Effect of Additional Double Bonds on the Mass Spectrometric Fragmentations of Δ^4-3-Keto Steroids[1-3]

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The introduction of additional conjugated double bonds into the medicinally and biologically important Δ^4-3-keto steroids alters their mass spectrometric behavior. Carbon atoms 2, 4, 8, 9, 11, 12, 14–17, and 19–21 of the $\Delta^{4,6}$- and $\Delta^{1,4,6}$-3-keto steroids were individually labeled with deuterium in order to elucidate the course of the diagnostic and mechanistically surprising ring B cleavages of these compounds. Such information is essential for both clinical analyses and structure elucidations of steroids by mass spectrometry. The 8β-hydrogen atom consistently proved to be the major contributor to the hydrogen migrations accompanying B-ring scission. The labeling data also demonstrated that two characteristic C-ring cleavages both proceed with reciprocal hydrogen transfers. Mechanisms are postulated to explain these fragmentations as well as the unexpected elimination of C_2H_4O instead of ketene from the $\Delta^{4,6}$-3-keto steroids.

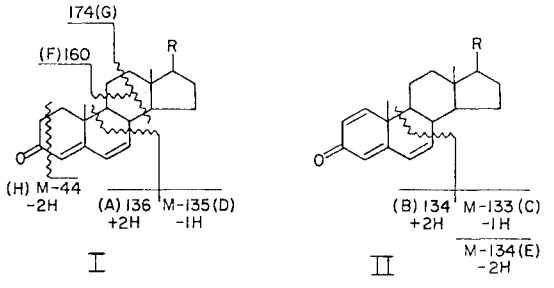
The enormous utility of mass spectrometry for the analysis of samples of biological origin is universally recognized. The mass spectrometer not only is of paramount importance in the identification of novel natural products[4] but also serves as a powerful tool in clinical pharmacology.[5] The availability of only minute quantities or the presence of complex mixtures often precludes other spectral or physical characterization of a sample, and thus the analysis depends solely upon gas chromatography–mass spectral measurements. However, the ability to make an unambiguous structural assignment on the basis of such data demands a knowledge of the mechanisms by which particular structural features of a molecule give rise to characteristic fragmentations.

Since the steroids constitute such an important class of natural products, considerable attention has been devoted to their mass spectrometric behavior. We previously reported[6] a mechanistic study of the highly diagnostic B-ring cleavage of Δ^4-3-keto steroids. A cursory examination of the mass spectra of some $\Delta^{4,6}$-3-keto steroids isolated from marine organisms[7] and insects[8] revealed that these compounds also suffer facile fragmentation of ring B—despite the a priori prediction that the Δ^6 double bond would inhibit such a cleavage. Scant attention has been given to the electron impact induced decompositions of such steroidal dienones[9] even though the $\Delta^{4,6}$-3-ketone moiety is a characteristic feature of several medicinally important progestogens and is also present in the potassium sparing steroid diuretic potassium canrenoate.[10,11]

We had previously found[6] that the introduction of a Δ^1 double bond into the skeleton of Δ^4-3-keto steroids simplified and enhanced the characteristic B-ring fragmentation occurring upon electron impact. Thus we found it interesting that the heretofore unstudied $\Delta^{1,4,6}$-3-keto steroids exhibited markedly different mass spectral behavior from their $\Delta^{4,6}$ counterparts. These trienones serve as valuable intermediates for the synthesis of vitamin D and its metabolites.[12] Furthermore, recent studies have demonstrated that corticosteroids possessing the $\Delta^{1,4,6}$-3-ketone moiety[13] show promising activity as antiinflammatory drugs.[14]

We were thus prompted to initiate a comprehensive investigation into the electron impact induced cleavages of the $\Delta^{4,6}$- and $\Delta^{1,4,6}$-3-keto steroids. The mass spectra of three representative dienones and trienones are presented in Figures 1 and 2, respectively. The spectra in each figure demonstrate that the characteristic fragmentations of these compounds are dictated primarily by the α,β-unsaturated ketone group and are thus consistent from one class of steroid to another. Hence the conclusions reached from an investigation of these relatively simple steroids should also facilitate an analysis of the mass spectra of at least some of the more highly oxygenated analogues which might be encountered in practice.

Spectra of Compounds. The most prominent peaks in the mass spectrum of 4,6-androstadien-3-one (1)[15] are m/z 135 (M − 135) and m/z 136. These ions result from scission of the 9–10 and 7–8 bonds of ring B with charge retention by either the hydrocarbon or oxygen-containing fragment, respectively (drawing I). Less pronounced but



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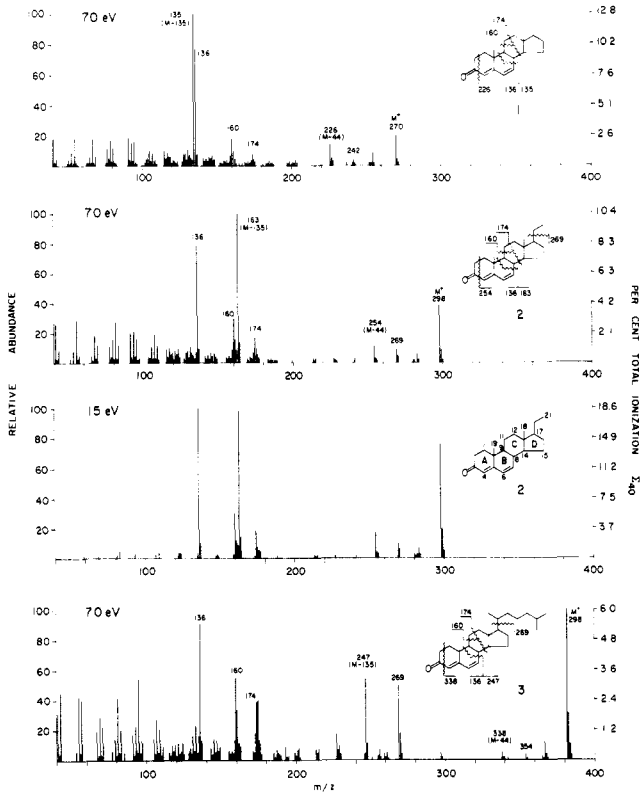

Figure 1. Mass spectra of $\Delta^{4,6}$-3-keto steroids.

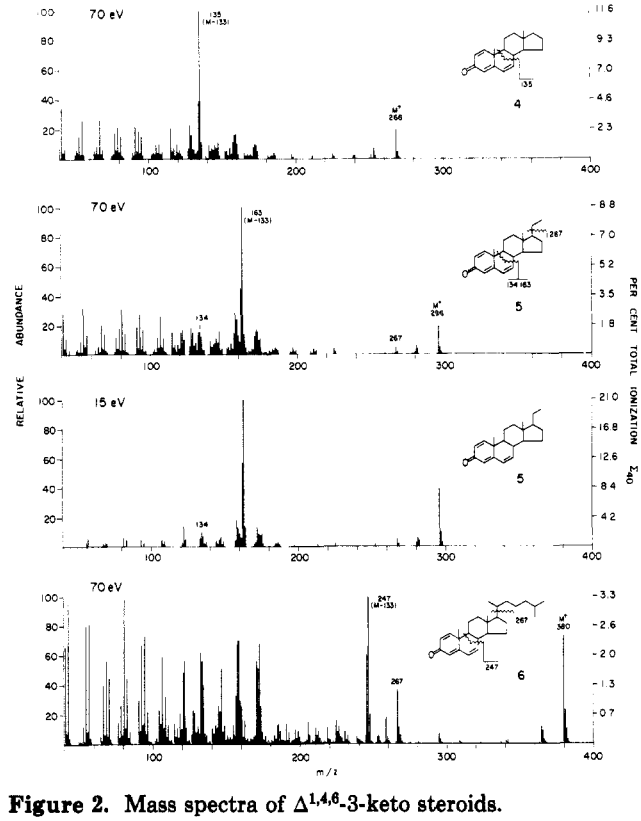

Figure 2. Mass spectra of $\Delta^{1,4,6}$-3-keto steroids.

still significant peaks, which increase in intensity at low voltages, are present at m/z 160 and 174. These fragments arise from loss of the D ring by scission of the 8–14 bond and either the 9–11 or 11–12 bonds, respectively. In the high-mass region of the spectrum a characteristic ion is observed at m/z 226 (M – 44; loss of C_2H_4O). All of these diagnostic peaks also appear in the spectra of the heretofore unknown 4,6-pregnadien-3-one (**2**) and of 4,6-cholestadien-3-one (**3**),[16] with the additional appearance of ions due to the loss of the respective C-17 side chains.

Table I. Metastable Defocusing Data[a]

compd	m/z for daughter ion	m/z for parent ion[b]
4,6-cholestadien-3-one	338	382, M
	247	382 (90), M 367 (10), M – CH_3
	174	382 (80), M 269 (10), M – C_8H_{17}
	160	382 (60), M 175 (20), $C_{12}H_{15}O$ 269 (10), M – C_8H_{17}
	136[c]	382 (70), M 247 (20), fragment D[d] 269 (10), M – C_8H_{17}
4,6-androstadiene-3,17-dione	136	284, M
1,4,6-cholestatrien-3-one	246, 247	380, M

[a] The data were obtained with an AEI MS-9 spectrometer by scanning the accelerating voltage with fixed electric sector voltage and fixed magnetic field. The results are indicative only of processes occurring in the first field-free region of the mass spectrometer. [b] The figures in parentheses (in percent) indicate the approximate relative intensities of the metastable peaks for each daughter ion. Peaks of intensity < 5% are not given. [c] The multiple origins of this ion were corroborated on a Varian MAT-711 spectrometer. [d] This ion must be the progenitor of the hydrocarbon species $C_{10}H_{16}$ shown by high-resolution measurements to compose 1% of the m/z 136 ion.

The most striking differences in the spectra of the trienones are the absence of significant peaks at m/z 134 (B-ring cleavage with charge retention by the oxygen-containing fragment) and M – 44 (loss of C_2H_4O) which were characteristic of the $\Delta^{4,6}$ analogues. The base peak at m/z 135 (M – 133) in the spectrum of 1,4,6-androstatrien-3-one (**4**)[17] is due to rupture of the 9–10 and 7–8 bonds with charge retention by the hydrocarbon portion (drawing II). An analogous ion, m/z 134 (M – 134), containing one less hydrogen atom, is also present. These two peaks are also observed in the spectra of 1,4,6-pregnatrien-3-one (**5**)[18] and 1,4,6-cholestatrien-3-one (**6**)[19] and constitute essentially the only diagnostic fragment ions of the trienones. The identities and origins of these species were corroborated by high-resolution mass measurements and metastable defocusing experiments (Table I).

Hydrogen migrations play a pivotal role in the B-ring cleavages of both the $\Delta^{4,6}$- and the $\Delta^{1,4,6}$-3-ketones and in the formation of the M – 44 ion. These complex processes were probed by means of extensive deuterium labeling of the steroid nucleus. The labeling work also demonstrated, quite unexpectedly, that the two ring C fragmentations observed for the dienones (m/z 160 and 174) do not occur by simple, direct cleavage but rather involve reciprocal hydrogen transfers.

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Table II. Peak Shifts[a] in the Mass Spectra of Deuterated $\Delta^{4,6}$-3-Keto Steroids

compd	isotopic comp, %	m/z values for ions (% shift[b] resulting from labeling)				
		$C_9H_{12}O$ (A)	M − $C_9H_{11}O$ (D)	$C_{11}H_{12}O$ (F)	$C_{12}H_{14}O$ (G)	M − C_2H_4O (H)
3-d_0		136	247	160	174	338
3-2,2,4-d_3	76 d_3	139	247	163	177	338 (96)
	14 d_2					339 (4)
	10 d_4					
3-8β-d_1	88 d_1	137 (76)	247 (48)	160 (59)	174 (50)	339
	11 d_2	136 (24)	248 (52)	161 (41)	175 (50)	
	1 d_0					
3-9α-d_1	87 d_1	136	248	160 (87)	174 (72)	338 (76)
	13 d_0			161 (13)	175 (23)	339 (24)
3-11,11-d_2	94 d_2	137 (15)	249	160	176	340
	3 d_1	136 (85)				
	3 d_0					
3-12,12-d_2	69 d_2	137 (12)	249	160	174	340
	28 d_1	136 (88)				
	3 d_0					
3-14α-d_1	95 d_1	137 (22)	247 (51)	161 (54)	175 (52)	339
	5 d_0	136 (78)	248 (49)	160 (46)	174 (48)	
3-19,19-d_2	90 d_2	138	247	162	176	340
	6 d_0					
	4 d_1					
2-d_0		136	163	160	174	254
2-15,15,17α-d_3	95 d_3	137 (53)	166	160	174	257
	5 d_2	136 (47)				
2-16,16-d_2	75 d_2	137 (4)	165	160	174	256
	18 d_1	136 (96)				
	7 d_1					
2-17α,21,21,21-d_4	79 d_4	137 (36)	167	160	174	258
	16 d_3	136 (64)				
	4 d_2					
	1 d_1					
2-17α-d_1	97 d_1	137 (12)	164	160	174	255
	3 d_0	136 (88)				
2-20,20-d_2	40 d_2	137 (4)	165	160	174	256
	29 d_3	136 (96)				
	19 d_4					
	7 d_5					
	3 d_1					
	2 d_6					
total % of migrating hydrogens[c] accounted for		210	99	200	174	172

[a] The shift values have been corrected for natural isotope abundance and deuterium composition. [b] These figures are reliable to ±5%. [c] A maximum value of 200% is expected for a process involving two hydrogen transfers.

Synthesis of Deuterated Compounds. During the course of this investigation steroids labeled with deuterium at carbons 2, 4, 8, 9, 11, 12, 14–17, and 19–21 were prepared. The isotopic incorporation for each of the final α,β-unsaturated ketones is given in Tables II and III.

4,6-Cholestadien-3-one-14α-d_1 (3-14α-d_1) was prepared by dehydrobromination of the correspondingly labeled 2,4-dibromocholestanone as we previously described.[6] The C-11 and C-12 labeled cholestadienones (3-11,11-d_2 and 3-12,12-d_2) were synthesized in exactly the same manner from the deuterated cholestanones,[20] which were available from an earlier investigation. Dehydrogenation of 4-cholesten-3-one-8β-d_1[6] with chloranil[21] afforded 3-8β-d_1. The preparation of 4,6-cholestadien-3-one-2,2,4-d_3 has been described previously.[22]

The C-9 and C-19 deuterated 4,6-cholestadien-3-ones (3-9α-d_1 and 3-19,19-d_2) and all of the labeled 4,6-pregnadien-3-ones (2-16,16-d_2, 2-15,15,17α-d_3, 2-17α,21,21,21-d_4, 2-17α-d_1, and 2-20,20-d_2) were obtained by manganese dioxide oxidation[23] of the corresponding Δ^5-3β-alcohols.[24] In addition, these labeled Δ^5-3β-alcohols could be trans-

Scheme I

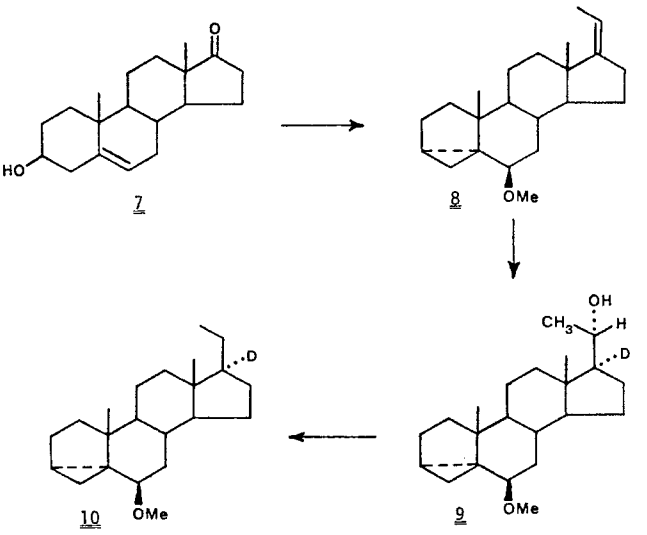

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Table III. Peak Shifts[a] in the Mass Spectra of Deuterated $\Delta^{1,4,6}$-3-Keto Steroids

compd	isotopic comp, %	m/z values for ions (% shift[b] resulting from labeling) M − C_9H_9O (C)	M − $C_9H_{10}O$ (E)
6-d_0		247	246
6-8β-d_1	88 d_1	247 (57)	246 (73)
	8 d_2	248 (43)	247 (27)
	4 d_0		
6-*11,11*-d_2	95 d_2	248 (7)	247 (11)
	3 d_1	249 (93)	248 (89)
	2 d_0		
6-*12,12*-d_2	69 d_2	249	247 (6)
	28 d_1		248 (94)
	3 d_0		
6-*14*α-d_1	94 d_1	247 (39)	246 (27)
	6 d_0	248 (61)	247 (73)
5-d_0		163	162
5-*15,15-17*α-d_3	94 d_3	166	164 (53)
	6 d_2		165 (47)
5-*17*α-d_1	97 d_1	164	162 (18)
	3 d_0		163 (82)
5-*17*α,*21,21,21*-d_4	79 d_4	167	165 (18)
	16 d_3		166 (82)
	4 d_1		
	1 d_2		
5-*20,20*-d_2	40 d_2	165	164
	29 d_3		
	19 d_4		
	7 d_5		
	3 d_1		
	2 d_6		
total % of migrating hydrogens[c] accounted for		103	170

[a-c] See corresponding footnotes in Table II.

formed with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to the desired $\Delta^{1,4,6}$-3-ketones.[25] Compounds 6-*12,12*-d_2, 5-*15,15,17*α-d_3, 5-*17*α-d_1, and 5-*20,20*-d_2 were obtained in this manner. The syntheses of 5-pregnen-3β-ol-*16,16*-d_2, -*15,15,17*α-d_3, and -*17*α-*21,21,21*-d_4 are outlined in our previous paper,[6] and 5-pregnen-3β-ol-*20,20*-d_2 has been made by Throop and Tökés.[26] Other reports from this laboratory describe the preparations of cholesterol-9α-d_1 and -*12,12*-d_2[20] as well as cholesterol-*19,19*-d_2.[27]

A 17α-deuterium atom was introduced (Scheme I) via deuterioboration–oxidation[28] of 3α,5-cyclo-5α-pregn-17-en-6β-yl methyl ether (8)[29] which was obtained by protecting the Δ^5-3β-alcohol moiety of dehydroisoandrosterone (7) as the *i*-methyl ether[30] and introducing the ethylidene group with a Wittig reaction.[31] The resulting C-20 alcohol in 9 was removed[32] by conversion to the mesylate[33] and reduction with lithium aluminum hydride.[34] Regeneration of the Δ^5-3β-hydroxy system[35] gave the desired 5-pregnen-3β-ol-*17*α-d_1.

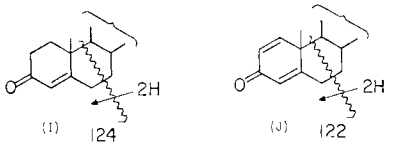
The C-8-, C-14-, and C-11-labeled 1,4,6-cholestatrien-3-ones and 1,4,6-pregnatrien-3-one-*17*α,*21,21,21*-d_4 were made from the correspondingly deuterated Δ^4-3-ketones[6] by dehydrogenation with DDQ.[36]

Results and Discussion

The bond cleavages giving rise to the major diagnostic ions seen in the mass spectra of $\Delta^{4,6}$- and $\Delta^{1,4,6}$-3-keto steroids (Figures 1 and 2) are summarized schematically in drawings I and II. The origins of the itinerant hydrogen atoms involved in each of these fragmentations were established by observing the shift of the *m/z* value for each key peak in the spectra of the deuterated derivatives (Tables II and III).

***m/z* 136 Ion (A).** The *m/z* 136 fragment generates one of the most prominent peaks in the mass spectra of each of the $\Delta^{4,6}$-3-keto steroids (Figure 1). High-resolution mass measurements established that this highly diagnostic peak is primarily due to an ion of composition $C_9H_{12}O$, with very small contributions being made by a $C_{10}H_{16}$ species (13% for **1**, 3% for **2**, and 1% for **3**). The peak is shifted to *m/z* 137 in the mass spectrum of 4,6-androstadiene-3,17-dione-7-d_1[37] and to *m/z* 122 for 19-nor-6-dehydrotestosterone.[7] Furthermore, the mass spectra of 3-*19,19*-d_2 and 3-*2,2,4*-d_3 illustrate that ion A retains these labels (Table II). Metastable defocusing (Table I) demonstrated that the molecular ion is the predominant progenitor of this species. Hence the *m/z* 136 ion must arise by expulsion of the C and D rings from the molecular ion (scission of carbon–carbon bonds 7–8 and 9–10) with the concurrent migration of two hydrogen atoms to the charged A-ring fragment.

An a priori analysis would not have predicted this important fragmentation of $\Delta^{4,6}$-3-keto steroids to be particularly facile. The Δ^4- and $\Delta^{1,4}$-3-keto steroids both exhibit characteristic B-ring cleavage ions (*m/z* 124 and *m/z* 122, respectively, for I and J) resulting from rupture of the



9–10 and 6–7 bonds.[6] As pointed out by Audier et al.[38] and the Spitellers,[39] the presence of a Δ^6 double bond prevents this particular decomposition. Furthermore, one would have expected the Δ^6 double bond to completely block *any* type of B-ring cleavage. Obviously this is not the case. In order to explain the seemingly unfavorable scission of a vinylic bond, we postulate that some sort of hydrogen migration or skeletal rearrangement, resulting in the sp^3 hybridization of C-7, occurs prior to cleavage of the 7–8 bond. Such a change in hybridization would decrease the electron density at C-7, thereby leaving the 7–8 bond more susceptible to homolysis.[40]

The deuterium-labeling data (Table II) indicate that the itinerant hydrogens have multiple origins. As was observed

(24) Although this reaction results in a low conversion, it obviates the extremely difficult separation of Δ^4-3-ketones from the desired $\Delta^{4,6}$-3-ketones, a problem encountered in most other methods of effecting the analogous transformation.
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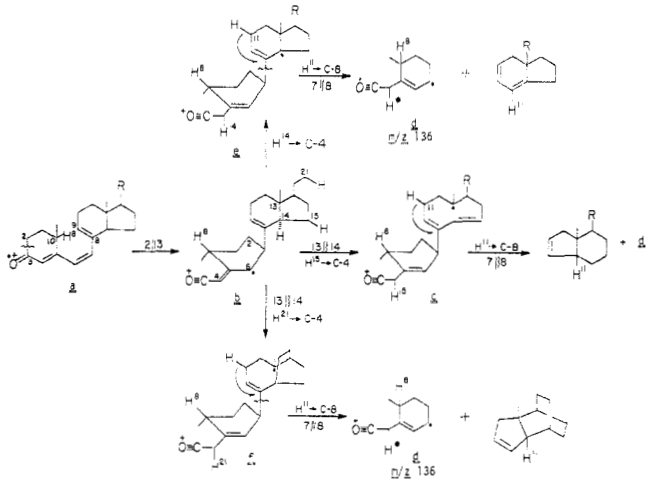
Scheme II



for the m/z 124 and 122 ions of the Δ^4- and $\Delta^{1,4}$-3-ketones,[6] C-8 is once again the most important hydrogen atom source (76%) despite the fact that it must now also suffer scission of a second bond (the 7–8 linkage). Significant contributions are also made by carbon atoms 15 (41%), 21 (24%), 11 (15%), 12 (12%), and 17 (12%). The extent of hydrogen transfer from sites quite removed from the reaction center, especially C-21, is indicative of the occurrence of significant fragmentation or rearrangement of the rigid steroid skeleton. Such extensive rearrangement may well be a reflection of the extended carbonyl conjugation, which stabilizes the molecular ion and increases its lifetime.

The migration of more than the requisite 2.0 atoms of hydrogen, as demonstrated by Table II (210% total transfer), is probably an artifact due to the use of both pregnane and cholestane substrates rather than an indication of the operation of an alternate, competing mechanism. Certainly the extent of hydrogen migration from C-21 might be expected to vary between these two different systems. The fact that 4,6-androstadien-3-one still gives an intense m/z 136 ion is evidence that the other active nuclear loci can make higher contributions that those indicated in Table II in order to compensate for the absence of the C-21 site.

In accord with our previous mechanistic investigation of the B-ring cleavage of Δ^4- and $\Delta^{1,4}$-3-keto steroids,[6] we propose that this fragmentation is initiated by homolysis of the allylically activated 9–10 bond and transfer of the allylic C-8 hydrogen atom to ring A (Scheme II). Such a process relieves the steric compression of the fused A/B/C ring system and generates the energetically favorable, conjugated trienone a. Subsequent rupture of the 2–3 linkage (the well-documented[41] α fission of a ketone) and bond formation between C-7 and the resulting radical at C-2 (a → b) generates an oxonium ion in which the hybridization at C-7 has been transformed from sp^2 to sp^3—a step which is crucial to the ultimate cleavage of the 7–8 bond. At this point many sites in the C/D ring portion of b have become accessible for hydrogen abstraction by the radical cation system (appropriate interatomic distances were measured with Dreiding models). Fission of the allylic 13–14 bond of the strained hydrindan moiety activates the C-15 protons for transfer to C-4 (b → c). Alternatively, the allylic C-14 hydrogen or the readily accessible C-21 protons can migrate to C-4 (b → e or b → f, respectively). In each case (c, e, and f) a subsequent [1,3] sigmatropic shift[42] of a hydrogen from C-11 to C-8 relieves the vinylic nature of the 7–8 bond and triggers its homolysis to yield the m/z 136 ion d and a neutral olefinic species. Similar mechanisms starting from the common species b can be visualized to explain the somewhat less frequent hydrogen migrations from carbon atoms 11, 12, and 17.

Thus this B-ring fragmentation proceeds by a rather complex process in which a key skeletal rearrangement and hydrogen migrations are postulated to activate the initially vinylic 7–8 bond for homolysis.

m/z 134 Ion (B). Our earlier investigation[6] of the mass spectrometric behavior of α,β-unsaturated 3-keto steroids demonstrated that the introduction of a Δ^1 double bond into Δ^4-3-ketones both simplified and enhanced the diagnostic B-ring cleavage (loss of rings C and D plus C-7). The two hydrogen migrations involved in the formation of the m/z 122 ion J were considerably more site specific (C-8 94%; C-11, 92%) than those responsible for the generation of the m/z 124 ion I (C-8, 82%; C-11, 48%; C-15, 42%; C-14, 20%). In addition, the percentage of the total ion current (Σ_{40}) carried by this B-ring-scission ion increased from 8% for 4-cholesten-3-one to 36% for 1,4-cholestadien-3-one.

A priori, we expected to observe a similar trend upon the introduction of a Δ^1 double bond into the $\Delta^{4,6}$-3-keto steroid nucleus. In the case of the trienones the B-ring cleavage process analogous to A would produce an ion at m/z 134. Although such ions do appear in the spectra of Figure 2, they are certainly not very prominent.[43] This is especially true at low ionizing voltages. Whereas the m/z 136 ion of **3** accounted for 11% of the total ion current at 12 eV, the m/z 134 ion of **6** carried only 2% of the ion current at 15 eV. Thus B-ring cleavage with charge retention by the oxygen-containing fragment is not an important process for these $\Delta^{1,4,6}$-3-keto steroids.

An examination of the mechanism (Scheme II) postulated to explain the genesis of the m/z 136 ion provides a rationale for the absence of the analogous m/z 134 ion in the spectra of the $\Delta^{1,4,6}$-3-ketones. The salient feature of this mechanism is homolysis of the 2–3 linkage and subsequent bond formation between C-2 and C-7. This skeletal rearrangement achieves the sp^3 hybridization at C-7 which is required for the ultimate rupture of the 7–8 bond to complete the scission of ring B. However, this mechanism is inoperative for the trienones because the presence of the Δ^1 double bond impairs the requisite 2–3 bond cleavage. Consequently the 7–8 bond remains vinylic, and cleavage of ring B is inhibited.

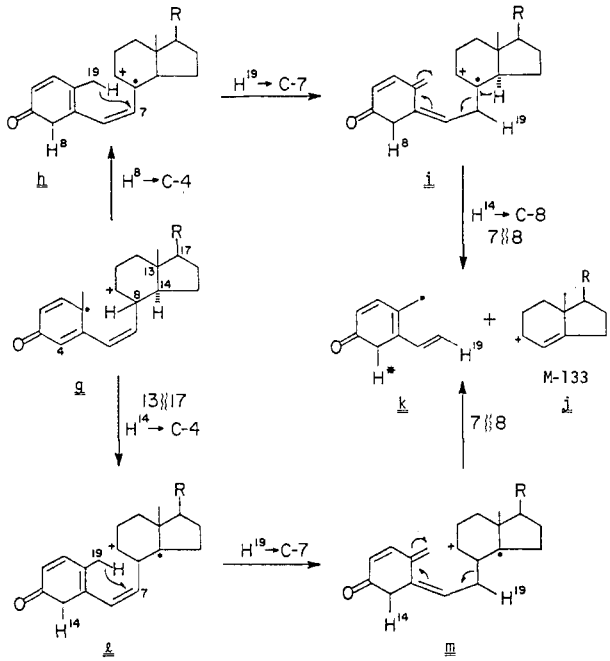
M − 133 Ion (C). Instead of the initially expected m/z 134 ion, an ion at M − 133 dominates the spectra of Figure 2. High-resolution mass measurements proved the ion to be a hydrocarbon, and metastable defocusing (Table I) indicated the molecular ion to be the sole progenitor. This fragment arises from the by now familiar rupture of the 7–8 and 9–10 bonds of ring B, but with the positive charge being retained by the hydrocarbon portion of the molecule. A single hydrogen atom is displaced from the charged fragment. Deuterium labeling (Table III) proved that the itinerant hydrogen has highly specific origins, coming from C-8 57% of the time and from C-14 39% of the time.

(41) Budzikiewicz, H.; Djerassi, C.; Williams, D. H. "Mass Spectrometry of Organic Compounds"; Holden-Day: San Francisco, 1967, pp 134–135.

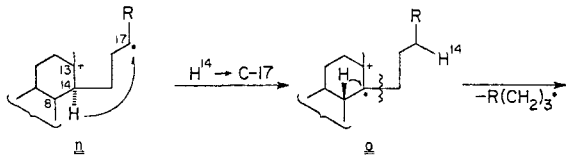
(42) For evidence supporting such shifts in the mass spectrometer, see ref 20a.

(43) A high-resolution spectrum of **4** proved that 74% of the peak at m/z 134 is due to a $C_{10}H_{14}$ species (ion D), with the remainder being contributed by a $C_9H_{10}O$ species (ion B).

Scheme III



The fragmentation (Scheme III) is most likely initiated by ionization of the now doubly allylically activated 9–10 bond to form the radical cation g. The positive charge should preferentially reside at C-9 since the alternative possibility (the radical at C-9 and the carbonium ion at C-10) creates the unfavorable situation of having a positively charged center in conjugation with the electropositive carbonyl. Migration of the hydrogen atom from C-8 to C-4 generates the highly conjugated species h. Transposition of an allylic hydrogen from C-19 to C-7 (a [1,5] sigmatropic shift) via a six-membered cyclic transition state (h → i) accomplishes the change in hybridization at C-7, which we postulate to rationalize subsequent rupture of the 7–8 bond. Consideration of the fission of the 7–8 bond of intermediate i raises a question previously encountered[20a] during a mechanistic investigation of the ubiquitous D-ring cleavage of steroids (n → o).



In both cases (i and o) immediate scission of the indicated carbon–carbon bond generates an ionized carbene. Alternatively, if the designated 1,2 hydrogen shift (1,3 hydrogen shifts are also plausible) occurs prior to bond homolysis, an allylic carbonium ion is obtained. In the absence of any substantial evidence for the existence of ionized carbenes in the mass spectrometer, we postulate that a hydrogen shift from C-14 (or another nearby locus) to C-8 precedes cleavage of the 7–8 bond (i → j). The alternative transfer of the C-14 hydrogen out of the charged fragment can be explained in a similar manner by starting from molecular ion g. Migration of the tertiary hydrogen from C-14 to C-4 (g → l) followed by the displacement of a hydrogen from C-19 to C-7 (l → m) triggers the 7–8 bond rupture to yield the even-electron allylic cation j.

M – 135 Ion (D). As well as exhibiting the previously discussed m/z 136 ion (A), the mass spectra of the $\Delta^{4,6}$-3-ketones (Figure 1) also display a prominent peak at M – 135. High-resolution mass spectra, metastable defocusing experiments (Table I), and deuterium-labeling studies (Table II) all confirm that this ion is also formed by cleavage of the 9–10 and 7–8 bonds. In this case the charge is held by the C/D ring system, which suffers the loss of one hydrogen atom. The labeling data established that the sources of the migrating hydrogen are C-8 (48%) and C-14 (51%). The similarity of the hydrogen-transfer figures for this fragmentation and the M – 133 ion (C) of the $\Delta^{1,4,6}$-3-ketones suggests that an identical mechanism is responsible for both processes. Scheme III (minus the Δ^1 double bond) therefore rationalizes the formation of the M – 135 fragment.

Thus B-ring cleavage with charge retention by the hydrocarbon portion of the molecule is characteristic of both the $\Delta^{4,6}$- and $\Delta^{1,4,6}$-3-keto steroids. The greater intensity of this ion in the mass spectra of the trienones is probably due to two factors. First of all, the presence of a Δ^1 double bond impedes the operation of the alternative B-ring cleavage pathway (Scheme II) which competes for a share of the total ion current in the case of the dienones. Second, the mechanism of Scheme III is probably less favorable energetically for the dienones since the expelled neutral fragment cannot achieve aromaticity. In contrast, the trienones yield neutral fragment k which is simply a tautomer of a hydroxymethylstyrene radical.

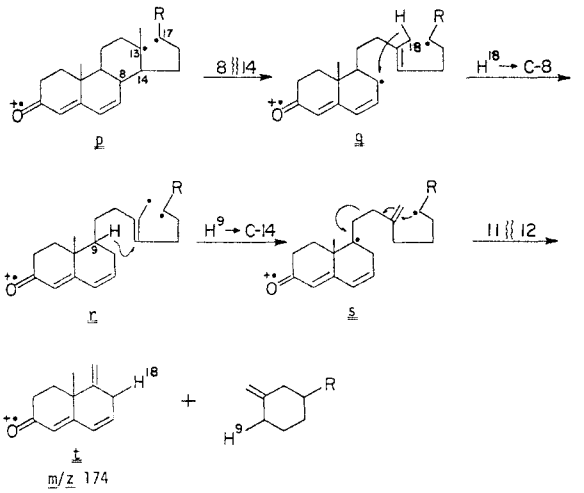
M – 134 Ion (E). In addition to the M – 133 ion, which forms the base peak in the spectra of the $\Delta^{1,4,6}$-3-keto steroids, these compounds also exhibit an ion at M – 134. The molecular ion is the sole parent of this species, which again results from B-ring cleavage with charge retention by the C/D ring system.[43] However, in this case *two* hydrogen atoms are transferred away from the charged fragment. This is an unprecedented fragmentation since an analogous decomposition is not observed for $\Delta^{4,6}$-, $\Delta^{1,4}$-, or Δ^4-3-keto steroids.[6] In all three of these compounds, B-ring cleavage with charge retention by the right-hand portion of the molecule proceeds with the migration of only a single hydrogen atom to generate an even-electron allylic cation such as j.

Deuterium labeling (Table III) proved that once again C-8 was a major source (76%) of a transposed proton. The second itinerant hydrogen, however, had multiple origins: C-15 (35%), C-14 (27%), C-17 (18%), C-11 (11%), C-12 (6%), C-21 (0%). Carbons 9, 16, or 18, being the only loci remaining unlabeled in the C/D ring system, must be responsible for the 30% unaccounted hydrogen migration. Although the origins of the displaced protons bear some resemblance to those observed for the m/z 136 ion (A) of the dienones, the signal lack of any transfer from C-21 (an important site in the case of process A) and the significant contribution which must come from either C-9, -16, or -18 (essentially inactive loci for process A) suggest the operation of different mechanisms for these two fragmentations. The similarities in the hydrogen-transfer data are most likely a reflection of the general accessibility of certain hydrogen-containing sites to centers of high electron density in ring A. It would require too much speculation at this time to propose a detailed mechanism for this obviously complex breakdown of ring B.

However, its existence does reinforce the point that there are several alternate pathways by which these α,β-unsaturated keto steroids can undergo B-ring cleavage. In certain cases the choice of which fragmentation route a molecule will follow may depend on rather subtle and ill-defined factors. For example, the mass spectra of the compound 1,4,6-androstatriene-3,17-dione,[44] the corre-

(44) Smith, H. E.; Smith, R. G. *Org. Mass Spectrom.* **1973**, *7*, 1019–1026.

Scheme IV



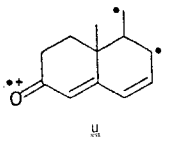
sponding 17β-alcohol,[44] and the analogous 17β-acetate[7] all exhibit a strong peak at m/z 134 (fragmentation B, which does *not* give a significant signal in the spectra of Figure 2) that remains prominent even at low ionizing voltages.[7] However, all these compounds do still show the characteristic B-ring cleavage with charge retention by the C/D ring protion (process C). Thus, although the unsubstituted $\Delta^{1,4,6}$-3-keto steroids of Figure 2 demonstrate consistent behavior, the presence of a heteroatomic substituent at C-17 can alter the appearance of the mass spectrum by affecting the relative energetics of the various pathways for ring-B cleavage. The $\Delta^{4,6}$-3-keto steroids appear to be a more well-behaved class since 4,6-androstadiene-3,17-dione,[37] the analogous 17β-alcohol,[39] and 4,6-pregnadiene-3,20-dione[7] all portray mass spectrometric characteristics in accord with the spectra of Figure 1.

***m/z* 174 Ion (G).** A smaller but still significant peak, which increases in relative intensity at low ionizing voltage,[45] is observed at m/z 174 in the mass spectra of each of the $\Delta^{4,6}$-3-keto steroids in Figure 1. High-resolution mass measurements proved a composition of $C_{12}H_{14}O$ for this ion, and metastable defocusing indicated the major parent to be the molecular ion. This species must therefore be formed by fission of the 8–14 and 11–12 bonds of ring C with charge retention by the oxygen-containing fragment and no apparent hydrogen transfer.

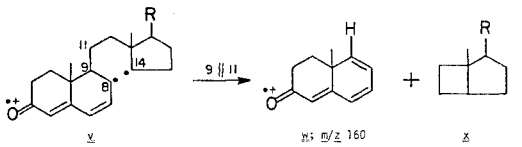
However, deuterium labeling (Table II) revealed that reciprocal hydrogen migrations are involved in this fragmentation. The major hydrogen sources in the left-hand portion of the molecule are C-9 (72%) and C-8 (50%). In the right-hand portion the only active site discovered by the labeling studies was C-14 (52%). No hydrogen transfer originated from carbon atoms 11, 12, 15–17, 20, or 21. Since 1.2 atoms of hydrogen are positively identified as moving out of the charged fragment and only 0.5 atom of hydrogen is demonstrated to migrate back into the charged portion, a major source of itinerant hydrogen in the right half of the molecule must remain undiscovered. The only possible candidate is C-18 since every other locus has already been labeled. If ~1.0 atom of hydrogen is assumed to originate from C-18, the relative percentages of hydrogen transferred from each site suggest the existence of two distinct and equally important mechanisms: one involving *two* reciprocal hydrogen migrations (from carbon atoms 8, 9, 14, and 18) and one proceeding with only a single reciprocal transfer (from carbon atoms 9 and 18).

(45) At 12 eV the m/z 174 peak is twice as strong as the m/z 175 peak in the spectrum of 3.

A possible mechanism is postulated for the latter, simpler case in Scheme IV. Scission of the frangible 13–17 bond initiates homolysis of the allylic 8-14 bond (p → q) which leads to allylic activation of the C-18 protons. Migration of a C-18 hydrogen atom to C-8 (appropriate interatomic distances were measured with Dreiding models) generates the trienone r. Subsequent transfer of the tertiary C-9 hydrogen to C-14 via a six-centered cyclic transition state (r → s) triggers rupture of the 11–12 bond to give the trienone radical cation t. This species is certainly more energetically favorable than fragment u (or its equivalent) which would result from direct cleavage of the 8–14 and 11–12 bonds.



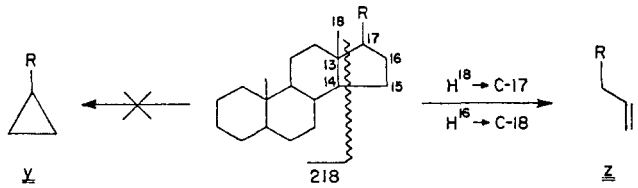
***m/z* 160 Ion (F).** A second ion, containing one less carbon atom than the previously discussed m/z 174 ion, occurs at m/z 160 in the spectra of all the dienones. High-resolution mass measurements confirmed that it was due to an analogous fragmentation of ring C—scission of the 8–14 and 9–11 bonds. According to metastable defocusing (Table I), the molecular ion is the predominant progenitor. A priori, such C-ring cleavage is quite easily rationalized since direct fission of the allylic 8–14 bond followed by rupture of the 9–11 bond forms the conjugated trienone w and a neutral bicyclic hydrocarbon, x.



However, the labeling studies revealed that reciprocal hydrogen migrations are also involved in this fragmentation. The origins of the itinerant hydrogen atoms (C-9, 87%; C-8, 59%; C-14, 54%; and presumably C-18) are almost identical in relative importance with those observed for the m/z 174 ion, so similar mechanisms must be responsible for both types of C-ring cleavage. It is difficult to imagine how a reciprocal hydrogen transfer could generate a charged fragment more energetically favorable than w, the direct cleavage product. Thus we assume that in the simpler process analogous to p → t (reciprocal transfer of H^9 and H^{18}) the transposed C-9 hydrogen is replaced by the return migration of a hydrogen atom from C-18 to yield w, but with the illustrated proton now being H^{18} instead of H^9. Certainly the fact that two bonds to C-9 must be broken in the course of this C-ring cleavage suggests that such a rearrangement occurs. Since the identical charged species w would presumably be obtained via either a direct scission or a rearrangement process, the actual course of the cleavage reaction must be dictated by the relative stabilities of the resulting neutral fragments.

An analogous situation occurs in the D-ring cleavage process which produces an m/z 218 ion for steroidal hydrocarbons.[46] Ionization of the 13–17 bond followed directly by fission of the 14–15 bond would result in expulsion of the neutral, substituted cyclopropane y. However, it is known that reciprocal hydrogen transfers from C-18 and C-16 play an integral part in the fragmentation. These rearrangements allow the molecule to eject the D-ring as the olefinic species z. The lower activation

(46) Djerassi, C. *Adv. Mass Spectrom.* **1968**, *4*, 199–210.



energy required for olefin expulsion outweighs the higher frequency factor of direct cleavage. Hence the molecule fragments by the more complicated rearrangement pathway. Furthermore, a study of *D*-homo steroids[47] demonstrated that direct scission to now expel a substituted cyclobutane still has too high an activation barrier to compete with the rearrangement process.

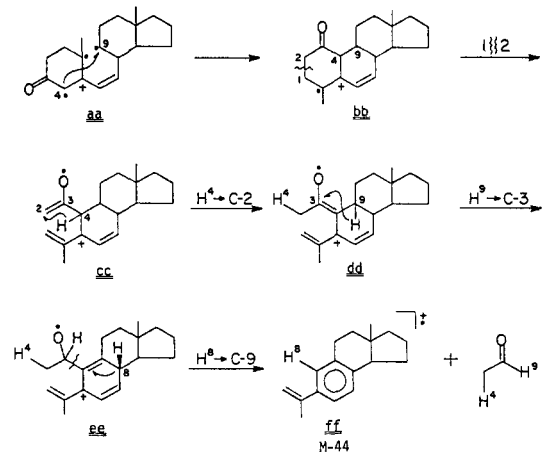
Therefore, in fragmentation F of the $\Delta^{4,6}$-3-keto steroids we conclude that the driving force for the complicated hydrogen rearrangements involved in the formation of w must be the extrusion of a neutral fragment of greater stability than the strained bicyclic hydrocarbon x. For the present it would require too much speculation on the nature of the neutral species x to postulate a detailed mechanism for this process.

Ions arising from C-ring fission are also present at m/z 158 and 172 in the spectra of the $\Delta^{1,4,6}$-3-keto steroids. However, several competing processes, differing only in the number of net hydrogen migrations, seem to be of approximately equal importance. Although these cleavages are not discussed further, they most probably proceed by routes similar to those summarized above.

M − 44 Ion (H). The observation of a M − 42 peak (loss of ketene) in the mass spectra of both Δ^1- and Δ^4-3-keto steroids[22] and of the analogous cyclohexenones[48] and Δ^3-octalones[49] has led to the conclusion[50] that a $R_1R_2C{=}CHCOCH_2$ moiety is required for this fragmentation. However, the $\Delta^{4,6}$-3-keto steroids do *not* exhibit this characteristic cleavage (Figure 1). Instead, a peak is observed in the mass spectra at M − 44: m/z 226 for **1**, m/z 254 for **2**, and m/z 338 for **3**. Exact mass measurements and metastable defocusing proved that these ions were hydrocarbons resulting from the expulsion of C_2H_4O from the molecular ion. The mass spectrum of **3**-*2,2,4-d*$_3$ (Table II) showed the loss of all three deuterium labels from the charged fragment, indicating that C-2 is undoubtedly expelled. The M − 44 ion therefore results from scission of the 1–2 and 3–4 bonds of ring A, just as is observed for the loss of ketene from Δ^4-3-keto steroids. However, the introduction of a Δ^6 double bond causes two hydrogen atoms, in particular those from C-4 (96%) and C-9 (76%), to be displaced from the charged fragment. This unexpected result can be rationalized by the mechanism postulated in Scheme V.

Initial ionization of the Δ^4 double bond and the familiar rupture of the 9–10 bond generates ion aa which can then rearrange to a new intact molecular ion bb by bond formation between C-9 and C-4. Scission of the 1–2 bond gives species cc in which both the radical and the positive charge are resonance stabilized. A subsequent series of [1,3] sigmatropic shifts[42] moves the 2–3 double bond into conjugation in ring C (cc → ee) and then triggers the final homolysis of the 3–4 bond to eliminate acetaldehyde and generate the aromatic species ff. While the exact sequence

Scheme V



of steps in this mechanism is highly speculative, Scheme V does illustrate that the impetus for this complex breakdown of ring A could be the ensuing aromatization of the charged fragment. Such aromatization requires the presence of a Δ^6 double bond in the molecule. Since Δ^4-3-keto steroids would therefore not benefit energetically by such a series of rearrangements, they instead undergo direct cleavage to eliminate ketene.

M − 14 Ion. The mass spectra of the $\Delta^{1,4,6}$-3-keto steroids (Figure 2) and the previously studied $\Delta^{1,4}$-3-keto steroids[6] measured on an AEI MS-9 spectrometer exhibit a peak at M − 14 which is too large to be simply an isotope contribution from the M − CH_3 ion. Although the elimination of :CH_2 has sometimes been postulated to explain mass spectral peaks,[51] such interpretations have usually proved erroneous.[52] Budzikiewicz et al.[53] have briefly reviewed this problem and have suggested alternative factors which may be responsible for the appearance of M − 14 peaks in mass spectra. In view of the above considerations, and since significant M − 14 peaks were not observed when the spectra of the $\Delta^{1,4}$- and $\Delta^{1,4,6}$-3-ketones were recorded on either a Varian MAT-44 or MAT-711 instrument, we consider these M − 14 peaks to be artifacts of the particular spectrometer rather than true examples of electron impact induced carbene eliminations.

Conclusions

Our previous investigation[6] of the electron impact induced decomposition of Δ^4- and $\Delta^{1,4}$-3-keto steroids has now been extended to include the $\Delta^{4,6}$- and $\Delta^{1,4,6}$-3-keto steroids. Despite the presence of a Δ^6 double bond, B-ring cleavage is still a very important fragmentation reaction for these latter steroids, which exhibit diagnostic ions resulting from scission of the 9–10 and 7–8 bonds with concomitant hydrogen migrations. On the basis of extensive deuterium labeling, mechanisms involving intramolecular rearrangements to effect a change in hybridization at C-7 are postulated to explain this a priori unlikely rupture of the vinylic 7-8 bond. As was observed for the Δ^4- and $\Delta^{1,4}$-3-ketones, C-8 again proved to be a major source of an itinerant hydrogen atom.

The mass spectra of the $\Delta^{4,6}$-3-keto steroids display two prominent B-ring-cleavage ions corresponding to charge retention by either the oxygen-containing fragment or the

(47) Eadon, G., Popov, S.; Djerassi, C. *J. Am. Chem. Soc.* **1972**, *94*, 1282–1292.
(48) Burlingame, A. L.; Fenselau, C.; Richter, W. J.; Dauben, W. G.; Shaffer, G. W.; Vietmeyer, N. D. *J. Am. Chem. Soc.* **1967**, *89*, 3346–3347.
(49) Harris, R. L. N.; Komitsky, Jr., F.; Djerassi, C. *J. Am. Chem. Soc.* **1967**, *89*, 4765–4775.
(50) Zaretski, Z. V. "Mass Spectrometry of Steroids"; Israel Universities Press: Jerusalem, 1976; p 29.
(51) (a) Willhalm, B.; Thomas, A. F.; Gautschi, F. *Tetrahedron* **1964**, *20*, 1185–1209. (b) Grover, P. K.; Anand, N. *Chem. Commun.* **1969**, 982.
(52) Trudell, J. R.; Woodgate, S. D. S.; Djerassi, C. *Org. Mass Spectrom.* **1970**, *3*, 753–776.
(53) Budzikiewicz, H.; Roth, G.; Vogel, E. *Org. Mass Spectrom.* **1979**, *14*, 140–144.

hydrocarbon portion. In the former process, two hydrogen atoms are transferred to the charged species from multiple sites in the C and D rings. A mechanism involving a skeletal rearrangement and the ultimate formation of a conjugated oxonium ion radical is postulated to explain this complex decomposition. A simpler process is responsible for charge retention by the C/D ring system. A single hydrogen atom is displaced from either C-8 or C-14 to generate an even-electron, allylic cation.

An identical mechanism accounts for the single most salient fragment ion observed in the mass spectra of the $\Delta^{1,4,6}$-3-keto steroids. In surprising contrast to the analogous $\Delta^{1,4}$-3-ketones, charge retention by the A ring is not significant for these trienones. This observation is rationalized by an analysis of the mechanisms postulated for B-ring scission.

The presence of a Δ^6 double bond in these molecules gives rise to some new fragmentation reactions which were not observed for the Δ^4- and $\Delta^{1,4}$-3-ketones. Two C-ring cleavages, both involving reciprocal hydrogen migrations, become important. Quite surprisingly the expected elimination of ketene from the $\Delta^{4,6}$-3-keto steroids is not observed. Instead, highly site-specific hydrogen transfers from C-4 and C-9 result in the loss of a C_2H_4O species. A possible explanation for this anomaly is suggested.

The introduction of additional conjugated double bonds into the medicinally and biologically important Δ^4-3-keto steroids alters the mass spectrometric behavior of these compounds. The effects can now be understood on mechanistically plausible grounds.

Experimental Section

General Methods. Mass spectra were obtained on either an AEI MS-9 spectrometer by Mr. R. G. Ross or on a Varian MAT-711 high-resolution mass spectrometer by Ms. A. Wegmann using, in both cases, direct-inlet systems. ^{1}H NMR spectra were measured on Varian T-60 and XL-100-15 instruments using deuteriochloroform as solvent and tetramethylsilane as an internal standard. Chemical shifts for the C-18 and C-19 angular methyl resonances were calculated by the method of Zürcher.[60] Infrared spectra were recorded on a Perkin-Elmer 700A spectrometer. Optical rotations were determined on an Autopol III polarimeter using a thermostated 1.00 dm microcell. Ultraviolet absorptions were measured on a Hewlett-Packard 8450A UV/VIS spectrometer. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Chromatography was performed on TLC grade (Type 60) silica gel H (E. Merck).

4,6-Cholestadien-3-one (3). A solution of 50.5 mg of 2,4-dibromo-5α-cholestan-3-one and 22 mg of lithium chloride in 1 mL of DMF was refluxed under nitrogen for 1 h.[6] Water was added, and the mixture was extracted with benzene. Evaporation of the dried organic layer gave an amber oil which was chromatographed on 10 g of silica gel with 10% ethyl acetate in hexane. Early fractions yielded 13.2 mg (38%) of **3**: mp 83.5–85 °C (MeOH) (lit.[16] mp 80–81 °C); NMR δ 6.12 (s, 2 H, C-6, C-7), 5.67 (s, 1 H, C-4), 1.10 (s, 3 H, C-19), 0.75 (s, 3 H, C-18). Later fractions contained 7.7 mg (22%) of 1,4-cholestadien-3-one.

4,6-Cholestadien-3-one-14α-d_1, -11,11-d_2, and -12,12-d_2. These dienones[54,55] were synthesized from the analogous labeled 2,4-dibromo-5α-cholestan-3-ones[6] in the same way as **3**.

4,6-Cholestadien-3-one-8β-d_1. A mixture of 98.6 mg of 4-cholesten-3-one-8β-d_1[6] and 380 mg of recrystallized chloranil in 6 mL of *tert*-butyl alcohol was stirred and refluxed under nitrogen for 4.5 h.[21] Undissolved chloranil was removed by filtration. The filtrate was placed on a 6-g column of neutral alumina (activity stage 1) and eluted with 100 mL of methylene chloride followed by 100 mL of a 4:1 mixture of methylene chloride–acetone. Evaporation of the eluant gave a dark brown oil which was chromatographed on 12 g of silica gel with 10% ethyl acetate in hexane to yield a yellow oil. The color was removed by preparative TLC to furnish 66.8 mg (67%) of 4,6-cholestadien-3-one-8β-d_1[54,55] as a clear oil which crystallized under vacuum.

4,6-Pregnadien-3-one (2). A solution of 1.0 g of 5-pregnen-3β-ol[56] in 150 mL of benzene was treated with 5.0 g of activated manganese dioxide (Sterling Organics).[23] The stirred mixture was refluxed under nitrogen for 20 h and then filtered through Celite while still hot. Evaporation of the filtrate gave a yellow solid which was chromatographed on 50 g of silica gel with 20% ethyl acetate in hexane. Early fractions yielded 0.22 g (22%) of **2**, which formed clumps of stout needles in methanol: mp 115.5–116.5 °C; $[\alpha]^{20}_D$ +51° (*c* 0.105, $CHCl_3$); UV (EtOH) 286 nm (ϵ 17 000); IR 1590, 1610, 1670 cm^{-1}; NMR δ 6.12 (s, 2 H, C-6, C-7), 5.66 (s, 1 H, C-4), 1.12 (s, 3 H, C-19), 0.66 (s, 3 H, C-18); mass spectrum, *m/z* 298.2298 (M^+, calcd for $C_{21}H_{30}O$ 298.2296). Unchanged starting material (0.46 g, 46%) was recovered from later fractions.

4,6-Cholestadien-3-one-9α-d_1 and -19,19-d_2. These steroids[54,55] were prepared from the corresponding labeled cholesterols[20,27] in exactly the same manner as **2**.

4,6-Pregnadien-3-one-16,16-d_2, -15,15,17α-d_3, -17α,21,21,21-d_4, -17α-d_1, and -20,20-d_2. The preparation of these compounds[54,55] from the analogous deuterated 5-pregnen-3β-ols[26,6] proceeded exactly as for **2**.

4,6-Cholestadien-3-one-2,2,4-d_3. This compound[54,55] was prepared by exchange of the active protons of the unlabeled compound in alkaline deuteriomethanol as described by Shapiro and Djerassi.[22]

1,4,6-Cholestatrien-3-one (6). After the method of Djerassi et al.,[19] a 65-mL etheral solution of 2.0 g of 4-cholesten-3-one[57] was cooled to 0 °C and was treated with 2 drops of 4 N hydrogen bromide in glacial acetic acid followed by a solution (rapid dropwise addition) of 1.75 g of bromine in 18 mL of glacial acetic acid. The clear yellow solution was stirred at 0 °C for 10 min, and then the ether was evaporated. The crystals which subsequently formed in the acetic acid solution were collected by filtration and washed with ethanol to yield 2,6-dibromo-4-cholesten-3-one as a powdery white solid: yield, 1.69 g (60%); mp 159–160 °C dec (lit.[19] mp 163 °C).

The dibromo compound was refluxed under nitrogen in dry *s*-collidine for 30 min. After the precipitated collidine hydrobromide was removed, the dark brown filtrate was taken up in ether and washed repeatedly with dilute hydrochloric acid and then water. Evaporation of the dried organic layer furnished a viscous brown oil which was chromatographed twice on silica gel to give a 44% yield of **6**. The yellow **6** recrystallized from aqueous methanol as slightly yellow prisms: mp 87–89 °C (lit.[19] mp 82–83 °C, petroleum ether); NMR δ 7.08 (d, 1 H, C-1, J = 10 Hz), 6.18 (m, 4 H, vinylic), 1.19 (s, 3 H, C-19, calcd 1.20), 0.79 (s, 3 H, C-18, calcd 0.79).

1,4,6-Pregnatrien-3-one (5). This compound was prepared in the same manner as **6**. Silica gel chromatography yielded **5** as a clear, colorless oil:[18] NMR[58] δ 7.08 (d, 1 H, C-1, J = 10 Hz), 6.26 (d, 1 H, C-7, J = 10 Hz), 6.24 (d, 1 H, C-2, J = 10 Hz), 6.05 (d, 1 H, C-6, J = 10 Hz), 6.00 (s, 1 H, C-4), 1.21 (s, 3 H, C-19, calcd 1.21), 0.69 (s, 3 H, C-18, calcd 0.70); 2,4-DNP hydrazone, mp 187–188 °C.

6β-Methoxy-3α,5-cyclo-5α-androstan-17-one. A solution of 5.5 g of 5-dehydroisoandrosterone (**7**) and 7 g of *p*-toluenesulfonyl chloride in 50 mL of pyridine was stirred for 8 h at room temperature and worked up in the usual manner to give 8.45 g of the tosylate. This material was combined with 7 g of freshly fused potassium acetate in 220 mL of anhydrous methanol and refluxed under nitrogen for 8 h.[30] When the mixture was cooled, potassium acetate crystallized out of the homogeneous solution and was removed by filtration. The filtrate was evaporated and the resulting residue was partitioned between ether and water. Evaporation of the dried organic layer afforded the *i*-methyl ether of dehydroisoandrosterone as a slightly yellow oil: IR 3080 (cyclopropyl σ_{C-H}), 1730 (C=O) cm^{-1}; NMR δ 3.38 (s, 3 H, OCH_3),

(54) Physical and spectral data were in agreement with those of authentic unlabeled material.
(55) The isotopic composition is given in Table II or III.
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2.85 (br t, 1 H, C-6), 1.30 (s, 3 H, C-19), 0.95 (s, 3 H, C-18), 0.58 (m, 2 H, C-4). This material was used in the next reaction without further purification.

***cis*-6β-Methoxy-3α,5-cyclo-5α-pregn-17-ene (8).** A 3.5-g quantity of a 50% dispersion of sodium hydride in oil was washed three times with hexane and treated with 50 mL of freshly distilled Me_2SO.[31] The stirred mixture was kept under nitrogen and heated at 75 °C in an oil bath until hydrogen evolution ceased (~1 h). The resulting cloudy, light green mixture was cooled to room temperature and 30.4 g of vacuum-dried ethyltriphenylphosphonium iodide was added to generate a red solution, which, after being stirred for 10 min, was treated with 5.5 g of 6β-methoxy-3α,5-cyclo-5α-androstan-17-one dissolved in 80 mL of Me_2SO. The now deep red solution was stirred under nitrogen at 55 °C in an oil bath for 12 h, cooled to room temperature, and poured into 300 mL of ice–water. The resulting milky suspension was extracted three times with ether. The combined organic layers were washed repeatedly with water, dried, and evaporated to yield a dark brown oil, which was chromatographed on 200 g of silica gel with 30% benzene in hexane. Evaporation of later fractions gave 3.14 g (55%) of 8[29] as a slightly yellow oil: IR 3080 (cyclopropyl σ_{C-H}); NMR δ 5.12 (q, 1 H, C-20, J = 7 Hz), 3.37 (s, 3 H, OCH_3), 2.78 (br t, 1 H, C-6), 1.05 (s, 3 H, C-19), 0.95 (s, 3 H, C-18), 0.53 (m, 2 H, C-4); mass spectrum, m/z (relative intensity) 314.2607 (M^+, 58, calcd for $C_{22}H_{34}O$ 314.2609), 299 (M – CH_3, 44), 282 (M – CH_3OH, 76), 267 (M – (CH_3OH, CH_3), 36), 259 (M – C_4H_7, ring A fission, 100).

6β-Methoxy-3α,5-cyclo-5α-pregnan-20-ol-*17α-d*$_1$ (9). Deuterated diborane, generated by the addition of 22.2 mL of boron trifluoride etherate in 50 mL of anhydrous diglyme to a stirred slurry of 5.77 g of lithium aluminum deuteride in 200 mL of diglyme,[59] was bubbled into an ice-cold solution of 2.88 g of 8 in 250 mL of anhydrous THF.[28] Upon completion of the diborane generation, the THF solution was warmed to room temperature and stirred for 4 h. Excess diborane was hydrolyzed by cautious addition of 3 mL of water. The solution was cooled to 0 °C, and 120 mL of 10% sodium hyroxide and then 80 mL of 30% hydrogen peroxide were added dropwise. The biphasic mixture was stirred for 1 h and then warmed to room temperature. The organic layer was separated, washed with 10% sodium bisulfite followed by water, dried, and evaporated. The residue was eluted from an 80-g silica gel column with 20% ethyl acetate in hexane. Evaporation of later fractions yielded 2.34 g (76%) of 9 as a viscous oil which became a waxy solid upon standing: IR 3400 (OH), 3080 (cyclopropyl σ_{C-H}); NMR δ 3.73 (q, 1 H, C-20, J = 6 Hz), 3.37 (s, 3 H, OCH_3), 2.80 (br t, 1 H, C-6), 1.05 (s, 3 H, C-19), 0.75 (s, 3 H, C-18), 0.52 (m, 2 H, C-4); mass spectrum, m/z (relative intensity) 333 (M^+, 55), 318 (M – CH_3, 50), 301 (M – CH_3OH, 74), 278 (M – C_4H_7, A-ring fission, 100).

5-Pregnen-3β-ol-*17α-d*$_1$ (11). To a solution of 2.5 g of 9 and 1.6 mL of triethylamine in 40 mL of methylene chloride was slowly added 0.64 mL of freshly distilled methanesulfonyl chloride dissolved in 2 mL of methylene chloride.[33] The resulting solution was stirred for 15 min and then washed successively with ice-cold water, cold 10% hydrochloric acid, saturated sodium bicarbonate solution, and brine. Evaporation of the dried organic layer gave the oily mesylate which was dissolved in anhydrous ether and added dropwise to a stirred slurry of lithium aluminum hydride in ether. After being stirred for 15 min, the reaction mixture was hydrolyzed with saturated sodium sulfate solution. Evaporation of the dried etheral solution furnished the oily *i*-ether 10.[34]

The Δ^5-3β-ol moiety was regenerated by dissolving this ether in 125 mL of dioxane, diluting the solution with 45 mL of water, adding several crystals of *p*-toluenesulfonic acid hydrate, and refluxing the mixture for 1 h.[35] An additional 50 mL of water was added, and the faintly cloudy solution was cooled in ice. The resulting precipitate was collected by filtration. Spectral analysis indicated the presence of substantial amounts of a diene resulting from elimination during the hydride displacement of the mesylate. Careful chromatography of the precipitate on 110 g of silica gel impregnated with 12% silver nitrate furnished 0.13 g of 5-pregnen-3β-ol-*17α-d*$_1$.[54]

1,4,6-Pregnatrien-3-one-*17α-d*$_1$ (12). A stirred solution of 71.7 mg of 11 and 200 mg of DDQ in 5 mL of *p*-dioxane was refluxed under nitrogen for 40 h.[25] The dark red-brown mixture was allowed to cool, and the precipitated hydroquinone was removed by filtration. The filtrate was placed on a 6-g column of neutral alumina (activity stage 1) and eluted with 100 mL of methylene chloride followed by 100 mL of a 4:1 mixture of methylene chloride–acetone. Evaporation of the latter eluent gave a yellow oil which was chromatographed on 12 g of silica gel with 7% ethyl acetate in hexane to yield 12[54,55] as a clear, colorless oil.

1,4,6-Pregnatrien-3-one-*15,15,17α-d*$_3$ and -*20,20-d*$_2$. These trienones[54,55] were obtained from the corresponding deuterated 5-pregnen-3β-ols[6,26] in the same manner as 12.

1,4,6-Cholestatrien-3-one-*12,12-d*$_2$. This compound[54,55] was prepared from the analogous labeled cholesterol[20] by the same method used for the synthesis of 12.

1,4,6-Cholestatrien-3-one-*8β-d*$_1$ (13). A solution of 49.0 mg of 4-cholesten-3-one-*8β-d*$_1$,[6] 60.7 mg of *p*-toluenesulfonic acid hydrate, and 75.2 mg of DDQ in 7 mL of *p*-dioxane was stirred and refluxed under nitrogen for 40 h.[36] The dark brown mixture was worked up by the same procedure used for 12. Chromatography gave 32.0 mg (65%) of 13[54,55] as a clear, colorless oil which solidified under vacuum.

1,4,6-Cholestatrien-3-one-*14α-d*$_1$ and -*11,11-d*$_2$. These trienones[54,55] were synthesized from the corresponding labeled 4-cholesten-3-ones[6] in the same manner as 13.

1,4,6-Pregnatrien-3-one-*17α,21,21,21-d*$_4$. The synthesis of this compound proceeded from the deuterated Δ^4-3-ketone[6] by the method used to prepare 13.

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Registry No. 2, 76010-32-7; **2-*15,15,17α-d*$_3$**, 76010-33-8; **2-*16,16-d*$_2$**, 76010-34-9; **2-*17α,21,21,21-d*$_4$**, 76010-35-0; **2-*17α-d*$_1$**, 76010-36-1; **2-*20,20-d*$_2$**, 76010-37-2; **3**, 566-93-8; **3-*2,2,4-d*$_3$**, 76010-38-3; **3-*8β-d*$_1$**, 76010-39-4; **3-*9α-d*$_1$**, 76010-40-7; **3-*11,11-d*$_2$**, 76010-41-8; **3-*12,12-d*$_2$**, 55116-25-1; **3-*14α-d*$_1$**, 73658-57-8; **3-*19,19-d*$_2$**, 76010-42-9; **5**, 58702-07-1; **5-*15,15,17α-d*$_3$**, 76010-43-0; **5-*17α,21,21,21-d*$_4$**, 76010-44-1; **5-*20,20-d*$_2$**, 76010-45-2; **6**, 3464-60-6; **6-*11,11-d*$_2$**, 76010-46-3; **6-*12,12-d*$_2$**, 76010-47-4; **6-*14α-d*$_1$**, 76010-48-5; **7**, 53-43-0; **7** tosylate, 2719-96-2; **8**, 76035-36-4; **9**, 76010-49-6; **9** mesylate, 76010-50-9; **10**, 76010-51-0; **11**, 76010-52-1; **12**, 76010-53-2; **13**, 76010-54-3; 5α-cholestan-3-one, 566-88-1; 1,4-cholestadien-3-one, 566-91-6; 4-cholesten-3-one-*8β-d*$_1$, 60816-29-7; 5-pregnen-3β-ol, 2862-58-0; 4-cholesten-3-one, 601-57-0; 2,6-dibromo-4-cholesten-3-one, 64313-97-9; 6β-methoxy-3α,5-cyclo-5α-androstan-17-one, 14425-92-4; 4,6-androstadiene-3,17-dione, 633-34-1.

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