Electron Impact Induced Fragmentation of Cholesterol and Related C-5 Unsaturated Steroids[1]

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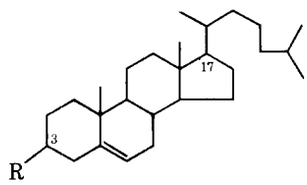
Comparison of the mass spectra of cholest-5-ene and various C-5 unsaturated 3β-hydroxy sterols indicates that the fragmentations leading to the characteristic $(M - 85)^+$ and $(M - 111)^+$ ions in these sterols are triggered solely by the double bond. With the aid of 11 deuterium labeled analogues all diagnostic cleavages of this biologically important class of compounds have now been identified. Both $(M - 85)^+$ and $(M - 111)^+$ ions of cholesterol are due to very complex fragmentations involving the loss of ring A and part of ring B by cleavages of the 1–10, 5–10 and 5–6 or 7–8 bonds, respectively, with a hydrogen transfer mainly from C-6 in the $(M - 85)^+$ ion. Fragmentations of such complexity can be revealed only with the aid of isotope labeling. The mechanisms of these fragmentations and the syntheses of the deuterium labeled compounds are discussed.

Mass spectroscopy, particularly in combination with gas chromatography, has proved to be a powerful and commonly used tool in the identification and structure elucidation of sterols.[2] Detailed knowledge of the fragmentation triggering behavior of the common functional groups on the steroid skeleton is essential, however, for reliable structure assignments by this technique.

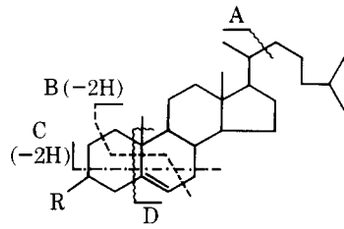
In view of our interest in the electron impact induced behavior of the unsubstituted steroid skeletons[3] and side chain unsaturated steroids[4] we decided to examine the fragmentation of cholesterol (I), which is the most common sterol lipid in tissues of fauna and a frequently used model compound for C-5 unsaturated 3β-hydroxy sterol homologues. Reports on the mass spectrum of cholesterol both as a natural product or as a material for testing instrumental performance span the history of organic mass spectroscopy. The high-mass range of its spectrum (see Figure 1) exhibits a number of diagnostically important peaks besides the common steroid fragments resulting from the loss of a methyl radical and/or water from the molecular ion. On the basis of comparative studies various interpretations have been proposed for the formation of these ions, but there has been no report on any isotope labeling study for the rigorous establishment of the cracking patterns and the mechanisms which lead to these ions.



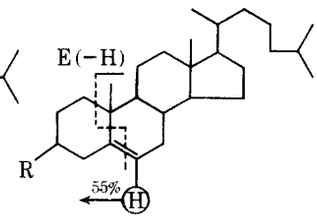
I, R = OH
II, R = H
III, R = OD

Some of the diagnostic peaks in Figure 1 are due to common steroid skeletal cleavages and have been correctly identified earlier. Such fragmentations are, for example, the loss of the C-17 side chain[5–8] (*m/e* 273), the loss of ring D with the side chain and an extra hydrogen[6–8] (*m/e* 231), and their respective dehydration products (*m/e* 255 and 213).

The prominent peaks at *m/e* 301 and 275 are more characteristic for cholesterol. At least four different cleavages have been proposed for the formation of the *m/e* 301 ion as shown on IV. The loss of C_6H_{13} from the side chain (cleavage A) was first proposed by Ryhage and Stenhagen,[6] then later by Wulfson et al.[7] and Zaretskii et al.[9] In 1967 Knights[8] suggested cracking patterns B and C without any mechanistic explanation. In 1968 the Spitellers[10] proposed cleavage C with a very reasonable mechanism to explain the loss of 85 mass units from androst-5-ene-3β,17β-diol. This was not correlated with the $M^+ - 85$ (*m/e* 301) cleavage in cholesterol, although these fragmentations are analogous. Recently, Mujtaba Naqvi[11] proposed pattern D, without any supporting evidence, mechanistic considerations, or evaluation against the earlier proposals. Our initial deuterium labeling efforts in trying to select the correct cleavage pattern indicated that, in fact, none of these proposals are valid, and in 1970 we reported[1] that the actual cleavage is E, as shown on V. The complexity of this a priori unexpected cleavage led us to examine its mechanism in more detail. While this manuscript was in preparation, Budzikiewicz and Ockels[12] confirmed this cleavage in an independent, detailed study of the fragmentation of androst-5-en-3β-ol with the aid of extensive deuterium labeling evidence. They, however, did not provide mechanistic explanation for any of the fragmentations.

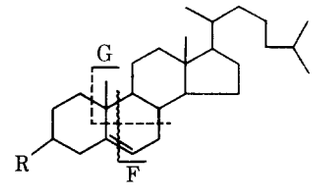


IV (*m/e* 301)



V (*m/e* 301)

The fragmentation patterns proposed to explain the genesis of the $(M - 111)^+$ ion are depicted in VI. Cleavage F was originally suggested by Friedland et al.[5] in 1959. They pointed out that "shifting of the double bond probably precedes the cleavage of the C_5–C_6 bond because a double bond would not be expected to cleave in preference to a single bond". In 1967, cleavage G was proposed independently by Knights[8] and by Zaretskii et al.[9] There was no further mechanistic explanation, isotope labeling, or other supporting evidence provided for this mode of cleavage. Our results confirmed[1] that cleavage



VI (*m/e* 275)

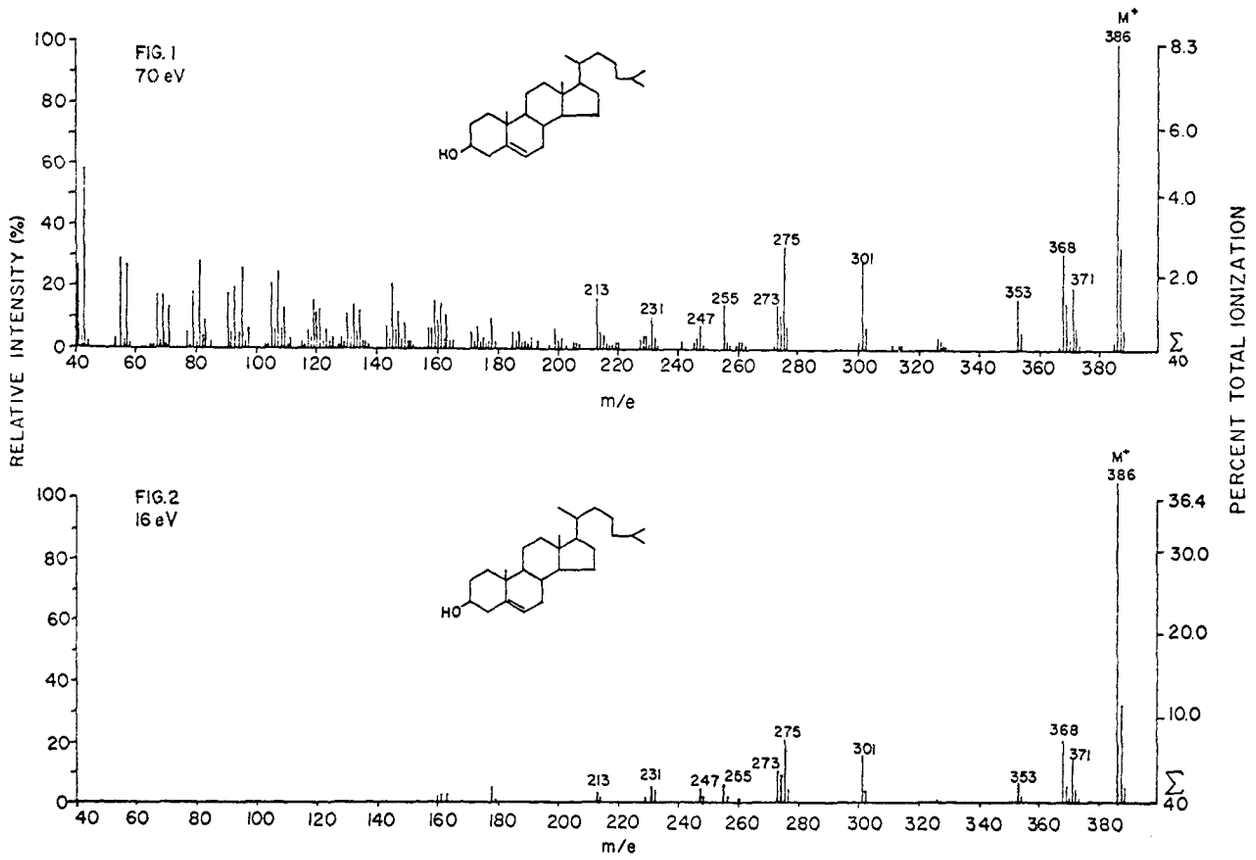
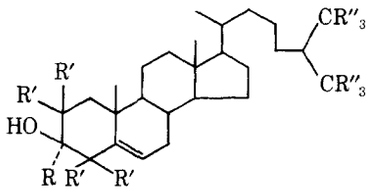


Figure 1. Mass spectrum of cholesterol (I), measured at 70 eV.
Figure 2. Mass spectrum of cholesterol (I), measured at 16 eV.

G is the correct one and this is now fully supported by the results of Budzikiewicz and Ockels in androst-5-en-3β-ol.[12]

To establish the exact cleavage patterns and to shed light on the mechanism of these fragmentations it was necessary to examine the mass spectra of various deuterium labeled analogues, which revealed some of the most complicated fragmentations encountered so far in the steroid field. The syntheses of these deuterated compounds and their spectral analyses are described in the following sections.

Syntheses of Labeled Compounds. During the course of this work the mass spectra of 11 site specifically deuterated analogues have been analyzed with labels at the following positions: C-1, 2, 3, 4, 6, 7, 8, 9, 19, 26, 27, and -OH. Of these, the preparations of 3α-d_1[13] (VII), 2,2,4,4-d_4[13] (VIII), and 26,27-d_6[14] cholesterols (IX) have been reported previously. Cholesterol-*O*-*d* (III) was readily accessible by recrystallization of cholesterol from methanol-*O*-*d*.



VII, R = D; R′ = R″ = H
VIII, R = R″ = H; R′ = D
IX, R = R′ = H; R″ = D

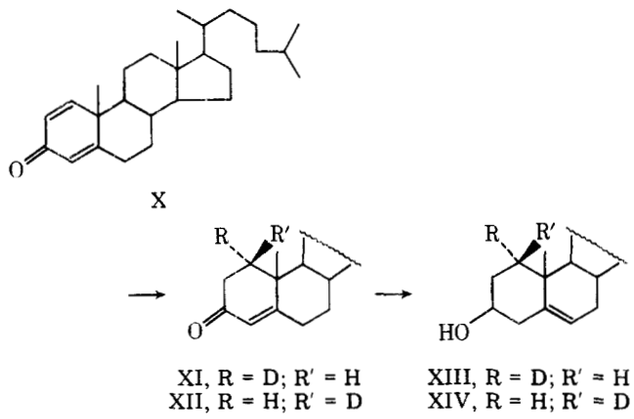
At C-1 both α and β positions had to be labeled to distinguish between the loss of this methylene group and a possible stereospecific deuterium transfer from either of these sites. Separate stereospecific labeling of the positions by homogeneous and heterogeneous catalytic deuterations of the C-1 double bond in cholesta-1,4-dien-3-one (X) proved to be the easier and ultimately more beneficial route than the preparation of cholesterol-*1,1*-d_2.

The stereochemical course of homogeneous catalytic saturation of the C-1 double bond has been established to proceed primarily (83–86%) from the α side in the tritiations of cholesta-1,4-dien-3-one[15] (X) and 17β-hydroxyandrosta-1,4-dien-3-one,[16] and in the deuteration of androsta-1,4-diene-3,17-dione.[17]

By analogy, cholest-4-en-3-one-*1*α-d_1 (XI) was prepared by the deuteration of X with Wilkinson's catalyst in benzene, followed by alkaline exchange of the C-2 deuterium. Conversely, in the presence of 5% palladium on charcoal catalyst this sequence provided the *1*β-d_1 analogue (XII) predominantly. The stereochemistry of the deuterium in XII was established by the over 80% retention of the label upon its reoxidation to cholesta-1,4-dien-3-one-*1*-d_1 with 2,3-dichloro-5,6-dicyanobenzoquinone, a step which is known to remove specifically the 1α and 2β axial hydrogens,[18] and by the characteristic C1–βD bond stretching[19] at 2180 cm^{-1} in the IR spectrum of XIV. These results are in agreement with the reported[20] 3:1 preference for β side tritiation of the C-1 double bond in the presence of palladium on charcoal in 17β-hydroxyandrosta-1,4-dien-3-one.[21]

The resulting d_1-cholestenones XI and XII on deconjugation and reduction with sodium borohydride yielded *1*α-d_1 (XIII) and *1*β-d_1 (XIV) cholesterols, respectively. These deuterio epimers showed a marked difference in the deuterium transfers from C-1 in association with the loss of water in their mass spectra (see Table I). This observation parallels the mechanism of the electron impact induced dehydration of 5α-cholestan-3β-ol[22] (vide infra), and provides supporting evidence for the stereochemical assignment of the deuteriums in XIII and XIV.

The ketone at the C-6 position in 3β-hydroxy-5α-choles-

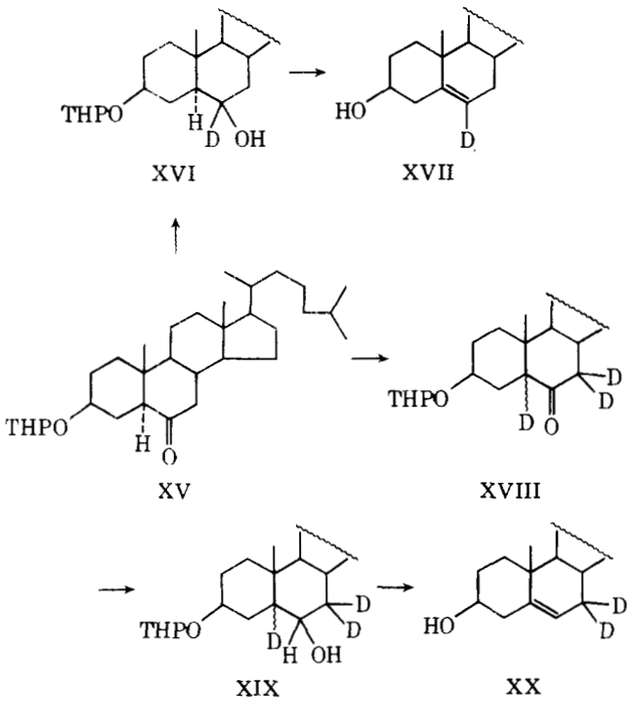


X

XI, R = D; R′ = H
XII, R = H; R′ = D

XIII, R = D; R′ = H
XIV, R = H; R′ = D

tan-6-one tetrahydropyranyl ether (XV) facilitated the deuteration of both C-6 and C-7 positions. Reduction of the carbonyl group with lithium aluminum deuteride, followed by dehydration of the resulting alcohol (XVI), and hydrolysis of the tetrahydropyranyl ether, gave cholesterol-6-d_1 (XVII) in 96% isotopic purity. This reaction sequence yielded cholesterol-7,7-d_2 (XX) when it was carried out on ketone XVIII in which the C-5 and C-7 hydrogens were exchanged with deuterium before reduction with lithium aluminum hydride.

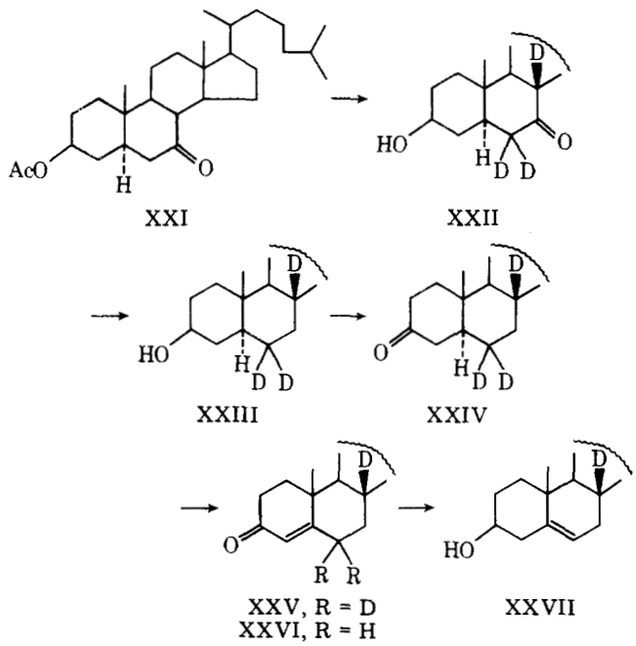


XVI XVII

XV XVIII

XIX XX

Deuteration of the 8β position was accomplished by base-catalyzed exchange of the C-6 and C-8 hydrogens in 3β-acetoxy-5α-cholestan-7-one (XXI). Owing to the slow exchange rate of the 8β hydrogen[23] this reaction required 3 days of heating to obtain sufficient isotopic purity. Reduction of the carbonyl function at C-7 in XXII was achieved without any deuterium loss by sodium borohydride treatment of its tosylhydrazone derivative.[3a,24] Oxidation of the hydroxyl function in XXIII gave cholestan-3-one-6,6,8β-d_3 (XXIV) which was converted into cholest-4-en-3-one-6,6,8β-d_3 (XXV) by bromination with pyridinium bromide perbromide,[25] followed by dehydrobromination of the 2α-bromo intermediate with lithium chloride in dry dimethylformamide.[26] Base-catalyzed back exchange of the deuteriums from C-6 yielded cholest-4-en-3-one-8β-d_1 (XXVI), which upon deconjugation and reduction with sodium borohydride gave cholesterol-8β-d_1 (XXVII) in 95% isotopic purity.



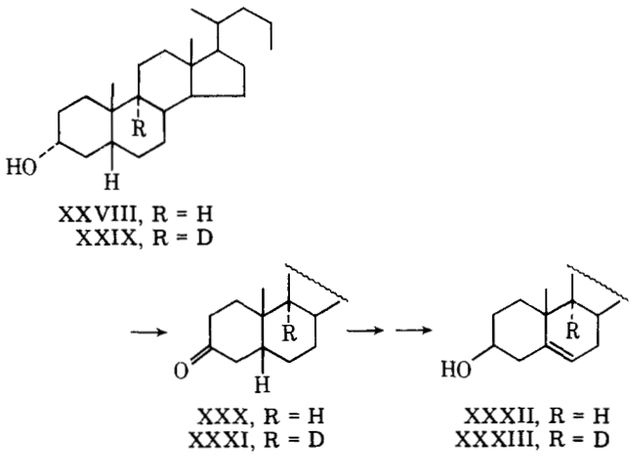
XXI XXII

XXIII XXIV

XXV, R = D
XXVI, R = H

XXVII

The preparation of a 9α-deuterated analogue was carried out in the cholane series, starting with 5β-cholan-3α-ol-9α-d_1 (XXIX) prepared by Klein and Djerassi.[27] Introduction of the C-5 double bond in both labeled (XXIX) and unlabeled (XXVIII) 5β-cholan-3α-ols was effected on their 3-keto derivatives (XXX and XXXI) by the usual bromination, dehydrobromination, deconjugation, and reduction sequence yielding chol-5-en-3β-ol-9α-d_1 (XXXIII) and the unlabeled reference compound XXXII, respectively.

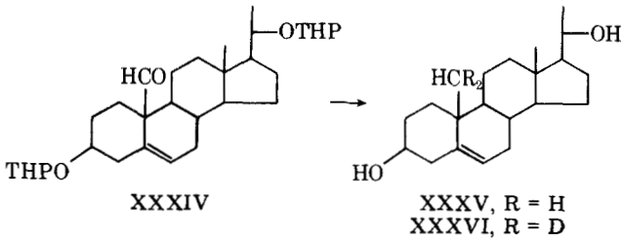


XXVIII, R = H
XXIX, R = D

XXX, R = H
XXXI, R = D

XXXII, R = H
XXXIII, R = D

Reduction of the aldehyde function in pregn-5-ene-3β,20β-diol-19-al ditetrahydropyranyl ether (XXXIV), which was available to us from other studies, provided an attractive route to a C-19 labeled analogue. Replacement of the carbonyl oxygen by two deuteriums in XXXIV, a conversion which is known to be difficult by conventional chemical means,[28] could be achieved easily by electrochemical reduction in strongly acidic medium.[29] The resulting pregn-5-ene-3β,20β-diol-19,19-d_2 (XXXVI) exhibited 94% isotopic purity, and was a



XXXIV

XXXV, R = H
XXXVI, R = D

Scheme I

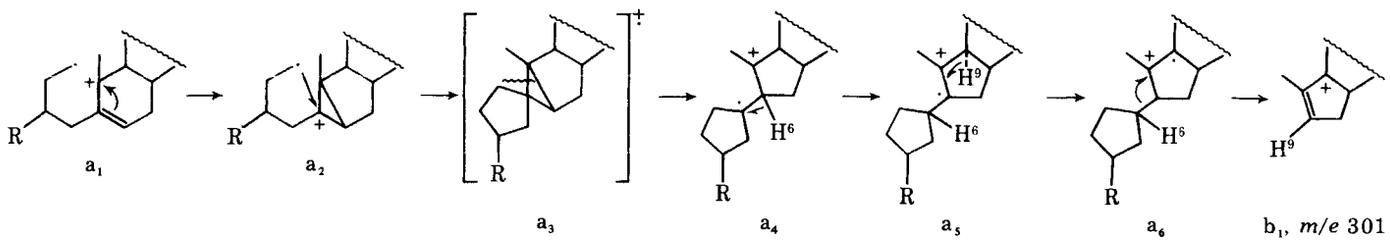


Table I. Shifts[a] of the Peaks in the Mass Spectra of Deuterium Labeled Compounds

		m/e (%)										
Registry no.	Compd	M^+	$(M - CH_3)^+$	$(M - H_2O)^+$	$[M - (CH_3, H_2O)]^+$	Ion b	Ion c	$(M -$ side chain$)^+$	$[M -$ (side chain, $H_2O)]^+$	Ion f	$[M -$ (ring D, H$)]^+$	$[M -$ (ring D, H, $H_2O)]^+$
	Cholesterols											
57-88-5	d_0 (I)	386	371	368	353	301	275	273	255	247	231	213
60816-12-8	*-O-d* (III)	387	372	368	353	301	275	274	255	247	232	213
60816-13-9	*1α-*d_1 (XIII)	387	372	369	354	301	275	274	256	247	232	214
60816-14-0	*1β-*d_1 (XIV)	387	372	369 (71) 368 (29)	354 (74) 353 (26)	301	275	274	256 (81) 255 (19)	247	232	214 (83) 213 (17)
7604-91-3	*2,2,4,4-*d_4 (VIII)	390	375	372	357	301	275	277	259	247	235	217
51467-57-3	*3α-*d_1 (VII)	387	372	369	354	301	275	274	256	247	232	214
16374-87-1	*6-*d_1 (XVII)	387	372	369 (75) 368 (25)	354 (90) 353 (10)	301 (55) 302 (45)	275	274	256 (75) 255 (25)	247	232	214 (80) 213 (20)
60816-15-1	*7,7-*d_2 (XX)	388	373	370	355	303	275	275	257	247[b]	233 (~90)	215 (~90)
60816-16-2	*8β-*d_1 (XXVII)	387	372	369 (94) 368 (6)	354 (80) 353 (20)	302	276	274	256 (83) 255 (17)	247 (55) 248 (45)	232 (~95)	214 (80) 213 (20)
60816-17-3	*26,27-*d_6 (IX)	392	377	374	359	307	281	273	255	253	231	213
5255-15-2	Chol-5-en-3β-ol (XXXII)	344	329	326	311	259	233	273	255	205	231	213
60816-18-4	*9α-*d_1 (XXXIII)	345	330	327 (75) 326 (25)	312 (83) 311 (17)	260 (93) 259 (7)	234	274	256 (84) 255 (16)	206 (56) 205 (44)	232 (92) 231 (8)	214 (67) 213 (33)
901-57-5	Pregn-5-ene-3β,20β-diol (XXXV)	318	303	300	285	233	207	273	255		231	213
20810-63-3	*19,19-*d_2 (XXXVI)	320	303 (58) 305 (42)	302 (96) 301 (4)	287 (30) 285 (70) 286[c]	235	209	275	257[b]		233[b]	215[b]

[a] Shift values are corrected for isotopic impurity as well as for ^{13}C contributions and are reliable to ±5% unless otherwise indicated. [b] Mainly at the indicated value but exact calculations were not possible owing to the low intensity of these peaks and overlap by other fragment ions. [c] A few percent of the *m/e* 285 peak was shifted to 286. The exact shift value for this peak was not calculated owing to the complexity of this group of peaks, and the uncertainty as to which of the two hydroxyl fractions is responsible for deuterium transfer from C-19.

legitimate C-19 labeled model compound for the $(M - 85)^+$ and $(M - 111)^+$ ions since these fragments were present in the mass spectra of both labeled (XXXVI) and unlabeled (XXXV) compounds.[30]

Discussion of Mass Spectral Results. The 70- and 16-eV spectra of cholesterol are reproduced in Figures 1 and 2. The shift values of the diagnostic peaks in the mass spectra of the deuterium labeled analogues are summarized in Table I.

As apparent from Figures 1 and 2, the diagnostic $(M - 85)^+$ and $(M - 111)^+$ peaks (*m/e* 301 and 275) are prominent at both high and low ionizing voltages. High-resolution analysis of these ions revealed that their elemental compositions are $C_{22}H_{37}$ and $C_{20}H_{35}$, respectively. This finding eliminates cleavage A (see IV) for the *m/e* 301 ion since the hydroxyl group is lost in both fragments. Furthermore, comparison of the mass spectra of various side chain analogues of cholesterol (see, for example, compounds IX, XXXII, and XXXV in Table I) shows that the C-17 side chain (if present) remains fully intact in both $(M - 85)^+$ and $(M - 111)^+$ ions.

Two other observations are important concerning the nature of these fragmentations. One is the presence of both *m/e* 301 and 275 peaks in the spectrum of cholest-5-ene (II),[31] in about the same relative intensity as in the spectrum of cholesterol. These peaks are absent in the spectra of the saturated analogues, 5α and 5β-cholestanes,[3c] indicating that *the cleavages leading to these ions are triggered solely by the C-5 double bond.* The other observation is that the relative significance of the $(M - 85)^+$ and $(M - 111)^+$ peaks is not decreased in the absence of the C-17 side chain (*m/e* 189 and 163 in the spectrum of androst-5-en-3β-ol[12]). This shows that the 13–17 bond, which is very cleavage prone in the presence of a C-17 side chain[3a] and is known to influence fragmentations at remote sites,[3c] remains intact during the formation of these ions.

The *m/e* 301 Ion. According to the labeling results shown in Table I, this ion lost all deuteriums at C-1, 2, 3, and 4, and retained them completely at C-7, 8, 19, 26, and 27. These results are incompatible with all four cleavages (A–D on IV) proposed earlier and are indicative of a complex cracking pattern which involve scissions of the 1–10, 5–10, and 5–6 bonds with a hydrogen transfer from the charge retaining side as shown in V. To complicate matters even further, this hydrogen transfer involves several sites, the most important (55%) being C-6. This is a priori the least expected site since it involves a vinylic hydrogen on a carbon which formally also has to cleave its double bond to C-5. An additional 7% transfer was observed from the 9α position, leaving 38% unaccounted for. This may come from one or more of the hitherto unlabeled positions, C-14 being a very likely one.

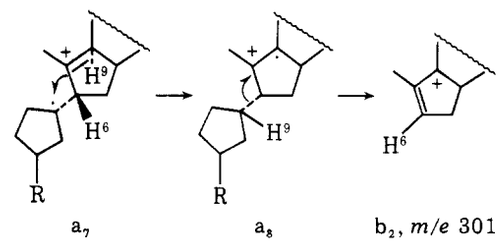
On mechanistic grounds this cleavage poses some problems. Formally, it involves the ruptures of two carbon–carbon bonds of C-10, and double bond as well as single bond of C-5, in addition to the unexpected hydrogen transfer from C-6. A fragmentation of such complexity can be considered as prima facie evidence for the participation of extensive skeletal rearrangements.[32] A possible mechanistic explanation for the major fragmentation pathway which involves the transfer of the C-6 hydrogen is depicted by Scheme I.

The sequence $a_1 \rightarrow a_4$ in Scheme I is apparently common for the formation of both $(M - 85)^+$ and $(M - 111)^+$ ions (vide infra). In essence, the transformation of a_1 into a_3 involves a shift of the 1–10 bond to the carbonium ion at C-5 and bond formation between the isolated radical and ionic sites (C-6 and C-10). This transformation can be synchronous[33] or stepwise as shown in Scheme I. Spirostane skeletons with three- and five-membered rings, similar to molecular ion a_3, are well-known photolysis products of conjugated steroidal dienones and trienes.[34]

The final steps in Scheme I, involving scissions of the 5–10 and 5–6 bonds with a concomitant hydrogen transfer from the charge retaining side ($a_3 \rightarrow b_1$), is a typical fragmentation of cyclopropyl hydrocarbons.[35] Opening of the most substituted bond in a_3 (5–10 bond) and a 1,2 hydrogen shift from the doubly activated C-6 position to C-5 in ion radical a_4 lead to an ionized olefin a_5. Such ionized olefins are well-documented intermediates in the fragmentation of steroidal hydrocarbons.[36] The loss of the cyclopentyl radical in a_5 is analogous to the loss of vinylic alkyl substituents from cyclic olefins,[35-37] and is probably preceded by a 1:3 hydrogen shift from the activated 9α position to facilitate the homolysis of the 5–6 bond.

The minor part of this fragmentation, involving the 7% hydrogen transfer from the 9α position, may proceed also via molecular ion a_4. Steps $a_1 \rightarrow a_4$ can lead to two stereoisomers at C-6, both of which can yield a_5 with equal facility. In the stereoisomer of a_4 with the cyclopentyl group on the α side (a_7) hydrogen transfer to C-5 may occur also from the activated 9α position. This route yields fragment ion b_2, via an ionized olefin a_8 (Scheme II). These are essentially identical with ions

Scheme II

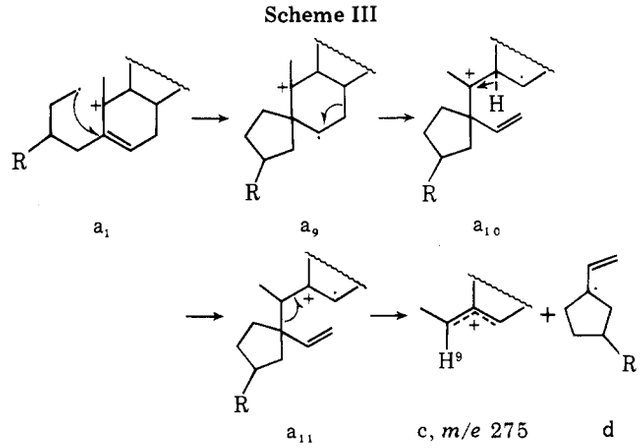


a_7 a_8 b_2, *m/e* 301

a_6 and b_1 in Scheme I, the difference being only the origin of the hydrogens at C-5 and C-6. The eightfold preference for the longer fragmentation sequence $a_4 \rightarrow b_1$, as compared to $a_7 \rightarrow b_2$, is probably due to the relative facility of the hydrogen transfers involved. Judging from Dreiding models, the minimum internuclear distance between the 9α hydrogen and the carbon radical is less in a_5 (2.8 Å) than in a_7 (3.2 Å). Actually, both of these distances are quite large compared to the <1.8 Å internuclear distance criterion for bond formation between hydrogens and carbonyl oxygens in the McLafferty rearrangement,[38] but analogous internuclear distance requirements for carbon–hydrogen bond formation have not been established as yet.

The *m/e* 275 Ion. The labeling results in Table I indicate that the $C_7H_{11}O$ fragment expelled in forming this ion encompasses C-1 to C-7, without any detectable hydrogen transfer. These findings are in complete agreement with cleavage G (see VI) and they invalidate cleavage F. A possible mechanism for this fragmentation is shown in Scheme III.

Scheme III



a_1 a_9 a_{10} a_{11} c, *m/e* 275 d

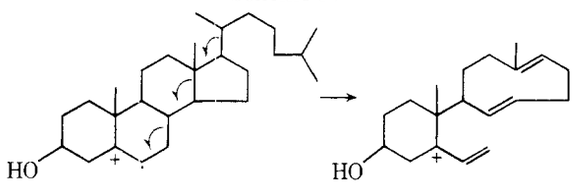
Bond formation between C-1 and C-5 in molecular ion a_1 relieves the C-1 primary radical while forming an isolated

secondary radical in a_9. This radical can either form a bond with C-10 (see a_3), or can trigger the cleavage of the 7–8 bond, forming an olefin a_{10} with a new isolated radical at C-8. Migration of the doubly activated 9α hydrogen to the adjacent carbonium ion gives an ionized diene a_{11} in which homolysis of the 5–10 bond yields the allylic ion c (*m/e* 275) and a conjugated radical d.

The origin of the *m/e* 301 and 275 fragments from the molecular ion has been confirmed by the observed intense metastable peaks corresponding to these transitions in the pure metastable spectrum of cholesterol, using the method of Jennings.[39] Weak metastable peaks corresponding to the formation of these fragments from the $(M - H_2O)^+$ peak were also detected.

The *m/e* 273 and 255 Ions. The labeling results (Table I) substantiate earlier proposals[5-8] that these ions are due to the losses of the C-17 side chain (*m/e* 273) and the combination of the side chain and a molecule of water (*m/e* 255). These are common steroid fragmentations, although they are relatively insignificant in the spectra of 5α- and 5β-cholestanes,[3a,c] or 5α-cholestan-3β-ol.[22] The considerable intensity of these ions in Figure 1 indicates that, by analogy to Djerassi's proposal[31] for cholest-5-ene (II), the side chain loss in cholesterol is enhanced by the C-5 double bond as shown in Scheme IV.

Scheme IV

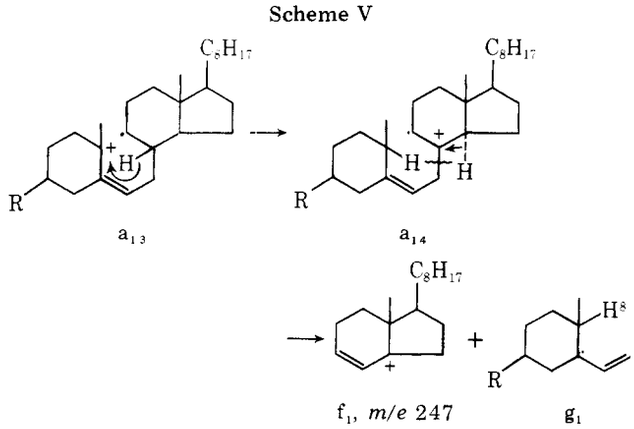


a_{12} e, *m/e* 273

The *m/e* 247 Ion. Friedland et al.[5] attributed this ion to the loss of rings A and B plus an extra hydrogen by cleavage of the 7–8 and 9–10 bonds. This proposal has been generally accepted and is confirmed now by our labeling results, which also establish that the sources of the extra hydrogen are the 8β (55%) and 9α (44%) positions. It is noteworthy that once again the hydrogen transfer sites are carbon atoms which undergo skeletal bond cleavage as well.

Apparently, two different cleavages are participating in the formation of this ion. One cleavage may be initiated by the rupture of the allylic 9–10 bond, forming molecular ion a_{13} which can undergo facile hydrogen shift from the activated 8β position to C-10 in a six membered transition state. By analogy to the fragmentation of other ionized olefin intermediates,[36] a 1,2 shift of the 14α hydrogen to C-8, followed by fission of the 7–8 bond leads to ion f_1 (*m/e* 247) and radical g_1, both of which are allylically stabilized (Scheme V).

Scheme V

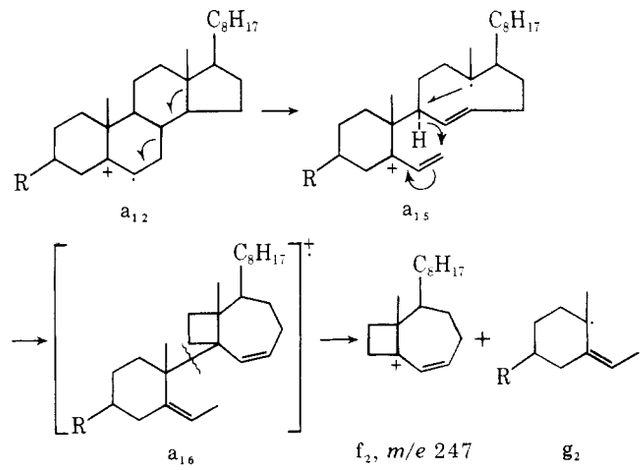


a_{13} a_{14}

f_1, *m/e* 247 g_1

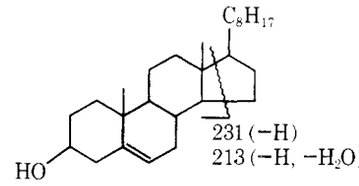
The second cleavage, which involves the loss of the 9α hydrogen, may start with the simultaneous rupture of both 7–8 and 13–14 bonds as shown in Scheme VI. In this case, however, instead of losing the C-17 side chain as in $a_{12} \rightarrow$ e, vide supra, hydrogen transfer occurs through a six-membered transition state from the allylic 9α position to C-7. This step may be further assisted by bond formation between the isolated C-13 radical and C-9 yielding a_{16}. Cleavage of the doubly allylic 9–10 bond then leads to ion f_2 (*m/e* 247) and radical g_2.

Scheme VI



a_{12} a_{15} a_{16} f_2, *m/e* 247 g_2

The *m/e* 231 and 213 Ions. The *m/e* 231 ion and its dehydration product (*m/e* 213) are generated by the loss of ring D with the side chain and an extra hydrogen (see XXXVII), which is a characteristic fragmentation of C-17 side chain bearing steroids.[3a,40] Consequently, these ions are of negligible intensity in the spectrum of androst-5-en-3β-ol.[12] Detailed study of this cleavage in the hydrocarbon series revealed that the origin of the extra hydrogen is random, involving the 7, 8β, 9α, 12, 14α, and 18 positions, the 14α position being the major contributor.[3a] The present labeling results (Table I) are in agreement with these earlier reports.



XXXVII

The Loss of Water and Methyl Radical. The labeling results in Table I shed light also on the complexity of the mechanism of electron impact induced loss of water in the genesis of the *m/e* 368 $(M - H_2O)^+$, 353 $[M - (CH_3, H_2O)]^+$, 255 $[M - (\text{side chain}, H_2O)]^+$, and 213 $[M - (\text{ring D}, H_2O)]^+$ ions. Karliner, Budzikiewicz, and Djerassi reported[22] that 5α-cholestan-3β-ol lost 28% deuterium from the 1β position and 11% from the 5α position in the $[M - (CH_3, H_2O)]^+$ fragment while all labels were retained at the 1α, 2, 3α, and 4 positions. The remaining 61% hydrogen transfer was unaccounted for. By contrast, the corresponding 3α-hydroxy compound lost mainly the 5α label (73%) and only small amounts from the 1α (4.5%) and, possibly, from the 1β (2.5%) positions.

In cholesterol the stereospecific loss of 26–29% deuterium from the 1β position and the lack of any other transfer from positions 1–4 resemble closely those reported for 5α-cholestan-3β-ol. It is evident, however, from our results that in the presence of a C-5 double bond the major transfer sites include remote positions also, such as the 6 (10–25%), 8β (6–20%), 9α (17–25%), and probably the C-19 positions. The sum of these transfers in cholesterol accounts for about 89% and over 73% of the hydrogen losses in the $(M - H_2O)^+$ and $[M - (CH_3,$

$H_2O)]^+$ ions, respectively. Similar transfers were also apparent in the *m/e* 255 and 213 ions.

Extensive hydrogen transfer (80%) from the distant 9α position during the dehydration of a C-3 hydroxyl group has been reported with 5β-cholan-3α-ol by Klein and Djerassi.[27] There was no transfer from this site in the corresponding 3β hydroxy epimer. These observations were readily explainable by the spatial relationship between the hydroxyl group and the potential hydrogen transfer sites in the intact molecules.[41] In the 5β series with ring A in the boat conformation only the 3α hydroxyl group is in the proper spatial arrangement for hydrogen transfer from the 9α position. In cholesterol, however, there is no possibility for the 3β-hydroxyl group to approach the 6, 8β, and 9α positions and, therefore, skeletal cleavages must precede the hydrogen transfers from these sites.

The complete retention of the deuterated C-26 and C-27 terminal methyl groups in IX and the 58% loss of the labeled C-19 methyl function in XXXVI provide supporting evidence for the earlier reports that the 18 and 19 angular methyl groups are the exclusive source of the expelled methyl radicals in the $(M - CH_3)^+$ process in androstane[3b] and pregnane.[3a]

In conclusion, the characteristic $(M - 85)^+$ and $(M - 111)^+$ fragmentations of 3β-hydroxy Δ^5-steroids are triggered by the C-5 double bond and are present in the spectra of compounds lacking any strong fragmentation triggering functions in ring A or other parts of the molecule. Consequently, the corresponding fragment ions are detected in the spectra of Δ^5-steroids with or without a hydroxy or keto group at C-3, but are absent or very weak when an acetoxy,[8,42] silyloxy,[8,13] or double bond[6,42] function is present at C-3. These fragmentations are also hindered by the presence of alkyl substituents at C-4. In these compounds the molecular ions which are analogous to a_1 undergo a different rearrangement which involves migration of the hydroxyl group to C-6.[43]

Experimental Section

The mass spectra were measured on Varian MAT CH-7 and CH-4 (equipped with an EFO-4B ion source) and AEI MS-902 mass spectrometers at 70 eV ionizing voltage unless otherwise stated. The high-resolution measurements were carried out on the AEI MS-902 mass spectrometer at a resolution of 10 000 (10% valley) and ±5 ppm accuracy. The IR spectra were recorded on a Perkin-Elmer Model 237 Infracord spectrometer. The NMR spectra were measured on a Varian HA-100 spectrometer using tetramethylsilane as internal reference. The melting points are uncorrected.

Isotope composition of labeled compounds follows.

Cholesterol-1α-d_1 (XIII): d_0 3%, d_1 87%, d_2 10%. Cholesterol-1β-d_1 (XIV): d_0 23%, d_1 76%, d_2 1%. Cholesterol-2,2,4,4-d_4[13] (VIII): d_1 4% d_2 15%, d_3 42%, d_4 39%. Cholesterol-3α-d_1[13] (VII): d_0 2%, d_1 98%. Cholesterol-6-d_1 (XVII): d_0 4%, d_1 96%. Cholesterol-7,7-d_2 (XX): d_0 5%, d_1 55%, d_2 38%, d_3 2%. Cholesterol-8β-d_1 (XXVII): d_0 3%, d_1 95%, d_2 2%. Chol-5-en-3β-ol-9α-d_1 (XXXIII): d_0 11%, d_1 86%, d_2 3%. Pregn-5-ene-3β,20β-diol-19,19-d_2 (XXXVI): d_1 6%, d_2 94%. Cholesterol-26,27-d_6[14] (IX): d_2 1%, d_3 5%, d_4 8%, d_5 23%, d_6 63%. Cholesterol-O-d (III): d_0 16%, d_1 84%.

Acknowledgments. We wish to thank Professor C. Djerassi of Stanford University for the generous supply of labeled and unlabeled 5β-cholan-3α-ol samples (XXVIII and XXIX), and Dr. A. F. Hofmann of Mayo Clinic for a sample of cholesterol-26,27-d_6 (IX).

Registry No.— X, 566-91-6; XI, 60816-19-5; XII, 60816-20-8; XV, 58704-09-9; XVI, 60816-21-9; XVIII, 60816-22-0; XIX, 60816-23-1; XXI, 6038-71-7; XXII, 60816-24-2; XXII tosylhydrazone, 60816-25-3; XXIV, 60816-26-4; XXIV 2-bromo, 60816-27-5; XXV, 60816-28-6; XXVI, 60816-29-7; XXVIII, 5352-77-2; XXIX, 42921-50-6; XXXI, 60816-30-0; XXXIV, 60816-31-1; cholest-4-en-3-one-1,2-d_2, 60816-32-2; cholesta-1,4-dien-3-one-1-d_1, 60816-33-3; cholesterol THP ether-6-d_1, 60840-36-0; *p*-toluenesulfonhydrazide, 1576-35-8; pyridinium bromide perbromide, 39416-48-3; chol-4-en-3-one-9α-d_1, 60816-34-4.

Supplementary Material Available. Experimental part for the preparation of deuterium labeled compounds (4 pages). Ordering information is given on any current masthead page.

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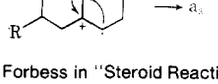

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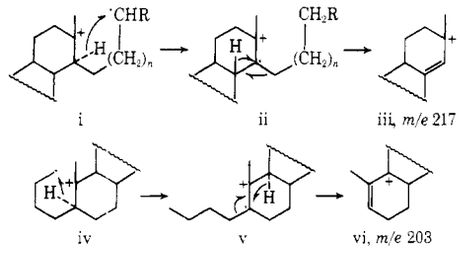

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The Effect of the Trimethylsilylmethyl Substituent on Ketene Cycloadditions

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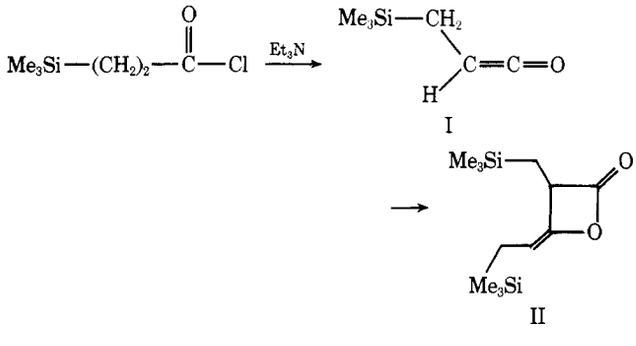
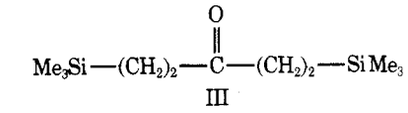
(Trimethylsilylmethyl)ketene is prepared by the dehydrochlorination of β-trimethylsilylpropionyl chloride. The 2-oxetanone dimer of the ketene is readily converted to 1,5-bis(trimethylsilyl)-3-pentanone. The ketene readily undergoes in situ cycloaddition to cyclopentadiene to yield only the *endo*-trimethylsilylmethylcyclobutanone and cycloaddition to ethyl vinyl ether yields only the trans cyclobutanone. Vinyltrimethylsilane would not undergo cycloaddition with a variety of ketenes. However, allyltrimethylsilane readily underwent cycloaddition with methylchloro- and dichloroketenes. An interpretation of these results is offered.

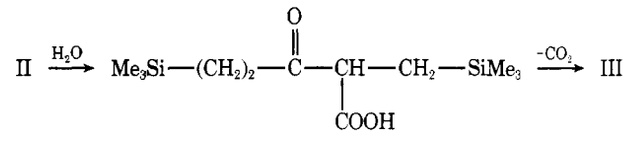
The effect of the trimethylsilyl substituent on the properties and chemistry of trimethylsilylketene is truly remarkable. This aldoketene is very stable, does not dimerize upon heating, and can be stored for long periods of time.[1,2] Numerous efforts to effect cycloaddition of trimethylsilylketene with a variety of unsaturated compounds have been mostly unsuccessful; the only cycloaddition which has been reported was with dimethyl and diethyl acetal of ketene under rather vigorous conditions for a ketene cycloaddition.[3] Also, condensation of trimethylsilylketene with benzaldehyde gave *cis*- and *trans*-trimethylsilylstyrene which presumably involved cycloaddition to form the 2-oxetanone which underwent decarboxylation to yield the olefins.[4] We have recently reported on the preparation and cycloaddition of trimethylsilylbromoketene and this ketene appears to be more reactive in cycloaddition reactions than trimethylsilylketene, although only cycloadducts with an imine and carbodiimide have been prepared.[5]

In this report we describe the effect of the trimethylsilylmethyl substituent on the properties and chemistry of (trimethylsilylmethyl)ketene and also describe the effect of the trimethylsilyl substituent and the trimethylsilylmethyl substituent on the reactivity of the olefin in ketene cycloaddition reactions.

(Trimethylsilylmethyl)ketene (I) was prepared by the triethylamine dehydrochlorination of β-trimethylsilylpropionyl chloride in hexane as evidenced by a band in the infrared at 2123 cm.$^{-1}$ The ketene was not isolable but underwent dimerization to yield the expected dimer, II. This dimer was accompanied by an unexpected product, 1,5-bis(trimethylsilyl)-3-pentanone (III). The formation of III was quite







puzzling; however, it was established that this ketone was formed from the 2-oxetanone dimer. The dimer was hygroscopic and would slowly react with atmospheric moisture yielding the keto acid which decarboxylated to the ketone. Normal drying tube precautions were not sufficient to keep II from being hydrolyzed.

The β-trimethylsilylpropionyl chloride was prepared from vinyltrimethylsilane by the addition of hydrogen bromide in the presence of benzoyl peroxide, Grignard formation, carbonation, hydrolysis, and acid halide formation with thionyl chloride. In some original preparations, II and III were also accompanied by 1,4-bis(trimethylsilyl)butane. This was the result of a coupling reaction in the Grignard step and this coupled product codistilling with β-trimethylsilylpropionyl chloride. Careful distillation of the β-trimethylsilylpropionic acid eliminated this problem.

An alternate route to I would be the zinc dehalogenation of α-halo-β-trimethylsilylpropionyl chloride. Attempts to α-halogenate β-trimethylsilylpropionyl chloride were unsuccessful owing to cleavage of the carbon–silicon linkage.