak ($\Sigma_{40} = 0.61$); thus, minor variations of conditions have an important effect on the mass spectra of trimethylsilyl ethers of steroids. In the light of the significance placed[4,5] upon the *m/e* 129 peak for identification purposes, it is very important to note these extreme variations. All spectra on which the present discussion is based were recorded on an AEI MS-9 spectrometer.

The most straightforward proof that the fragment of mass 129 is composed of carbon atoms 1, 2, and 3 (II) and *not* carbon atoms 2, 3, and 4 (I) comes from a substance where a methyl group is substituted for a C-4 hydrogen. Thus in the mass spectrum (Figure 2) of 4β-methyl-Δ^5-cholesten-3β-yl[21] trimethylsilyl ether (XIII) the *m/e* 129 peak expected on the basis of structure II, is 49% ($\%\Sigma_{40} = 5.31$) of the base peak, while the *m/e* 143 peak (see I) is only 3% ($\%\Sigma_{40} = 0.30$) of the base peak. The presence of a small *m/e* 143 peak (11%) in the mass spectrum (Figure 1) of cholesterol trimethylsilyl ether itself suggests that the fragment of mass 143 may not even involve C-4 or any substituents attached to it.

XIII

The results of deuterium labeling likewise substantiate the origin of the fragment of mass 129 according to the bond fissions indicated in II. Although the isotopic purity was not as high as might be desired, there was sufficient incorporation (see Table II) to obtain sound

TABLE II
EFFECT OF DEUTERIUM LABELING ON *m/e* 129 PEAK IN CHOLESTEROL TRIMETHYLSILYL ETHER

Compd	Isotopic compn, %	Fragment peaks: Peak	% Σ_{40}	Relative abundance,[a] %
Cholesterol trimethylsilyl ether		129	6.18	100
		130	1.05	17
		131	0.93	15
3α-d_1-Cholesterol trimethylsilyl ether	4, d_0; 94, d_1; 2, d_2	129	0.23	4
		130	5.95	100
2,2,4-d_3-Cholesterol trimethylsilyl ether	8, d_1; 29, d_2; 55, d_3; 6, d_4; 2, d_5	129	0.91	20
		130	6.86	100
		131	0.91	20
2,2,4,4-d_4-Cholesterol trimethylsilyl ether	3, d_0; 3, d_1; 8, d_2; 29, d_3; 54, d_4; 3, d_5	129	0.38	10
		130	1.92	50
		131	0.57	15
		132	0.38	10

[a] Corrected for natural isotope abundance and calculated deuterium isotope composition (column 2).

(18) W. G. Dauben and J. F. Eastman, *J. Am. Chem. Soc.*, **73**, 4463 (1951).
(19) S. H. Langer, S. Connell, and I. Wender, *J. Org. Chem.*, **23**, 50 (1958).
(20) L. Birkofer, A. Ritter, and F. Bentz, *Chem. Ber.*, **97**, 2196 (1964).
(21) The authors wish to thank Dr. S. Julia for samples of this compound; see S. Julia and J. P. Lavaux, *Bull. Soc. Chim. France*, 1223 (1963).

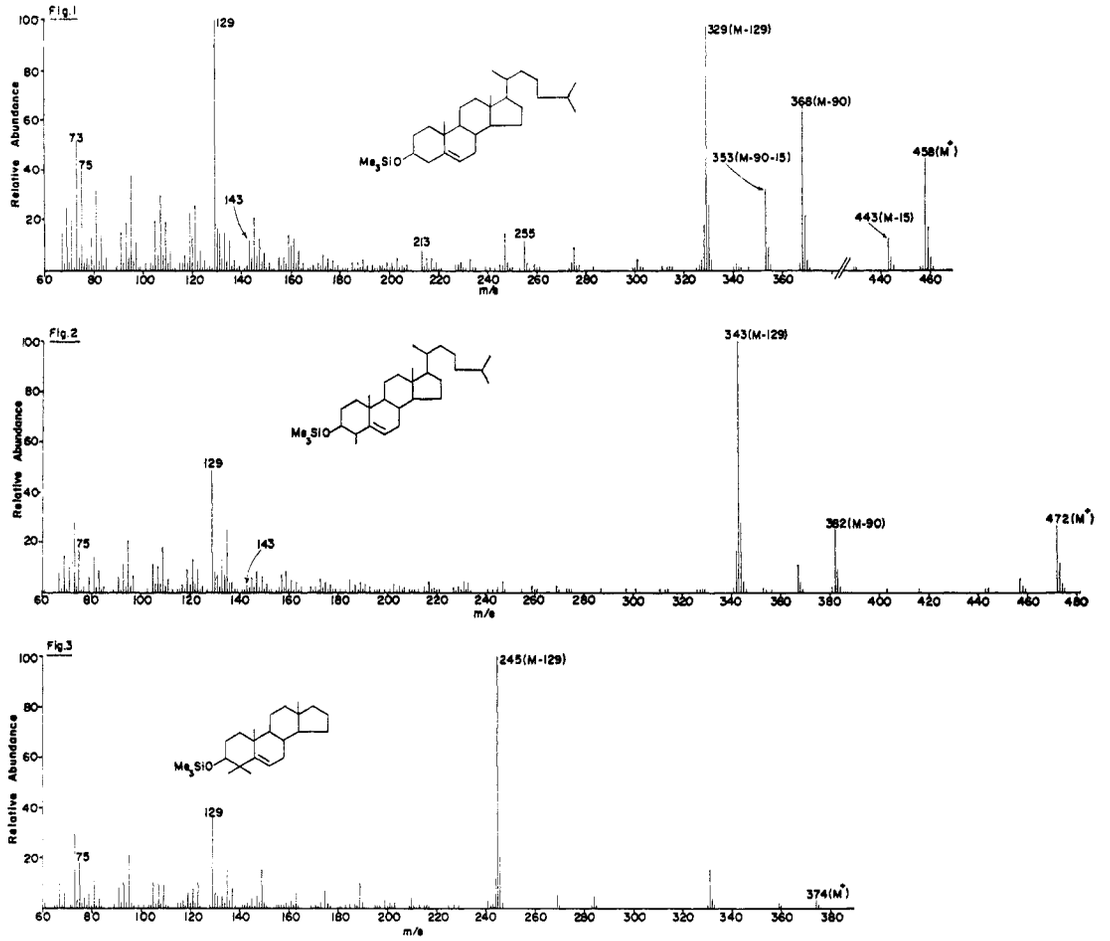


Figure 1.— Mass spectrum of Δ^5-cholesten-3β-ol trimethylsilyl ether (AEI MS-9 mass spectrometer; direct inlet system).
Figure 2.— Mass spectrum of 4β-methyl-Δ^5-cholesten-3β-ol trimethylsilyl ether (AEI MS-9 mass spectrometer; direct inlet system).
Figure 3.— Mass spectrum of 4,4-dimethyl-Δ^5-androsten-3β-ol trimethylsilyl ether (AEI MS-9 spectrometer; direct inlet system).

results. As expected, the *m/e* 129 peak is shifted to *m/e* 130 in 3α-d_1-cholesteryl trimethylsilyl ether because the 3α hydrogen (or deuterium) remains attached to C-3 throughout the fragmentation sequence. A similar observation was made in both 2,2,4-d_3- and 2,2,4,4-d_4-cholesteryl trimethylsilyl ethers in accord with the fragmentation sequence a → d.

Another intense peak (97% relative intensity) in the spectrum (Figure 1) of cholesteryl trimethylsilyl ether appears at *m/e* 329 (M − 129). This peak is complementary to the base peak (*m/e* 129) and its identity is substantiated by deuterium labeling (Table III). In 3α-d_1-cholesteryl trimethylsilyl ether this peak remains at *m/e* 329 (M − 130, 86%), and in the 2,2,4-d_3- and 2,2,4,4-d_4 labeled trimethylsilyl ethers this peak moves to *m/e* 331 (M − 130, 60%) and *m/e* 332 (M − 130, 59%), respectively.

Interestingly enough, *m/e* 343 (M − 129) becomes the base peak in the spectrum (Figure 2) of 4β-methyl trimethylsilyl ether (XIII) while *m/e* 129 substantially decreases in intensity (49%). One finds this relationship to be even more pronounced when C-4 has two methyl substituents as in 4,4-dimethyl-Δ^5-androsten-3β-yl trimethylsilyl ether (XIV). In this spectrum (Figure 3) M − 129 (*m/e* 245) is again the base peak and the *m/e* 129 peak is now only of 36% relative abundance.

One must propose a fragmentation sequence different from a → d in order to account for the charge retention

TABLE III
EFFECT OF DEUTERIUM LABELING ON *m/e* 329 (M − 129) PEAK IN CHOLESTEROL TRIMETHYLSILYL ETHER

		Fragment peaks		
Compd	Isotopic compn, %	Peak	% Σ_{40}	Relative abundance,[a] %
Cholesterol trimethylsilyl ether		329 (M − 129)	5.99	97
3α-d_1-Cholesterol trimethylsilyl ether	4, d_0; 94, d_1; 2, d_2	329 (M − 130)	5.12	86
2,2,4-d_3-Cholesterol trimethylsilyl ether	8, d_1; 29, d_2; 55, d_3; 6, d_4; 2, d_5	331 (M − 130)	2.74	60
2,2,4,4-d_4-Cholesterol trimethylsilyl ether	3, d_0; 3, d_1; 8, d_2; 29, d_3; 54, d_4; 3, d_5	332 (M − 130)	2.27	59

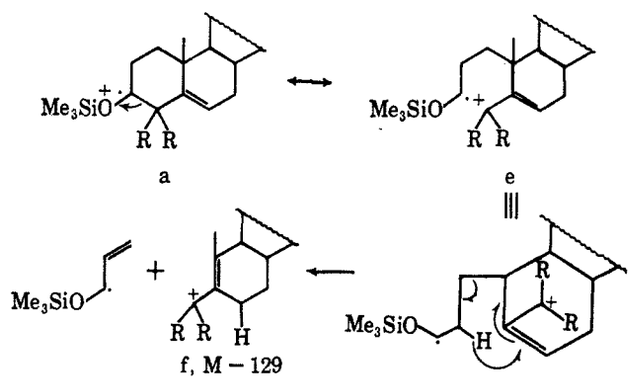
[a] Corrected for natural isotope abundance and calculated deuterium isotope composition (column 2).

on the silicon-free M − 129 species and for the fact that additional substitution at C-4 increases the intensity of this M − 129 peak. As was pointed out in an earlier study[22] on the electron-impact-induced retro-Diels–Alder reaction, one can rationalize and predict in which species the charge will be retained if one treats mass spectrometric fragmentation sequences

(22) H. Budzikiewicz, J. I. Brauman, and C. Djerassi, *Tetrahedron*, **21**, 1855 (1965).

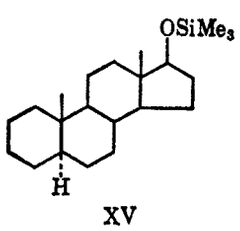
in a stepwise manner in which each hypothetical intermediate is analyzed for stabilizing features.

If one considers the molecular ion of cholesterol trimethylsilyl ether in terms of resonance froms a or e (or if one assumes direct production of e by loss of a σ electron), one can derive a fragmentation path (e → f) which rationalizes charge retention on the hydrocarbon moiety (f).



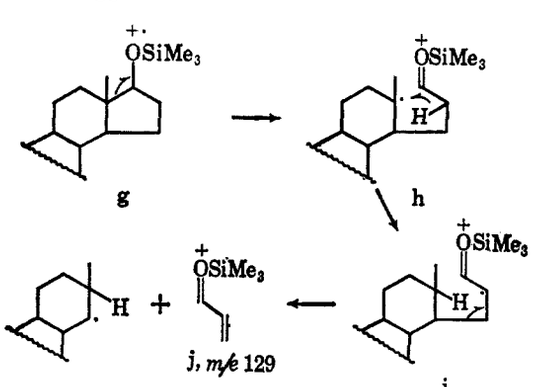
The occurrence of sequence e → f in lieu of a → d depends upon two factors: the polarization of the carbon 3,4 bond and the stability of the resulting carbonium ion (f). As electron-donating methyl groups (R = CH_3) are substituted on C-4, the (e → f) sequence becomes a more attractive process. More important is the observation that methyl substitution at C-4 greatly stabilizes the carbonium ion (e) and forces path e → f to predominate, thus intensifying the M − 129 peak.

Because the *m/e* 129 peak could possibly result in part from a ring-D fragmentation sequence in the mass spectrum of 5α-androstane-3β,17β-diolbistrimethylsilyl ether reported by Sjövall,[5] it was decided to synthesize and record the spectrum (Figure 4) of 5α-androstan-17β-yl trimethylsilyl ether (XV). High-resolution mass measurements showed the relatively weak *m/e*



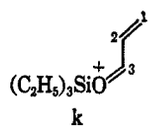
XV

129 peak (28% relative intensity; Σ_{40} = 2.01) to have the same composition, $C_6H_{13}OSi$, as ion d. This observation suggests a fragmentation sequence g → j,

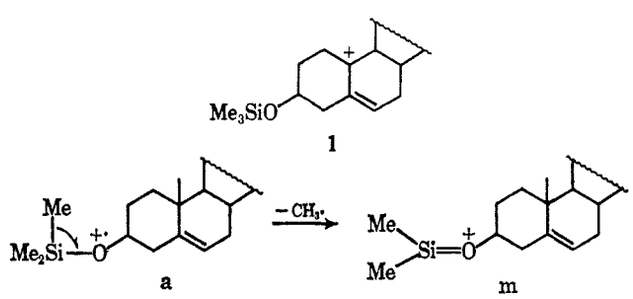


where cleavage of the 13,17 bond in the initial step is aided by the formation of a tertiary radical at the C-13 position completely analogous to the electron-impact-induced decomposition in ethylene ketals of 17-keto steroids.[13]

It is also pertinent to note that a prominent peak at *m/e* 171 (33% relative intensity; Σ_{40} = 4.59) occurs in the mass spectrum (Figure 5) of the triethylsilyl ether of cholesterol. This ion (k) results from a fragmentation sequence analogous to a → d which produced the species of mass 129 in the trimethylsilyl ether derivatives (II).



Inspection of the entire mass spectrum (Figure 1) of cholesterol trimethylsilyl ether reveals other prominent peaks which should be commented upon even though they do not contribute as much structural significance as the *m/e* 129 peak. The ion of mass 443 (M − CH_3, 13%) corresponds to loss of a methyl radical from the molecular ion. Although cleavage of the C-19 angular methyl group leaves an allylic carbonium ion (1), it appears that the most attractive site of methyl cleavage is at the silicon atom (a → m). This

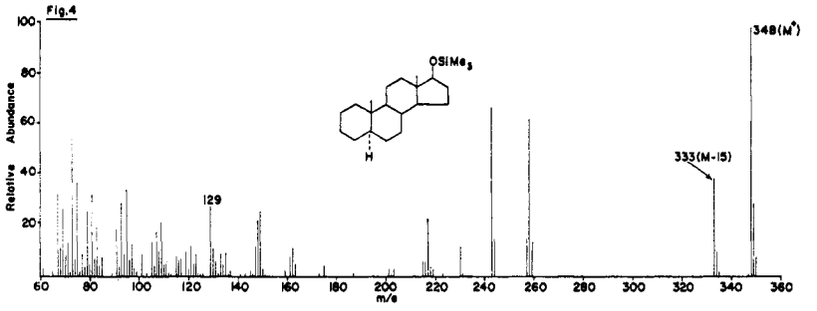


type of fragmentation is a very common occurrence in the mass spectral fragmentation of trimethylsilyl ethers and esters.[23] This prediction as to the site of cleavage is based upon the observation that the *m/e* 471 (M − C_2H_5) peak is the most intense one (Σ_{40} = 13.91) in the mass spectrum (Figure 5) of cholesteryl triethylsilyl ether. No peak corresponding to loss of a (C-19 angular) methyl group (*m/e* 485) appears. It is also very unlikely that a methyl radical is lost from the C-17 side chain[24] or the C-18 position.

High resolution mass determinations show the composition of the fragment ion of mass 368 (M − 90, 66%) to be due to the loss of trimethylsilanol, $(CH_3)_3SiOH$. The actual fragmentation sequence is somewhat difficult to elaborate owing to uncertainty regarding the site from which a hydrogen migrates to the trimethylsiloxy group. The existence of an *m/e* 369 (M − 90, 56%) peak in the spectrum of 3α-d_1-cholesteryl trimethylsilyl ether proves that this hydrogen atom does not originate from the 3α position. Incomplete deuterium labeling (Table II) makes difficult

(23) A. G. Sharkey, R. A. Friedel, and S. H. Langer, *Anal. Chem.*, **29**, 770 (1957); E. M. Teeter, Tenth Annual Conference on Mass Spectrometry, ASTM Committee E-14, New Orleans, La., 1962, p 51; J. B. Thomson and C. Djerassi, unpublished results.

(24) C. Beard, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **86**, 269 (1964).



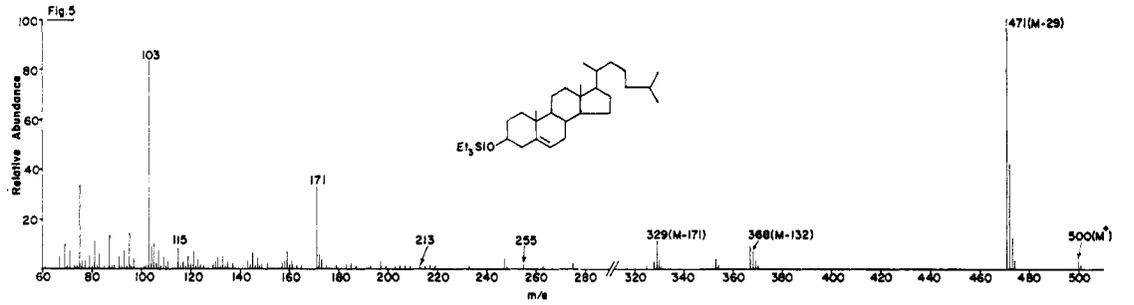
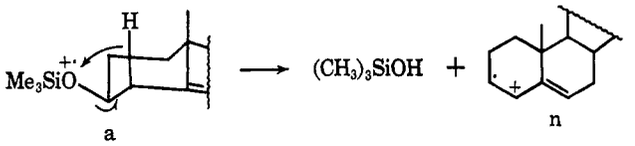


Figure 4.—Mass spectrum of 5α-androstan-17β-ol trimethylsilyl ether (AEI MS-9 mass spectrometer; direct inlet system).
Figure 5.—Mass spectrum of Δ⁵-cholesten-3β-ol triethylsilyl ether (AEI MS-9 mass spectrometer; direct inlet system).

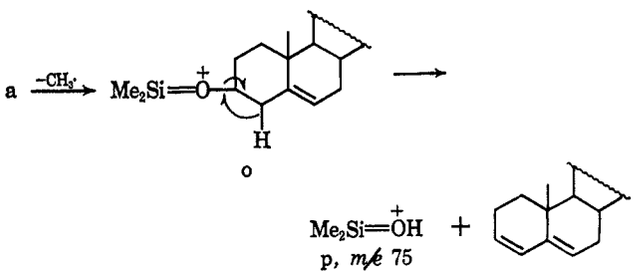
comparison of the M − 90 (or M − 91) peaks in 2,2,4-d_3- and 2,2,4,4-d_4-Δ⁵-cholesten-3β-yl trimethylsilyl ether, but careful scrutiny of these peaks provides the source of at least some of the migrating hydrogen. The ratio of the intensities of molecular ion peaks [*m/e* 461 (d_3): 460 (d_2)] is identical with the ratio of the M − 90 peaks [*m/e* 371:370] in the d_3 species. Thus, no M − 91 contributes to the *m/e* 370 peak and no hydrogen migrates from the C-2 position or one of the C-4 positions. In the d_4 species, however, the ratio of *m/e* 372:371 is about 40% greater than the *m/e* 462:461 ratio; this suggests that some of the migrating hydrogen does come from C-4. On the basis of stereochemical considerations it seems most likely that the 4β hydrogen is probably migrating (a → n) to give trimethylsilanol and an ionized diene. Further support is lent to this prediction when one considers the mass spectrum (Figure 2) of 4β-methyl-Δ⁵-cholesten-3β-yl trimethylsilyl ether (XIII) and finds the *m/e* 382 peak (M − 90, 25%) to be greatly reduced in per cent total ionization and relative intensity.



It is difficult to predict from where the remaining hydrogen originates, but most likely some of its comes from the C-1 position. This suggestion is based upon the observation that a substantial portion of the migrating hydrogen originates from that position in the mass spectrum of 5α-cholestan-3β-yl trimethylsilyl ether as proven by deuterium labeling.[25] One finds also an analogous mode of fragmentation when water is eliminated from 3-hydroxy steroids,[26,27] and where this loss appears to be preceded by ring opening (cleavage of either the 3,4 or 2,3 bonds). Expulsion of water is then accompanied by random migration of hydrogen from the open-chain species. One might be tempted to apply this same argument to loss of trimethylsilanol from Δ⁵-3-steryl trimethylsilyl ethers, but deuterium-labeling experiments disprove this alternative.

Directly related to the M − 90 peak (*m/e* 368) is one appearing at *m/e* 353 [M − (90 + 15)]. This fragment corresponds to the loss of trimethylsilanol and a methyl group from the parent compound. Here the ejected methyl radical most likely originated from C-19.

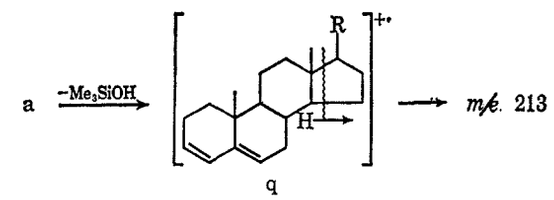
Another fragmentation sequence related to the M − 90 peak results in a fragment ion of mass 75 (40% relative intensity; Σ_{40} = 2.47). High-resolution mass measurements indicate the composition of this species to be $(CH_3)_2SiOH$, which would result from cleavage of a methyl radical from the trimethylsilyl function followed by loss of the dimethylsilanol moiety (a → p). Although the scheme (a → p) depicts the site of hydrogen migration to be C-4, deuterium labeling and methyl substitution at C-4 reveal a situation identical with the hydrogen transfer involved in the M − 90 fragmentation, namely, that only about 40% of the hydrogen originates from the C-4 position and that it is probably the axial 4β-hydrogen atom that is implicated.



Three peaks common to both cholesteryl silyl ethers studied were those of *m/e* 73, 255, and 213. High-resolution measurements showed that the ion (C_3H_9Si)

(25) Unpublished results.
(26) J. Karliner, H. Budzikiewicz, and C. Djerassi, *J. Org. Chem.*, **31**, 710 (1966).
(27) H. Egger and G. Spiteller, *Monatsh.*, **97**, 579 (1966).

of mass 73 (52% relative abundance; $\Sigma_{40} = 3.21$) corresponded to cleavage of the trimethylsilyl group from the parent ion. Loss of trimethylsilanol (mass 90) and the C-17 side chain (mass 113) gives a fragment of mass 255 (12% relative abundance; $\Sigma_{40} = 0.74$) and the observation of a metastable peak at *m/e* 176.5 (calcd $255^2/368 = 176.7$) suggests the fragmentation sequence M − 90 → M − (90 + 113) instead of M − 113 → M − (90 + 113). The peak at *m/e* 213 (8% relative abundance; $\Sigma_{40} = 0.49$) corresponds to loss of trimethylsilanol, the C-17 side chain, and three carbon atoms of ring D, accompanied by a hydrogen transfer[28] from the charge retaining species (q).



q

In conclusion, it is pertinent to point out that the trimethylsilyl function does *not* compare with the ethylene ketal group[13] in its ability to direct characteristic fragmentation patterns. Although it is quite useful in this respect in the case of Δ^5-3-hydroxy steroids, this utility disappears when the 5,6 double bond is reduced; in this case, the *m/e* 129 peak becomes buried in a multiplicity of surrounding low-intensity peaks.[25]

Experimental Section[29]

Cholesteryl Trimethylsilyl Ether (II). Procedure A.—A mixture of 100 mg of cholesterol and 0.05 ml of hexamethyldisilazane was dissolved in 20 ml of dry tetrahydrofuran (distilled from lithium aluminum hydride). One drop of chlorotrimethylsilane was added and the cloudy solution was allowed to stand for 30 hr in a nitrogen atmosphere at room temperature. Purification by tlc (dry benzene development) gave 79 mg of white, crystalline product: mp 121–123°; λ_{max}^{Nujol} 8.0, 11.9, and 13.3 (Me_3Si) and 9.2 μ (SiO).

Anal. Calcd for $C_{30}H_{54}OSi$: C, 78.51; H, 11.87; mol wt, 458.848. Found: C, 81.32; H, 12.01; mol wt, 458 (Figure 1).[31]

The following steryl trimethylsilyl ethers were synthesized using procedure A.

4,4-Dimethyl-Δ^5-androsten-3β-yl trimethysilyl ether (XIV) had mp 127–129°; λ_{max}^{Nujol} 7.9, 11.8, 13.3, and 9.1 μ.

Anal. Calcd: mol wt, 374.686. Found: mol wt, 374.

5α-Androstan-17β-yl trimethysilyl ether (XV) had mp 101–103°; λ_{max}^{Nujol} 8.0, 12.0, 13.4 and 9.2 μ. *Anal.* Calcd: mol wt, 348.648. Found: mol wt, 348.

Procedure B.—In a nitrogen atmosphere 100 mg of cholesterol and 238 mg of N-(trimethylsilyl)acetamide[32] were fused for 5 min in an oil bath maintained at 135°. The reaction mixture was cooled to room temperature and taken up in cold hexane (8 ml), and the side product (acetamide) was removed by filtration. The hexane was stripped to give a product whose infrared spectrum indicated the presence of some remaining acetamide; λ_{max}^{Nujol} 3.0, 3.2, and 5.7 μ. The mass spectrum also indicated the presence of acetamide, (*m/e* 59), and another impurity (*m/e* 147).[33] Purification by tlc (dry benzene development) gave 28 mg of the white product. The following steryl trimethylsilyl derivatives were synthesized using procedure B.

4β-Methyl-Δ^5-cholesten-3β-yl trimethylsilyl Ether (XIII) had mp 103–105°; λ_{max}^{Nujol} 8.1, 11.9, 13.3, and 9.1 μ. *Anal.* Calcd: mol wt, 472.875. Found: mol wt, 472.

4,4-Dimethyl-Δ^5-androsten-3β-yl trimethylsilyl Ether (XIV) had mp 129–132; λ_{max}^{Nujol} 8.0, 11.8, 13.4, and 9.2 μ. *Anal.* Calcd: mol wt, 374.686. Found: mol wt, 374.

N-(Triethylsilyl)acetamide.—To a mixture of 3.6 g of acetamide and 5.4 g of triethylamine in 25 ml of dry refluxing benzene, was added 8.1 ml of chlorotriethylsilane[34] over a period of 1 hr. The mixture was refluxed for 1 hr and cooled to room temperature, and the precipitated salt removed by filtration and washed with benzene. The benzene was evaporated and 3.8 g of N-(triethylsilyl)acetamide collected by fractional distillation: bp 84–86° (10 mm); λ_{max} 8.1, 11.7, and 13.3 μ.

Cholesteryl 3β-Triethylsilyl Ether.—Utilizing a method identical with procedure B, 100 mg of cholesterol and 315 mg of N-(triethylsilyl)acetamide yielded 81 mg of cholesteryl triethylsilyl ether: 88–90°, λ_{max}^{Nujol} 8.1, 11.6, 13.5, and 9.3 μ.

3α-d_1-Cholesterol (X) Trimethylsilyl Ether.—In a nitrogen atmosphere 5 ml of *t*-butyl alcohol was added to 200 mg of Δ^4-cholesten-3-one (III) and 500 mg of potassium *t*-butoxide, and the yellow solution was allowed to stir at room temperature for 2 hr. Cold acetic acid (10 ml, 10%) was rapidly added and the now milky solution was poured into ice–water. The aqueous solution was extracted twice with ether and the combined ethereal extracts washed with cold water, aqueous sodium bicarbonate, and twice more with cold water. The ethereal solution was then dried over anhydrous magnesium sulfate in a refrigerator for 15 hr. Analytical tlc (benzene–ethyl acetate, 17:3) indicated a mixture of Δ^4-(III)- and Δ^5-(VI)-cholesten-3-one. The solvent was evaporated and the product was dissolved in dry tetrahydrofuran.

In 6 ml of dry tetrahydrofuran was placed 80 mg of lithium aluminum deuteride and the suspension was stirred in an icebath for 10 min. While the slurry was stirring, 0.4 ml of *t*-butyl alcohol was added dropwise, followed by the dropwise addition of the mixture of Δ^4- and Δ^5-cholesten-3-one. The entire mixture was stirred for 0.5 hr at 0° and for 2 hr at room temperature. The system was again cooled, a few drops of water was added to decompose the excess lithium aluminum tri-*t*-butoxy deuteride, and then enough hydrochloric acid (5%) was added to turn the suspension white. The product was extracted twice with ether, the ether was stripped, and the product was allowed to stand over night at room temperature in a mixture (50 ml) of hydrochloric acid (15%) and concentrated acetic acid (1:1). The acid solution was extracted twice with ether, and the combined ethereal extracts washed twice with water, twice with aqueous potassium bicarbonate, and twice more with water, and dried over anhydrous magnesium sulfate. After evaporation of the ether, chromatography on silica (Davison, grade 950, 60–200 mesh) and elution with benzene gave 3α-d_1-cholesterol (X) which was converted by procedure B to 8.0 mg of the trimethylsilyl ether, mp 117–119°. The mass spectrum showed the product to have the following isotopic distribution: 4% d_0 (*m/e* 455), 94% d_1 (*m/e* 459), and 2% d_2 (*m/e* 460).

(28) Such a transfer in steroid hydrocarbons has been studied in detail by L. Tökés, Ph.D. Thesis, Stanford University, 1965.

(29) Melting points (uncorrected) were determined on the Kofler block. Ultraviolet absorption spectra were determined with a Bausch and Lomb Spectronic 505 spectrophotometer and the infrared absorption spectra were measured with a Perkin-Elmer Model 137 Infracord spectrophotometer. Optical rotations were performed in chloroform solution with a Zeiss polarimeter. Mass spectra recorded with a Consolidated Electrodynamics Corp. mass spectrometer No. 21-103C were run by Mr. Nelson Garcia. This spectrometer possesses an all-glass inlet system heated to 200°, while the isatron temperature was maintained at 270°. Mass spectra measured with an Atlas CH-4 mass spectrometer were run by Dr. A. M. Duffield and Dr. J. K. MacLeod. This spectrometer is equipped with a TO-4 ion source (temperature, 60°). Spectra measured on the AEI MS-9 instrument were run by Mr. Robert Ross. The sample was inserted using a direct inlet system with the ion source temperature at 200°. The ionizing energy was kept at 70 ev in all three spectrometers. Thin layer chromatography (tlc) was performed on silica gel G (Merck A. G. Darmstadt), followed by spraying with a 2% solution of ceric sulfate in 2 *N* sulfuric acid and heating for optimum development of colored spots. Preparative tlc was performed on silica gel H (Merck A. G., Darmstadt) having a thickness of 1.0 mm. The appropriate fractions were detected by exposure to ultraviolet light or by spraying with iodine vapors. All microanalyses were by Messrs. E. Meier and J. Consul.

(30) Hexamethyldisilazane was purchased from K & K Laboratories, Inc., Plainview, N. Y.

(31) Because of the extreme ease of hydrolysis, it was impossible to obtain satisfactory elemental analyses; therefore, all subsequent analyses of trimethylsilyl ethers are based upon molecular weights determined by mass spectrometry.

(32) N-(Trimethylsilyl)acetamide was purchased from Eastman Organic Chemicals, Distillation Products Industries, Rochester, N. Y.

(33) The composition of this fragment ion is $Me_2Si=O^+SiMe_3$: J. B. Thomson and C. Djerassi, unpublished results.

(34) Chlorotriethylsilane was purchased from Pierce Chemical Co., Rockford, Ill.

2,2,4,4-d_4-Cholesteryl Trimethylsilyl Ether.—This procedure is identical with that used in the synthesis of the 3α-d_1-labeled trimethylsilyl ether with a few variations. *t*-Butyl alcohol was replaced with *t*-butyl alcohol-O-d_1 and acetic acid-O-d_1 was added to the reaction mixture instead of unlabeled acetic acid. In the reduction of the keto group, lithium aluminum hydride was used instead of lithium aluminum deuteride. The labeled cholesterol analog (IX) was converted to its trimethylsilyl ether by procedure B giving 45 mg of 2,2,4,4-d_4-cholesteryl trimethylsilyl ether, mp 115–118°. The mass spectrum indicated the following isotopic distribution: 3% d_0 (*m/e* 458), 3% d_1 (*m/e* 459), 8% d_2 (*m/e* 460), 29% d_3 (*m/e* 461), 54% d_4 (*m/e* 462), and 3% d_5 (*m/e* 463).

2,2,4-d_3-$\Delta^{3,5}$-Cholestadien-3-yl Acetate (XII).—In a nitrogen atmosphere was placed 200 mg of Δ^4-cholesten-3-one (III) and 500 mg of potassium *t*-butoxide. *t*-Butyl alcohol-O-d_1 (5 ml) was added and the yellow solution allowed to stir at room temperature for 2 hr. After the addition of 1 ml of acetic anhydride, the solution was stirred for another hour. The mixture was poured into cold water, extracted with ether, washed twice with cold water, twice with aqueous sodium bicarbonate, and twice more with cold water, and dried for 16 hr at 0° over anhydrous magnesium sulfate. The ether was evaporated and the fluorescent, oily product was recrystallized twice from ethanol and once from methanol giving 123 mg of white needles: mp 79–81°; $[\alpha]^{25}D$ −99.0° (*c* 1.003); λ_{max}^{EtOH} 235 mμ (log ϵ 4.25), λ_{max}^{Nujol} 5.7, 6.0, and 6.2 μ. The nmr spectrum exhibited one vinylic proton signal at δ 5.4.[35]

2,2,4-d_3-Cholesteryl Trimethylsilyl Ether.—A solution of 121 mg of sodium borohydride in absolute ethanol (4 ml) was slowly added over a period of 30 min to an ice-cold stirring solution of 61 mg of 2,2,4-$\Delta^{3,5}$-cholestadien-3-yl acetate (XII) in 11 ml of absolute ethanol. Stirring was continued at 0° for 42 hr, at which time the mixture was heated under reflux for 1 hr. Concentrated hydrochloric acid (1.8 ml) was added dropwise and refluxing was continued for another hour. After cooling to room temperature, the mixture was extracted twice with ether and the combined ethereal extracts were washed four times with water. The ethereal solution was then dried over anhydrous magnesium sulfate. The ether was evaporated, and the mixture of products was dissolved in a minimum amount of benzene. Chromatography on silica (Davison, Grade 950, 60–200 mesh) with benzene as eluent gave a white powder which was recrystallized from methanol to give 22 mg of 2,2,4-d_3-cholesterol (XI), mp 141–143°.

Utilizing procedure B this deuterated analog was converted to 7.1 mg of 2,2,4-d_3-Δ^5-cholesten-3β-yl trimethylsilyl ether, mp 114–116°. The mass spectrum showed the following isotopic distribution: 8% d_1 (*m/e* 459), 29% d_2 (*m/e* 460), 55% d_3 (*m/e* 461), 6% d_4 (*m/e* 462), and 2% d_5 (*m/e* 463).

Registry No.—II, 1856-05-9; XIV, 7604-81-1; XV, 7604-82-2; XIII, 7604-83-3; N-(triethylsilyl)acetamide, 7604-84-4; cholesterol 3β-triethylsilyl ether, 7604-85-5; X trimethylsilyl ether, 7604-86-6; 2,2,4,4-d_4-cholesterol trimethylsilyl ether, 7604-87-7; XII, 7604-88-8; 2,2,4-d_3-Δ^5-cholesterol trimethylsilyl ether, 7604-89-9; XI, 7604-90-2; 2,2,4,4-d_4-cholesterol trimethylsilyl ether, 7604-91-3.

Acknowledgment.—Grateful acknowledgment is made to Dr. Peter Brown for stimulating discussion in connection with this work.

(35) This spectrum was recorded by Mr. D. McMillan with a Varian A-60 spectrometer, employing deuteriochloroform as solvent and tetramethylsilane as internal reference.

Mass Spectrometry in Structural and Stereochemical Problems. CXXVI.[1] Synthesis and Fragmentation Behavior of Deuterium-Labeled 17-Keto Steroids[2]

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Received October 19, 1966

Deuterium labeling of 5α-androstan-17-one and high-resolution mass spectrometry establish that the diagnostically significant *m/e* 230 peak arises from loss of carbon atoms 17 and 16. The two hydrogen atoms which migrate from the charged portion of the molecule do not originate from the C-18 angular methyl group but come from at least three positions, C-7, C-8, and C-12. These results are interpreted in terms of a fragmentation sequence involving breaking of the 13,17 bond followed by a random migration of one hydrogen to the radical center (C-17) and subsequent site-specific McLafferty migration of the second hydrogen. The lack of deuterium removal from positions other than C-15 and C-16 suggests that the hydrogen migration occurring in the formation of *m/e* 217 may be a random process as well. The genesis of each of the significant peaks in the high mass range of the mass spectrum of 5α-androstan-17-one is discussed.

Our preliminary study[4] of the mass spectra of steroidal ketones revealed the dependency of characteristic features of fragmentation on the position of the keto function. The complexity of the fragmentation process soon came to be appreciated as the result of further study in our laboratory.[5] Subsequently, our main interest in the mass spectral behavior of steroidal ketones centered on the reaction mechanisms involving hydrogen transfer. Detailed studies[6] on a number of deuterated steroidal ketones demonstrated further the significance of hydrogen rearrangements in the fragmentation mechanisms and illustrated the necessary steric requirements for these rearrangements. In continuing these studies, we now have investigated in a detailed manner the electron-impact fragmentation and accompanying hydrogen transfers in the only two remaining steroidal ketone types, the 17- and 20-keto steroids. This paper describes the synthesis of the deuterated 17-keto steroids, the mass spectral results, and the derived conclusions. Such studies seemed particularly relevant because of the biological importance

(1) Paper CXXV: J. Dickman and C. Djerassi, *J. Org. Chem.*, **32**, 1005 (1967).

(2) Financial assistance by the National Institutes of Health (Grants No. AM-04257 and CA-07195) is gratefully acknowledged.

(3) Recipient of a National Institutes of Health Special Fellowship, 1965–1966, while on leave from the College of Forestry, Syracuse University.

(4) H. Budzikiewicz and C. Djerassi, *J. Am. Chem. Soc.*, **84**, 1430 (1962).

(5) (a) D. H. Williams, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *ibid.*, **85**, 2091 (1963); (b) C. Beard, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *ibid.*, **86**, 269 (1964); (c) H. Powell, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *ibid.*, **86**, 2623 (1964); (d) R. Beugelmans, R. H. Shapiro, L. J. Durham, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *ibid.*, **86**, 2832 (1964).

(6) (a) R. H. Shapiro, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *ibid.*, **86**, 2837 (1964); (b) J. E. Gurst and C. Djerassi, *ibid.*, **86**, 5542 (1964); (c) C. Djerassi, G. von Mutzenbecher, J. Fajkos, D. H. Williams, and H. Budzikiewicz, *ibid.*, **87**, 817 (1965); (d) C. Djerassi, R. H. Shapiro, and M. Vandewalle, *ibid.*, **87**, 4892 (1965); (e) C. Djerassi and L. Tökés, *ibid.*, **88**, 536 (1966); (f) H. Gutzwiller and C. Djerassi, *Helv. Chim. Acta*, in press.