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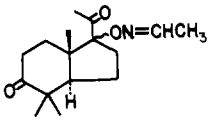
(29) In cases where products were isolated by solvent extraction, the procedure generally followed was to extract the aqueous layer with several portions of the indicated solvent; then the organic layers were combined and washed with water followed by saturated brine. The organic layer was dried over anhydrous sodium sulfate or magnesium sulfate and filtered, and the solvent was evaporated under reduced pressure (water aspirator) using a rotary evaporator. The use of the term "wash" indicates washing the combined organic layers with saturated aqueous sodium bicarbonate solution ("base wash"), with dilute aqueous hydrochloric acid ("acid wash"), or with the indicated solution prior to the aforementioned washing with water.
(30) In an experiment performed by R. J. Parry, oxidation of **3** with the ruthenium tetroxide solution for only 3 min afforded a product which was identified by IR, VPC, and mass spectrometry as i. Further treatment of i with ruthenium tetroxide for 3 h afforded a mixture of i and the dione **4**.



i

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Elucidation of the Course of the Electron Impact Induced Fragmentation of α,β-Unsaturated 3-Keto Steroids[1,2]

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Abstract: A series of deuterium-labeled Δ^4- and $\Delta^{1,4}$-3-keto steroids has been synthesized in order to investigate the diagnostic, electron impact-induced fragmentations, notably ring B cleavages, characteristic of these compounds. Such information is important in the structure elucidation of new steroids and is especially relevant in view of the recent isolation of Δ^4- and $\Delta^{1,4}$-3-keto steroids from marine organisms, in addition to the almost ubiquitous presence of these functionalities in adrenal and sex hormones. The introduction of a ketone functionality into various positions of the steroid nucleus permitted the labeling of carbon atoms 6, 7, 8, 9, 11, 12, 15, 16, and 17. In agreement with earlier studies, the 8β-hydrogen atom was shown to play a key role in the hydrogen migrations accompanying the ring B scissions. Hydrogen atoms from C-11, -14, and -15 were also implicated. Mechanisms are presented to explain the fragmentations. The differences between the mass spectra of the two types of α,β-unsaturated ketones, as well as among substituted analogues, are analyzed in terms of these mechanisms.

Recently there has been a significant resurgence in the search for new steroids from natural sources, most notably the marine environment.[4] Because of the extremely small quantities involved, the structure elucidation of these new compounds often relies solely upon gas chromatography–mass spectral measurements. Accurate interpretation of these data demands an adequate knowledge of the mechanisms of the principal fragmentation processes arising from a particular structural feature. The recent isolation of $\Delta^{1,4}$-3-keto steroids from marine organisms[5] and human urine[6] prompted us to examine the electron impact induced fragmentations of such dienones since very little attention has been given to their mass spectrometric behavior,[7] even though the $\Delta^{1,4}$-3-ketone moiety is also an important feature of many medicinally important corticosteroids. Deuterium labeling was required to establish the course of the diagnostic cleavages of the $\Delta^{1,4}$-3-keto steroids and, since these compounds are generally prepared from Δ^4-3-keto precursors, the key fragmentations of the latter steroids[8] were reinvestigated. The behavior of the Δ^4-3-keto steroids under electron impact has commanded considerable interest[7] because of the frequent occurrence of this functionality in the progestational and androgenic sex hormones as well as in the corticosteroids. More recently such steroids have been obtained from marine sources.[9] In contrast to their saturated counterparts, these α,β-unsaturated 3-keto steroids exhibit characteristic mass spectral fragmentations of a general type (i.e., consistent from one class of steroids to another) which are usually independent of ring substituents. These compounds are thus ideal candidates for mechanistic investigations.

The mass spectra of some representative Δ^4- and $\Delta^{1,4}$-3-keto steroids are presented in Figures 1 and 2. As demonstrated previously,[8] the prominent peaks in the high-mass region of the 4-androsten-3-one (**1**) spectrum are *m/z* 230 (M − 42; loss of ketene from ring A), *m/z* 215 (M − 57; loss of ketene plus a methyl radical), *m/z* 187 (M − 85; loss of C-1, 2, 3, 10, and 19), *m/z* 149 (M − 123) and *m/z* 124. The latter two ions result from fission of the 6–7 and 9–10 bonds of ring B with the charge remaining on either the hydrocarbon or oxygen-containing fragment, respectively. These characteristic peaks also prevail in the mass spectra of 4-pregnen-3-one (**2**) and 4-cholesten-3-one (**3**), with the additional appearance of ions resulting from loss of the respective C-17 side chains.

The most striking difference in the spectra of the $\Delta^{1,4}$-3-ketones is the very high percentage of the total ion current that is carried by the *m/z* 122 ion (ring B cleavage). The spectra do not display the M − 42 (loss of ketene) or M − 85 ions which are characteristic of the Δ^4 analogues. Small peaks are present in the spectrum of 1,4-androstadien-3-one (**4**) at *m/z* 229 (M − 41; loss of C_3H_5), *m/z* 149 (M − 121; fission of the 6–7 and 9–10 bonds of ring B), and *m/z* 135 (M − 135; rupture of the 7–8 and 9–10 bonds of ring B). These diagnostic ions are also seen in the spectra of the heretofore unknown 1,4-pregnadien-3-one (**5**) and of 1,4-cholestadien-3-one (**6**). The origins and identities of these ions were corroborated by metastable defocusing experiments (Table I) and high-resolution mass measurements.

Hydrogen migrations play an important role in the ring B cleavages of both the Δ^4- and $\Delta^{1,4}$-3-keto steroids as well as

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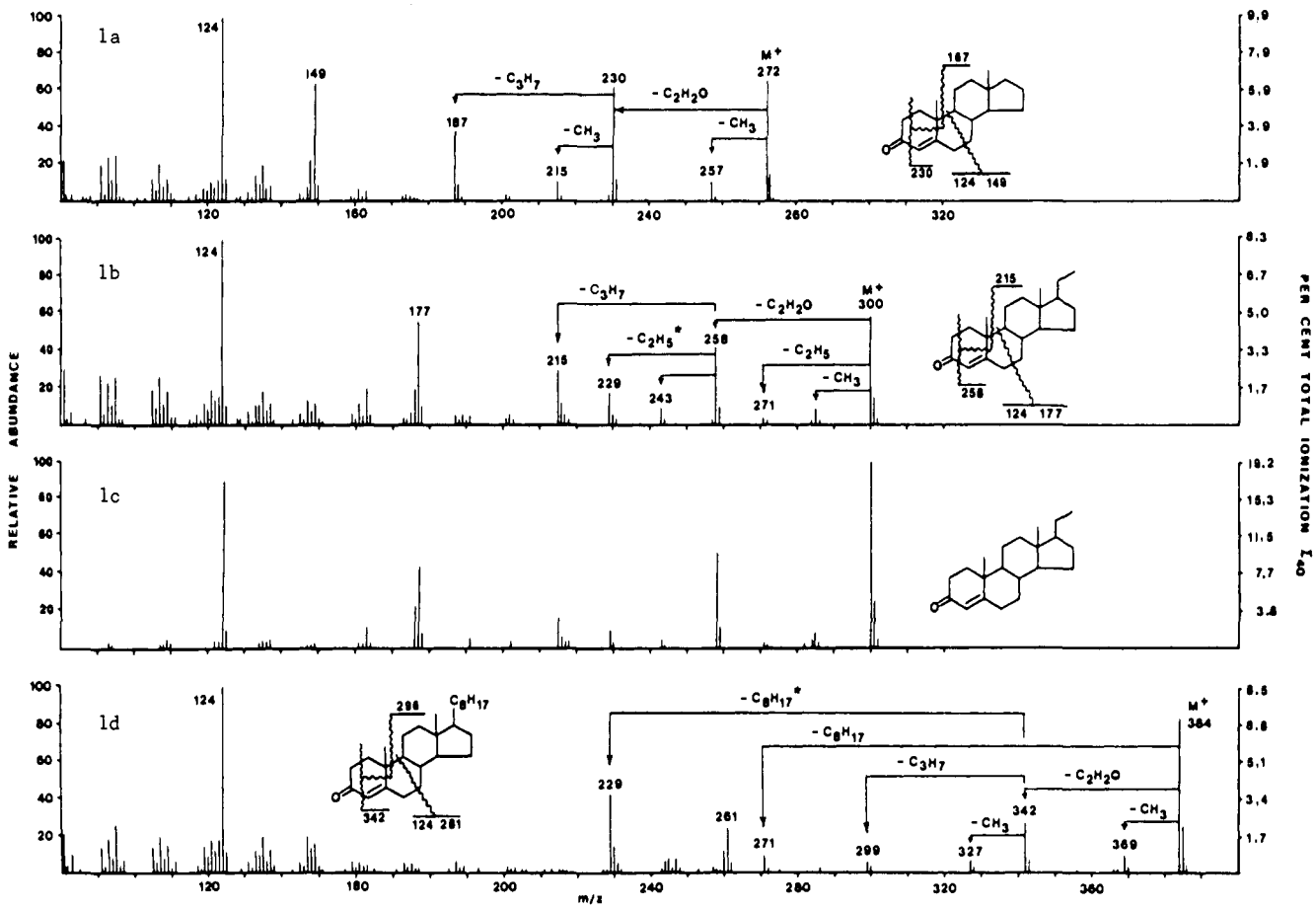


Figure 1. Mass spectra of Δ^4-3-keto steroids: (a) 4-androsten-3-one (**1**), 70 eV; (b) 4-pregnen-3-one (**2**), 70 eV; (c) 4-pregnen-3-one (**2**), 15 eV; (d) 4-cholesten-3-one (**3**), 70 eV; (*) approximately 20% of this peak arises from ring D cleavage.

Table I. Metastable Defocusing Data[a]

compound	daughter ion	parent ion	
4-cholesten-3-one	342, 261, 124	384	M
	299[b]	384 (10%)	M
		342 (90%)	M − ketene
4-pregnen-3-one	258, 177, 124	300	M
	215[c]	258 (90%)	M − ketene
		230 (5%)	
4-androsten-3-one	230, 149, 124	272	M
	187	230 (90%)	M − ketene
		202 (10%)	
1,4-cholestadien-3-one	367, 261, 247, 122	382	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] High-resolution mass measurements indicated that approximately 16% of the *m/z* 299 peak was attributable to a $C_{21}H_{31}O$ species (loss of C_6H_{13}—probably from the side chain). [c] 3% of the *m/z* 215 peak was due to a $C_{15}H_{19}O$ ion by high-resolution analysis.

in the M − 85 and M − 41 ions. These complex processes were unraveled by means of extensive deuterium labeling. Although some of the fragmentations of 4-androsten-3-one had been investigated previously in our laboratory,[8] the more elaborate labeling carried out in the course of this work revealed some surprising results and allowed a more detailed analysis of the cleavage mechanisms for Δ^4-3-ketones. The mass spectrometric fragmentations of $\Delta^{1,4}$-3-keto steroids have not been previously examined by isotopic labeling.

Synthesis of Deuterated Compounds. During the course of this investigation Δ^4- and $\Delta^{1,4}$-3-keto steroids labeled at C-6, 7, 8, 9, 11, 12, 14, 15, 16, and 17 were prepared. The syntheses were designed so as to achieve maximum deuterium incorporation specifically at the selected sites while minimizing isotopic scrambling. The isotopic purity of each of the final α,β-unsaturated ketones is given in Tables II and III.

A deuterium atom was introduced at C-14 (Scheme I) via deuterioboration[10] of 5α-cholest-14-en-3β-ol (**7**)[11] followed by hydrolytic cleavage of the boron adduct to give 5α-cholestan-3β-yl-*14*α-d_1 propionate (**8a**). Saponification and Jones oxidation furnished the 3-ketone which was brominated in acetic acid[12] to give the dibromide **9**. Treatment with sodium iodide and subsequent reduction of the resulting 2-iodo-Δ^4-3-ketone with chromous chloride[13] yielded the desired 4-cholesten-3-one-*14*α-d_1 (**10**). Alternatively, **9** could be dehydrobrominated with lithium chloride in dimethylformamide[14] to a mixture of the labeled $\Delta^{1,4}$- and $\Delta^{4,6}$-3-keto steroids (**11** and **12**, respectively) which were separable by column chromatography on silica gel.

The C-8 labeled 4-cholesten-3-one (**13**) and the *6,6,8*-d_3 analogue were prepared by the method of Tökés et al.[15] Dehydrogenation of **13** with selenium dioxide[16] furnished 1,4-cholestadien-3-one-*8*β-d_1. Oppenauer oxidation[17] of cholesterol-*7,7*-d_2[18] yielded 4-cholesten-3-one-*7,7*-d_2. The C-9, 11, and 12 labeled 4-cholesten-3-ones were available[19] from previous studies undertaken in our laboratory.[20] The corresponding $\Delta^{1,4}$-3-ketones were obtained from the respective deuterated 5α-cholestan-3α-ols[19] in the same manner as **11**.

Steroids deuterated at C-15, 16, and 17 were prepared in the pregnane series (Scheme II) since the appropriately functionalized 3β-acetoxy-5-pregnen-16-one (**17**)[21] could be obtained from 16-dehydropregnenolone (**14**)[22] by Wharton

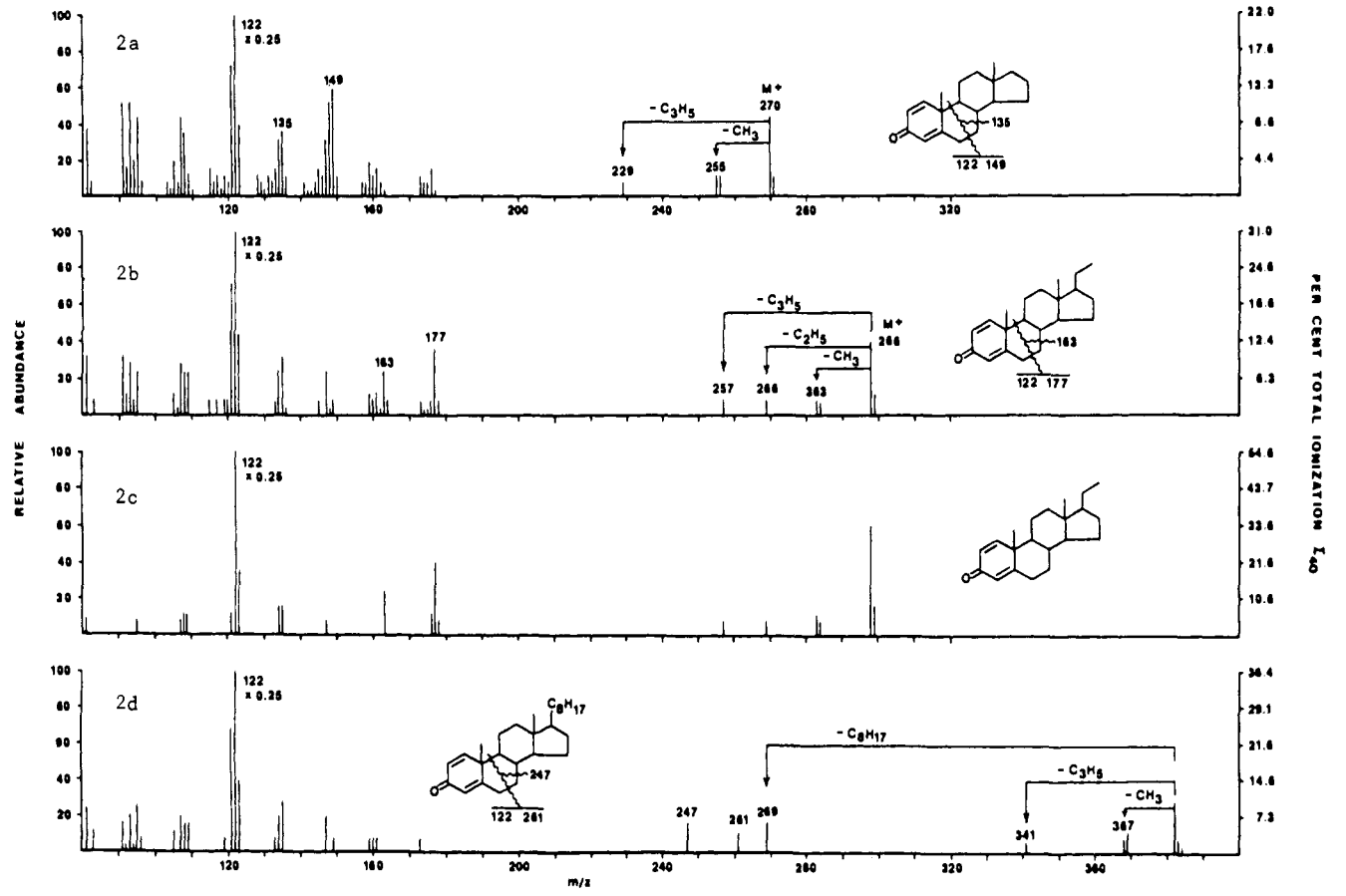
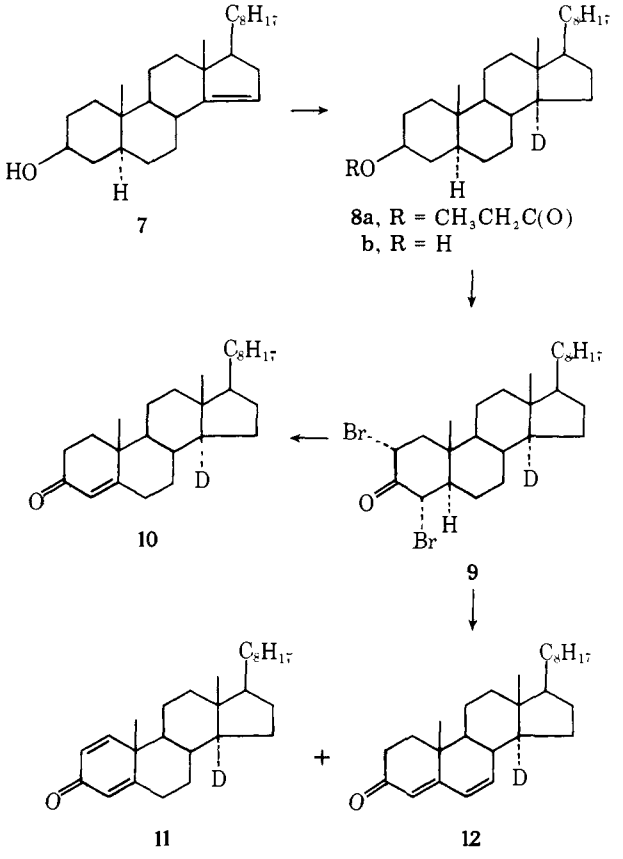


Figure 2. Mass spectra of $\Delta^{1,4}$-3-keto steroids: (a) 1,4-androstadien-3-one (**4**), 70 eV; (b) 1,4-pregnadien-3-one (**5**), 70 eV; (c) 1,4-pregnadien-3-one (**5**), 15 eV; (d) 1,4-cholestadien-3-one (**6**), 70 eV.

Scheme I



rearrangement[23] in the presence of acetic acid[24] of 16α,17α-epoxypregnenolone acetate (**15**)[25] to give a mixture of the *cis*- and *trans*-5,17-pregnadiene-3β,16α-diol 3-acetates (**16b**). Selective hydrogenation of the Δ^{17} double bond[26] followed by Collins oxidation provided the desired ketone **17**[27] which was electrochemically reduced[28] in a dioxane-D_2SO_4 solution to give 5-pregnen-3β-ol-*16,16-d*$_2$ (**18**). Base-catalyzed exchange of the active sites of **17** with deuteriomethanol prior to reduction in a dioxane-H_2SO_4 electrolyte furnished 5-pregnen-3β-ol-*15,15,17α-d*$_3$ (**19**). Oppenauer oxidation of **18** and **19** provided the corresponding labeled Δ^4-3-ketones, and subsequent selenium dioxide dehydrogenation gave the deuterated $\Delta^{1,4}$-3-ketones. In order to distinguish between deuterium transfers from C-15 and C-17, 4-pregnen-3-one-*17α,21,21,21-d*$_4$ was prepared by deuterium exchange of the active protons of pregnenolone followed by electrochemical reduction and Oppenauer oxidation.

Results and Discussion

The bond cleavages giving rise to the major diagnostic ions seen in the mass spectra of Δ^4- and $\Delta^{1,4}$-3-keto steroids (Figures 1 and 2) are summarized schematically below.

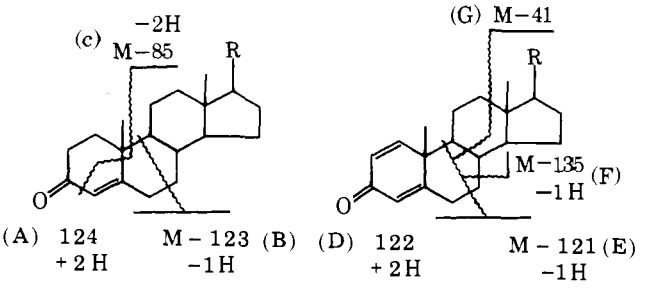


Table II. Peak Shifts[a] in the Mass Spectra of Deuterated Δ^4-3-Keto Steroids

compound	isotopic compn, %	*m/z* values for fragment ions (% shift[b] resulting from labeling) $C_8H_{12}O$ (A)	$M - C_8H_{11}O$ (B)	$M - C_5H_9O$ (C)
4-cholesten-3-one		124	261	299
-6,6,8β-d_3	92 d_3	127 (86%)	261 (31%)	300 (19%)
	8 d_2	126 (14%)	262 (69%)	301 (48%)
				302 (33%)
-7,7-d_2	98 d_2	124	263	301
	2 d_1			
-8β-d_1	93 d_1	125 (82%)	261 (33%)	299 (77%)
	6 d_2	124 (18%)	262 (67%)	300 (23%)
9α-d_1	87 d_1	125 (9%)	261 (17%)	299 (15%)
	13 d_0	124 (91%)	262 (83%)	300 (85%)
-11,11-d_2	95 d_2	125 (48%)	263	301
	3 d_1	124 (52%)		
	2 d_0			
-12,12-d_2	69 d_2	124	263	301
	29 d_1			
	2 d_0			
-14α-d_1	94 d_1	125 (20%)	261 (25%)	299 (51%)
	6 d_0	124 (80%)	262 (75%)	300 (49%)
4-pregnen-3-one		124	177	215
-15,15,17α-d_3	94 d_3	125 (42%)	179 (24%)	217 (13%)
	6 d_2	124 (58%)	180 (76%)	218 (87%)
-16,16-d_2	73 d_2	124	179	217
	17 d_3			
	6 d_1			
	4 d_0			
-17α,21,21,21-d_4	81 d_4	124	181	218 (10%)
	16 d_3			219 (90%)
	3 d_2			
total % of migrating hydrogens[c] accounted for:		201	99	185

[a] The % shift values have been corrected for ^{13}C contributions and effects due to isotopic composition. The spectra were measured at 12 or 15 eV. [b] These figures are reliable to ±5%. [c] A maximum value of 200% is expected for a process involving two hydrogen transfers.

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

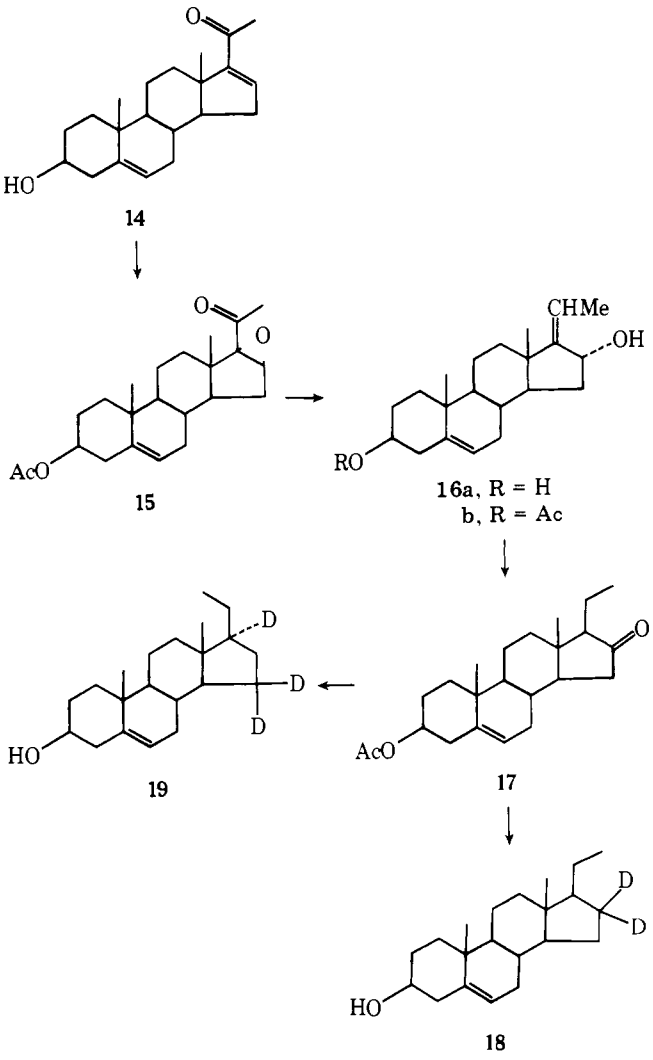
compound	isotopic cmpn, %	*m/z* values for fragment ions (% shift[b] resulting from labeling) $C_8H_{10}O$ (D)	$M - C_8H_9O$ (E)	$M - C_9H_{11}O$ (F)	$M - C_3H_5$ (G)
1,4-cholestadien-3-one		122	261	247	341
-8β-d_1	87 d_1	123 (94%)	261 (69%)	247 (42%)	341 (42%)
	10 d_2	122 (6%)	262 (31%)	248 (58%)	342 (58%)
	3 d_3				
-11,11-d_2	95 d_2	123 (92%)	262 (24%)	248 (64%)	342 (98%)
	3 d_1	122 (8%)	263 (76%)	249 (36%)	343 (2%)
	2 d_0				
-12,12-d_2	69 d_2	122	263	249	341
	29 d_1				
	2 d_0				
-14α-d_1	94 d_1	123 (12%)	261 (11%)	247 (24%)	342
	6 d_0	122 (88%)	262 (89%)	248 (76%)	
-2,4,6,6-d_4	3 d_1	126	261	247 (61%)	345
	10 d_2			248 (39%)	
	38 d_3				
	39 d_4				
	6 d_5				
	4 d_6				
1,4-pregnadien-3-one		122	177	163	257
-15,15,17α-d_3	94 d_3	122	180	165 (11%)	260
	6 d_2			166 (89%)	
total % of migrating hydrogens[c] accounted for:		198	104	180	140

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

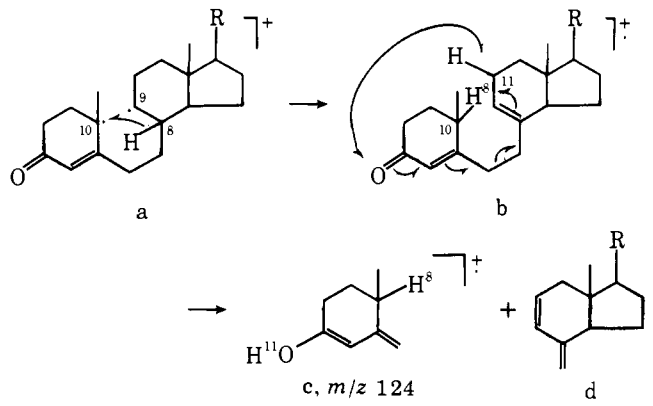
The origins of the itinerant hydrogen atoms (Tables II and III) were established by observing the shift of the *m/z* value for each key peak in the spectra of the deuterated derivatives.

***m/z* 124 Ion (A).** It is well established that the *m/z* 124 ion in the mass spectra of Δ^4-3-keto steroids is comprised of ring A plus carbons 6 and 19 and two additional hydrogen atoms. Shapiro and Djerassi[8b] have shown that C-8 and C-11 are major sources of the two itinerant hydrogens. Metastable defocusing (Table I) indicated that the molecular ion was the sole parent of the *m/z* 124 ion. This fragmentation can therefore

Scheme II

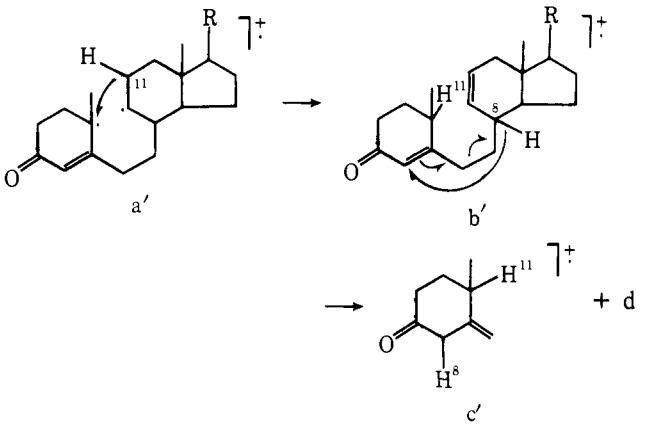


be visualized as occurring by the process illustrated below.



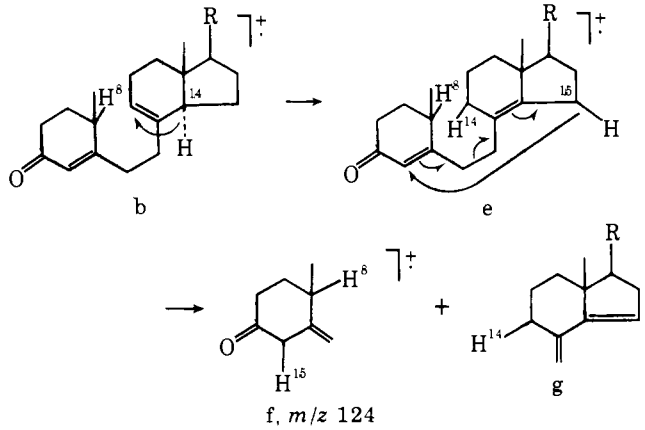
Fission of the allylically activated 9–10 bond of the ionized steroid relieves the steric compression of the fused A/B ring system and results in ion a, the most stable arrangement of radicals possible in this steroid nucleus. Migration of the hydrogen atom from C-8 to the radical site at C-10 is energetically favored by the resulting formation of the ionized diene b. Transfer of an allylic hydrogen atom from C-11 to the oxygen atom (appropriate interatomic distances were indicated with Dreiding models) triggers scission of the 6–7 bond to yield the observed *m/z* 124 ion c and the neutral fragment d.

A priori, this mechanism could be written with the hydrogen migration from C-11 preceding that from C-8 (a′ → c′).

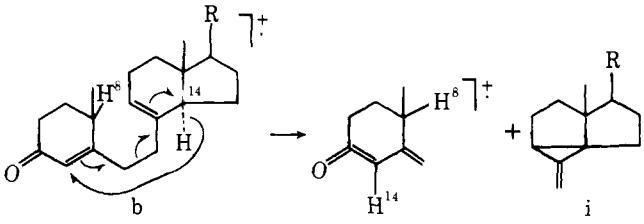


An attempt was made to differentiate between these two processes through direct analysis of the daughter ions (DADI)[29] of the *m/z* 124 ion. It was hoped that a key daughter ion could be found in the spectrum of a Δ^4-3-keto steroid labeled at C-8 or C-11 which might allow one to discern whether c or c′ had been the parent. Disappointingly, the major daughters resulted from loss of a methyl radical (~75%) and loss of carbon monoxide or ethylene (~20%).[30] As these ions were not deemed diagnostic for distinguishing between c and c′, we did not pursue this investigation. However, in view of the crucial migratory role played by the C-8 hydrogen in the M − 123 ring B cleavage and in both the *m/z* 122 and M − 121 B ring fragmentations of $\Delta^{1,4}$-3-ketones (vide infra), we propose that transfer of the hydrogen atom from C-8 is the primary initiating process in these transformations.

Although process a → c is indeed an important contributor to the *m/z* 124 ion, Table II indicates that hydrogen atoms from C-11 account for less than half of the second itinerant hydrogen. Quite surprisingly, C-15 proved to be the other major (42%) source. This result may be explained by a process involving the 1,3-sigmatropic shift[31] of a hydrogen from C-14 to C-9 in species b to yield the thermodynamically more stable ionized diene e.



Transfer of an allylically labilized hydrogen from C-15 to C-4 instigates the 6–7 bond fission to produce the *m/z* 124 ion f and the neutral diene g. (In this case consideration of interatomic distances with Dreiding models indicated that C-4 rather than the oxygen atom was more accessible to the second migrating proton.) Since the C-14 proton occupies an allylic position in species b and can come within close proximity of C-4, it is not surprising that it also is transferred to ring A (b → h) to a significant extent (20%). However, to the degree that the transition state for the final 6–7 bond fission in these ring B cleavage processes resembles the final products, this mechanism is energetically less favorable than those involving hy-



h, *m/z* 124

drogen migrations from C-11 or C-15 since the resulting neutral fragment i (or its equivalent) is a higher energy species than either d or g. This factor may well account for the smaller percentage of hydrogen transfer observed from C-14 compared with the seemingly less likely C-15 position. The 9% transfer of a hydrogen atom from C-9 to ring A corroborates the postulated 1,3-hydrogen shift (b → e) since the C-9 proton becomes allylically activated as a result of this isomerization.

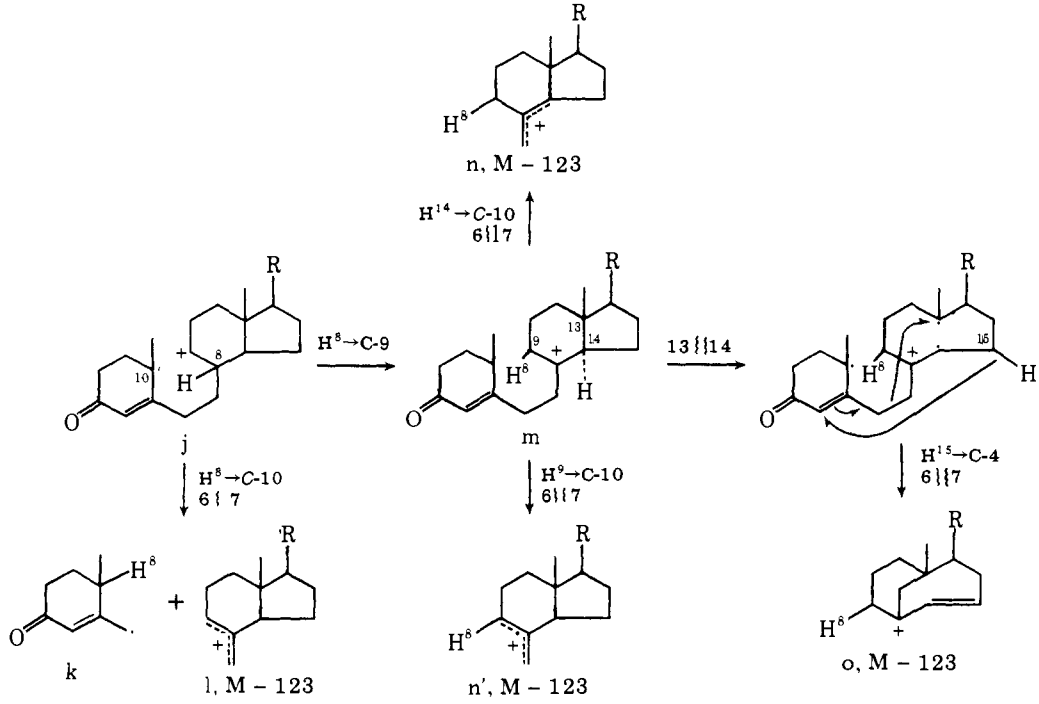
Zaretskii[32] has summarized data demonstrating a stereochemical influence upon the ring B cleavage in Δ^4-3-keto steroids—the "abnormal" 10-iso steroids exhibit more intense *m/z* 124 ions than their "natural" 10β-counterparts. Since the stereochemistry at C-9 (i.e., a cis vs. trans B/C ring juncture) has no appreciable influence[32] upon this fragmentation process, it is unlikely that the differing behavior of the 10α and 10β compounds is due simply to differences in internal energy. The important factor must then be the relative ease of hydrogen migration. The observed stereochemical sensitivity is readily rationalized when this hypothesis is examined in terms of the mechanistic representation presented earlier (a → c) for ring B cleavage. The initiating step is scission of the 9–10 bond and transfer of the C-8 hydrogen to C-10 (a → b). This transformation must be concerted with attachment of the migrating hydrogen to C-10 occurring synchronously with the rupture of the 9–10 bond and detachment of the hydrogen from C-8. Inspection of models clearly demonstrates that this process is more favorable when the hydrogen can approach C-10 from the β face and, hence, should occur more readily in the 10α-Δ^4-3-keto steroids. The diminished proclivity to ring B cleavage observed for the 8α isomer of 19-nortestosterone (compared with the "normal" 8β compound)[32] is also explained by this hypothesis. Ring C of the 8α steroid can adopt a stable boat conformation which moves the C-8 proton away from C-10, thereby denying the possibility of the hydrogen migration occurring synchronously with cleavage of the 9–10 bond.

***m/z* 122 Ion (D).** The introduction of a second double bond into ring A to give the $\Delta^{1,4}$-3-ketones causes a dramatic increase in the relative intensity of the ring B cleavage peak at *m/z* 122. This phenomenon is undoubtedly due to the now double allylic activation of the 9–10 bond, energetically favoring its scission to the near exclusion of any other. The lack of other competitive initiating fragmentations leads the molecule inevitably to cleavage of the B ring. The data of Table III indicate that the two hydrogen transfers are highly site-specific—C-8 and C-11 account for 93% of the itinerant hydrogens. Thus, *the ring B cleavage of these dienones is a considerably simpler process than that of the* Δ^4*-3-keto steroids* and may be almost solely accounted for by the sequence a → c (with the additional Δ^1 double bond). The *m/z* 122 ion is a tautomer of ionized 3,4-dimethylphenol. There is a small contribution (12%) to the migrating hydrogens from C-14, corresponding to operation of the b → h pathway. Apparently the energetics are such that the 1,3-sigmatropic shift necessary to activate the C-15 hydrogen atoms for transfer (b → e for Δ^4-3-ketones) cannot compete effectively in the case of the $\Delta^{1,4}$-3-keto steroids.

Thus, these two ring B cleavages are consistently initiated by concerted rupture of the 9–10 bond and transfer of the C-8 hydrogen to C-10. The final homolysis of the 6–7 bond is invariably triggered by the migration of a second hydrogen from an allylically activated position. The only difference between the mechanisms for the Δ^4- and $\Delta^{1,4}$-3-keto steroids is the specificity of the second hydrogen transfer.

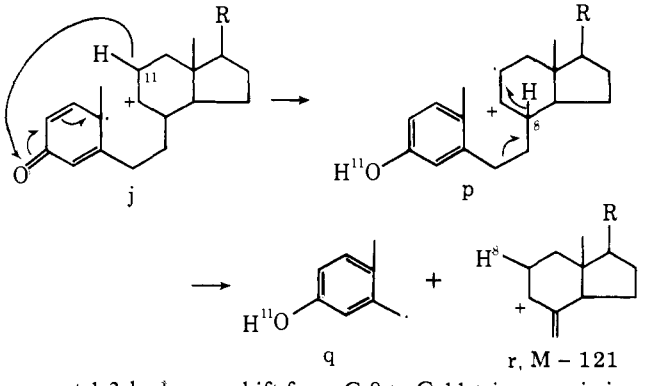
M − 123 Ion (B). An ion (*m/z* 149) resulting from fission of the 6–7 and 9–10 bonds, but with the charge now being retained by the hydrocarbon portion (rings C and D) of the molecule, is observed in the spectrum (Figure 1a) of 4-androsten-3-one. This M − 123 ion is characteristic of Δ^4-3-ketones; it is also observed in the spectra of 4-pregnen-3-one (*m/z* 177) (Figure 1b) and 4-cholesten-3-one (*m/z* 261) (Figure 1d). In each case the molecular ion is the sole progenitor, and one hydrogen atom is transferred away from the charged fragment. The data in Table II prove that this transfer is not very specific—significant contributions are made by C-8 (33%), 14 (25%), 15 (24%), and 9 (17%). The migration of the same

Scheme III

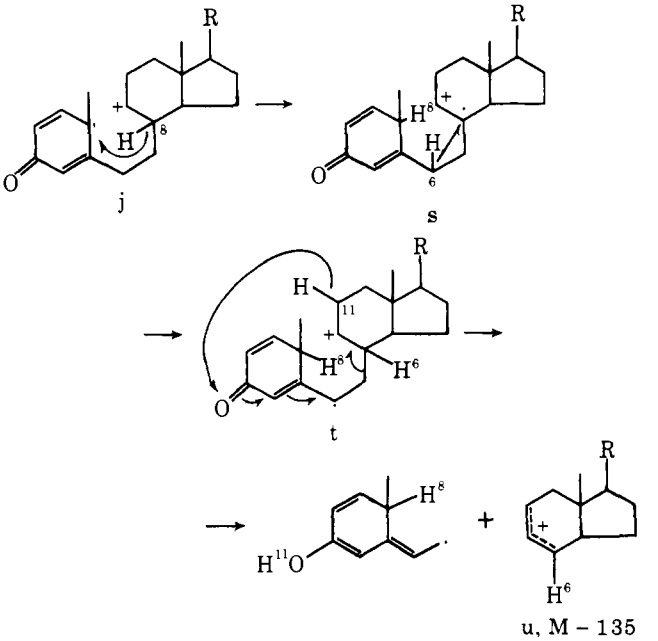


hydrogens (with the notable exception of the C-11 protons) as were involved in the formation of the *m/z* 124 ion suggests the operation of a similar mechanism. Initial ionization of the allylic 9-10 bond to place a positive charge on C-9 apparently competes to some degree with charge localization in ring A. Generation of the molecular ion j thus initiates the process leading to the M − 123 ion. Direct transfer of the proton from C-8 to C-10 facilitates fission of the 6-7 bond (j → l) to furnish the even-electron, allylically stabilized cation l. This is the simplest and major process (33% transfer of the C-8 hydrogen) contributing to the M − 123 ion. Since the direct scission of two bonds on the same carbon atom is an unfavorable reaction, the observed transfer of a hydrogen from C-9 is a strong indication that saturation of the C-9 radical site by a hydrogen shift is occurring. Such a 1,2-hydrogen shift[31] from C-8 to C-9 (j → m) generates a more stable molecular ion in which several bond fissions can be postulated so as to rationalize the observed results. Thus, subsequent migration of a hydrogen to C-10 from either C-14 (m → n) or C-9 (m → n′) would trigger the rupture of the 6-7 bond. Fission of the 13-14 bond of the strained hydrindan system in m could activate the protons on C-15 and allow them to approach C-4. Transfer of a C-15 hydrogen would then precipitate cleavage of the 6-7 bond to give the M − 123 ion o. So in each case migration of a hydrogen atom to ring A could initiate the 6-7 bond rupture to yield an allylic cation. *The C-11 hydrogens are not involved* since transfer from that site in j fails to provide a system in which the 6-7 bond becomes allylic and thereby prone to fission. Significantly, very little difference in intensity is observed between the M − 123 ion equivalents for 10α- and 10β-testosterone,[32] indicating that *this B-ring cleavage process, as implied in the above mechanism, is not concerted.* The intensity of the M − 123 ion is enhanced by alkyl substituents at C-6[33,34] since they facilitate the scission of the 6-7 bond. See Scheme III.

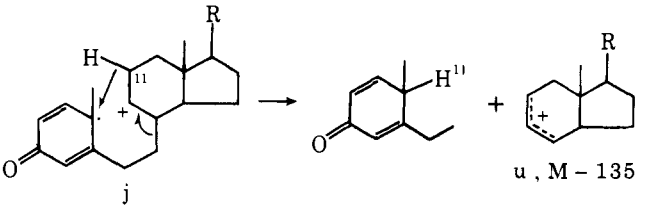
M − 121 Ion (E). The analogous fragmentation in $\Delta^{1,4}$-3-keto steroids gives rise to an ion at M − 121 at the same *m/z* values as observed for the three Δ^4-3-ketones. These peaks are smaller in the present case since here the overwhelming majority of the total ion current is carried by the *m/z* 122 ion. Again, the hydrogen transfer is considerably more site-specific than in the Δ^4-3-keto steroids. Table III demonstrates that C-8 accounts for almost 70% of the single transposed hydrogen, the remainder originating from C-11 (24%) and C-14 (11%). Mechanisms j → l and j → n (with the addition of a Δ^1 double bond) explain the transfers from C-8 and C-14. The migration of a C-11 hydrogen is quite surprising in light of its conspicuous absence in the Δ^4-3-ketone case. The most plausible mechanism would seem to involve initial transfer of a C-11 hydrogen to the oxygen atom in ion j (models demonstrate appropriate interatomic distances) to give the phenolic species p. A subsequent 1,3-hydrogen shift from C-8 to C-11 triggers scission of the 6-7 bond to give the very stable benzylic radical q and the M − 121 allylic cation r. Such a pathway would not be expected to make as significant a contribution to the M − 123 ion in the case of the Δ^4-3-keto steroids. These two ring B cleavages in which the positive charge is retained by the hydrocarbon portion of the steroid (fragmentations B and E) thus both proceed via migration of a single hydrogen atom to generate even-electron, allylic cations.



M − 135 Ion (F). The spectra (Figure 2) of all three $\Delta^{1,4}$-3-keto steroids exhibit an ion corresponding to a third type of ring B cleavage: *m/z* 135 for 1,4-androstadien-3-one, *m/z* 163 for 1,4-pregnadien-3-one, and *m/z* 247 for 1,4-cholestadien-3-one. This ion at M − 135 is comprised of rings C and D and results from fission of the 9-10 and 7-8 bonds with a *net* transfer of one hydrogen away from the charged fragment. However, Table III indicates that a *total of three hydrogen rearrangments* are involved in this fragmentation. The itinerant hydrogens originate mainly from C-11 (64%), C-8 (42%), and C-6 (39%). The following mechanism would account for these results.

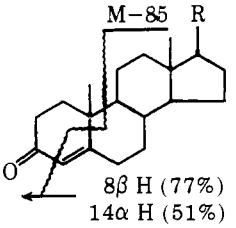


The initial transposition of a hydrogen from C-8 to C-10 (j → s) necessitates a reciprocal transfer back to C-8 (s → t) to alleviate the vinylic character of the 7-8 bond. Migration of a C-11 hydrogen to the oxygen atom and subsequent rupture of the doubly allylically activated 7-8 bond give a conjugated radical and the allylic cation u. It is quite likely that a single hydrogen transfer process (j → u′) also contributes to the formation of the M − 135 ion.



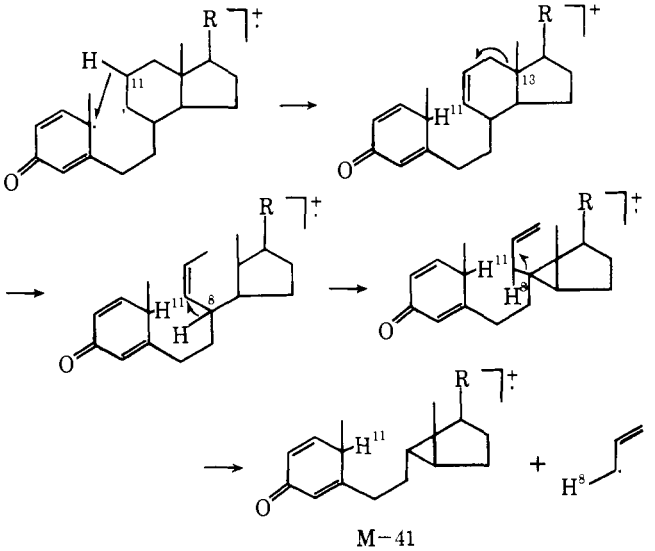
M − 85 Ion (C). The M − 85 ion (*m/z* 187 for 4-androsten-3-one, *m/z* 215 for 4-pregnen-3-one, *m/z* 299 for 4-cholesten-3-one) is quite characteristic of the Δ^4-3-ketone moiety. Shapiro and Djerassi[8b] established that C-2, C-3, and C-19 were lost while C-6 and C-7 were retained in this fragmentation. The mass spectrum of 4-cholesten-3-one-1β-d_1[35] indicated that C-1 is also expelled. Making the reasonable assumption that C-10 is lost, this ion must arise from a complicated process involving fissions of the 9-10, 5-10, and 3-4 bonds with two hydrogen atoms being transferred out of the charged hydrocarbon fragment. The labeling data (Table II) prove that once again C-8 is the predominant source (77%) of one of the transposed hydrogen atoms. The second migrating

hydrogen has multiple origins, but C-14 is the major (51%) contributing site.



The metastable defocusing data (Table I) indicate that the major progenitor of this ion is *not* the molecular ion but rather the M − 42 ion (loss of ketene). Metastable ions observed in the spectra presented in Figure 1 demonstrated that the M − 42 ion is the parent of not only the M − 85 ion but several other ions as well. It would require too much speculation to write a detailed mechanism for this complex process which must involve migration of hydrogen atoms from C-8 and C-14 to carbons 1, 19, or 10 and the eventual extrusion of these atoms from the M − 42 ion.

M − 41 Ion (G). One of the small but discernible peaks in the molecular-ion region of $\Delta^{1,4}$-3-keto steroids appears at M − 41. As indicated on the individual spectra of Figure 2, the observation of the appropriate metastable peaks combined with high resolution mass measurements proved that this ion was formed by the loss of $C_3H_5\cdot$ directly from the molecular ion. The loss of both of the isotopic labels of 1,4-cholestadien-3-one-*12,12-d*$_2$ (Table III) in this cleavage process indicated that C-12 was one of the expelled carbon atoms. A priori this ion would then most likely arise from loss of either the C-12, 13, and 18 or the C-9, 11, and 12 segment with no *apparent* hydrogen transfer in either case. However, for 1,4-cholestadien-3-one-*11,11-d*$_2$ the retention of only one deuterium atom by the charged fragment suggested the latter process, accompanied by a reciprocal hydrogen transfer. The observed displacement of the C-8 proton (42%) corroborates this explanation of the genesis of the M − 41 ion. A mechanism consistent with these data is presented below.



Effect of Substituents. Such mechanistic studies on the fragmentations of relatively simple steroids enable one to predict the cleavage patterns that will arise in considerably more complex analogues. For example, the observed influence of additional functionality on the diagnostic ring B cleavages of Δ^4- and $\Delta^{1,4}$-3-keto steroids (processes A and D, respectively) is readily rationalized in light of the mechanistic representations presented earlier.

The mass spectra of a number of Δ^4-3-keto steroids have been published due to the ubiquitous occurrence of the Δ^4-3-ketone moiety in steroids of biological importance. The characteristic *m/z* 124 ion is generally a prominent feature of the spectra. However, in certain cases substituents exert a pronounced effect. In a few instances (e.g., 17β-methoxy-4-pregnen-3-one,[36] corticosterone,[37] and 19-hydroxy-4-androstene-3,17-dione[38]), other fragmentations are so highly favored that the ring B cleavage ion is not seen. The presence of a ketone at C-11 blocks this fragmentation pathway by denying the possibility of the requisite second hydrogen transfer (b → c) and instead gives rise to a prominent *m/z* 122 ion by a different process.[8b] Predictably, a $\Delta^{9(11)}$ double bond inhibits formation of the *m/z* 124 ion[39] since two of the bonds which must be ruptured in the process (C-9–C-10 and H–C-11) are vinylic. Likewise, a Δ^7 double bond also suppresses ring B cleavage.[40]

The introduction of a ketone at C-6 causes a ring B scission process to occur in which only one hydrogen atom is transferred to the charged fragment.[41] This difference is most probably due to the fact that the 6-7 bond in the 6-keto analogue of b is sufficiently activated for direct cleavage (α-fission of a ketone) so that the second hydrogen transfer is not required to trigger its homolysis. For the same reason, alkyl substituents at C-6 allow some ring B cleavage to occur with the migration of only one hydrogen atom (however, the double hydrogen rearrangement process still predominates).[42] Hydroxyl substituents at C-11[43] or C-14[44] generate significant ions at *m/z* 123 by interfering with the transfer of hydrogen atoms from those sites.

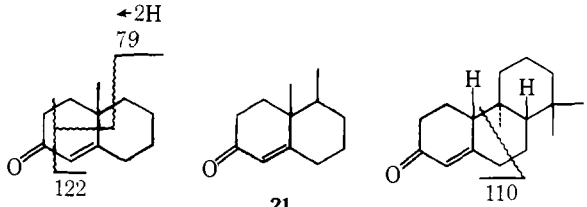
It has been suggested[44] that the 11β-hydrogen is preferentially transferred in the ring B cleavage. This conclusion was drawn from the observation[44] that the spectrum of 11β-hydroxyprogesterone showed a significantly smaller peak at *m/z* 124 than did the 11α epimer. However, since examination of Dreiding models reveals no preference for transfer of the 11β- over the 11α-hydrogen in process b → c, this result is probably attributable to simply an increased proclivity for dehydration in the 11β-hydroxy (axial) compound (the mass spectrum of this molecule does indeed show a much larger M − 18 ion than is observed for 11α-hydroxyprogesterone). Electron impact induced 1,3-elimination of water[45] from the 11β epimer would involve loss of the crucial 8β proton, thereby inhibiting formation of the *m/z* 124 ion. As expected, hydroxyl groups at the 7,[46] 9,[44] or 12[46] positions, a ketone at C-12,[39] or an alkyl substituent at C-16[47] do not impede the B ring fragmentation. Horváth and Ambrus[48] recently demonstrated that for 9α-hydroxy Δ^4-3-ketones an alternative mechanism for ring B cleavage involving migration of the hydroxyl proton makes a significant contribution to the *m/z* 124 ion. It has been reported[32] that the B ring fission is less pronounced in 19-nor steroids.[49]

The number of $\Delta^{1,4}$-3-keto steroids for which mass spectra have been reported is considerably smaller. However, similar substituent effects upon the *m/z* 122 ion have been observed. The introduction of a hydroxyl group at C-11[50] again interferes with the transfer of a hydrogen atom from that site and thus leads to the appearance of a substantial ion at *m/z* 121. In the case of a C-11 ketone,[51] the hydrogen migration is completely blocked and the base peak of the spectrum then appears at *m/z* 121. Hydroxyl functionalities at C-6[52] or C-17[53] or in a C-17 side chain[54] do not affect the B ring fragmentation. The mass spectrum of 1,4,7-cholestatrien-3-one[55] is considerably more complex than that of its $\Delta^{1,4}$ analogue. The Δ^7 double bond hinders the ring B fission process, causing other fragmentations to become competitive.

The ring B cleavage peak in the high resolution spectrum of 2-chloromercuri-1,4-androstadiene-3,17-dione is shifted in the expected manner to *m/z* 358.[56] This result is noteworthy from two standpoints. First, it is an indication of the extremely facile nature of ring B cleavage in these cross-conjugated di-

enones since fission of the B ring is apparently favored over rupture of the quite labile C-Hg bond. Second, it demonstrates that a mechanism, involving fission of the 9-11 and 8-14 bonds with charge retention in ring D, previously proposed[57] to explain the *m/z* 122 ion in the spectrum of 1,4-androstadiene-3,17-dione is almost certainly incorrect.

We were interested in ascertaining if some of the characteristic fragmentations discussed above also occurred in nonsteroidal α,β-unsaturated ketones. Thus, 10-methyl-1(9)-octal-2-one (**20**)[58] was prepared and its mass spectrum examined. The base peak at M − 42 (*m/z* 122; loss of ketene) was accompanied by significant peaks at M − 57 (*m/z* 107; loss of ketene plus a methyl radical) and M − 85 (*m/z* 79; from a process analogous to fragmentation C). The identity of the latter ion was confirmed by high resolution measurements and the observation that the M − 85 peak remained at *m/z* 79 in the spectrum of the *10*-d_3 labeled compound. The mass spectrum of *cis*-5,10-dimethyl-1(9)-octal-2-one (**21**)[59] displayed a very similar fragmentation pattern. This compound, although possessing the requisite hydrogens for transfer at C-8 and C-11 (steroid numbering), did not give the characteristic *m/z* 124 ion observed for the Δ⁴-3-keto steroids. On the other hand, the mass spectrum of the tricyclic diterpene **22**[60] exhibited an abundant ion at *m/z* 110 due to such ring B cleavage.



20 **21** **22**

Conclusions

The electron impact induced ring B cleavages of Δ⁴- and Δ¹,⁴-3-keto steroids generate abundant ions of great diagnostic utility. The facile process in which the charge is retained by ring A is initiated by concerted rupture of the 9-10 bond and migration of a hydrogen from C-8 to C-10. Subsequent transfer of an allylic hydrogen from C-11 or C-15 triggers cleavage of the 6-7 bond. In the alternative process where rings C and D retain the positive charge, the transfer of a single hydrogen atom (mainly from C-8, 11, 14, or 15) precipitates the 6-7 bond fission to generate an even-electron allylic cation. In both types of ring B cleavage the hydrogen migrations are considerably more site specific for the Δ¹,⁴-3-ketones. The effects of additional substituents at key positions in the steroid nucleus can now be rationalized on a mechanistically plausible basis.

Experimental Section

General. Low-resolution mass spectra were obtained by Mr. R. G. Ross on an AEI MS-9 spectrometer using a direct inlet system. Exact masses were determined on a Varian-MAT 711 high-resolution mass spectrometer by Ms. A. Wegmann. ^{1}H NMR spectra were obtained on a Varian T-60 spectrometer 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[61] Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected.

5α-Cholestan-3β-ol-*14α-d*$_1$ Propionate (8a). The addition of deuterated diborane (generated in situ as described by Sondheimer[62]) to 2.42 g of 5α-cholest-14-en-3β-ol[11] (**7**) and subsequent hydrolytic cleavage of the boron adduct with propionic acid[10] furnished **8a**. Recrystallization from ethanol gave clear, colorless leaflets:[63] yield 1.55 g, 56%; mp 123-125 °C; NMR δ 4.7 (m, 1 H, C-3 axial), 2.3 (quart, 2 H, propionate), 0.82 (s, 3 H, C-19), 0.67 (s, 3 H, C-18); isotopic composition 94% d_1, 6% d_0.

2α,4α-Dibromo-5α-cholestan-3-one-*14α-d*$_1$ (9). Hydrolysis of the ester **8a** gave 5α-cholestan-3β-ol-*14α-d*$_1$[63] (**8b**) which was quantitatively converted to 5α-cholestan-3-one-*14α-d*$_1$[63] by Jones oxidation and subsequently brominated according to the method of Djerassi and Scholz.[12] To a solution of 1.2 g of the ketone in 35 mL of glacial acetic acid was added 1.0 g of bromine dissolved in 15 mL of the same solvent. (For smaller scale reactions, the use of pyridinium hydrobromide perbromide[64] proved to be more advantageous.) The 2,2-dibromo steroid precipitated out of solution almost immediately. The stirred mixture was heated to 105 °C in an oil bath to give a homogeneous solution and then allowed to cool. The resulting crystalline material was collected by filtration and washed with ethanol to give **9**: yield, 0.93 g, 55%; mp 186.5-187.5 °C (d) (hexane) (lit.[12b] 188-191 °C (d)); isotopic composition 94% d_1, 6% d_0.

4-Cholesten-3-one-*14α-d*$_1$ (10). An acetone solution of 0.76 g of the dibromide **9** was refluxed with sodium iodide for 24 h and the resulting crude 2-iodocholest-4-en-3-one was dehalogenated directly with chromous chloride according to the procedure of Rosenkranz et al.[13] Silica gel chromatography of an ether extract of the reaction mixture gave **10**:[63,65] yield, 0.24 g, 44%; mp 81-81.5 °C (methanol) (lit.[66] 83-84 °C); NMR δ 5.7 (s, 1 H, vinyl), 1.2 (s, 3 H, C-19, calcd 1.2), 0.70 (s, 3 H, C-18, calcd 0.72).

1,4-Cholestadien-3-one-*14α-d*$_1$ (11). A mixture of 0.85 g of the dibromide **9** and 0.31 g of lithium chloride in 5 mL of DMF was refluxed under nitrogen for 1 h.[14] Water was added and the mixture was extracted with benzene. Evaporation of the dried organic layer yielded a yellow oil which was chromatographed on 120 g of silica gel with a 4% ether/hexane solution. Evaporation of an early fraction gave 0.31 g (52%) of 4,6-cholestadien-3-one-*14α-d*$_1$. A subsequent fraction furnished 1,4-cholestadien-3-one-*14α-d*$_1$[63,65] (**11**): yield, 0.14 g, 23%; mp 115-116.5 °C (methanol) (lit.[67] 110-112 °C); NMR δ 7.07 (d, 1 H, C-1), 6.23 (dd, 1 H, C-2), 6.07 (d, 1 H, C-4), 1.22 (s, 3 H, C-19, calcd 1.23), 0.74 (s, 3 H, C-18, calcd 0.74).

1,4-Cholestadien-3-one-*8β-d*$_1$. A mixture of 48 mg of 4-cholesten-3-one-*8β-d*$_1$ (**13**) (prepared by the method of Tökés et al.[15]) and 26 mg of selenium dioxide in 5 mL of *tert*-butyl alcohol containing one drop of acetic acid was heated under nitrogen at 70 °C for 24 h.[16] The reaction mixture was diluted with hot ethyl acetate and filtered through Celite. The filtrate was washed with dilute sodium hydroxide solution followed by water and then dried and evaporated to give a brown oil which upon chromatography on silica gel furnished 24 mg (51%) of 1,4-cholestadien-3-one-*8β-d*$_1$.[63,65]

4-Cholesten-3-one-*7,7-d*$_2$. Oppenauer oxidation[17] of 1.49 g of cholesterol-*7,7-d*$_2$ (prepared according to the procedure of Cunningham and Overton[18]) gave, after chromatography and recrystallization, 1.05 g (71%) of 4-cholesten-3-one-*7,7-d*$_2$.[63,65]

1,4-Cholestadien-3-ones: -*11,11-d*$_2$, -*12,12-d*$_2$. These steroids[63,65] were prepared from the corresponding labeled 5α-cholestan-3α-ols[19] in exactly the same manner as dienone **11**.

4-Pregnen-3-one (2). Oppenauer oxidation[17] of 6.0 g of 5-pregnen-3β-ol[68] followed by chromatography on silica gel gave **2**: yield 3.9 g, 65%; mp 101-103 °C (petroleum ether) (lit.[69] 91-92 °C); NMR δ 5.69 (s, 1 H, vinyl), 1.17 (s, 3 H, C-19, calcd 1.20), 0.60 (s, 3 H, C-18, calcd 0.62); $[\alpha]^{21}_D$ +108° (*c* 0.3436, $CHCl_3$).

1,4-Pregnadien-3-one (5). Dehydrogenation of 1.0 g of **2** with selenium dioxide as described for 1,4-cholestadien-3-one-*8β-d*$_1$ gave, after silica gel chromatography, **5**: yield, 0.48 g, 49%; mp 99-100.5 °C (hexane); $[\alpha]^{21}_D$ +22° (*c* 0.326, $CHCl_3$); UV 245 nm (ε 14 000); IR 1660, 1710 cm^{-1}; NMR δ 7.06 (d, 1 H, C-1, *J* = 10 Hz), 6.21 (dd, 1 H, C-2, *J* = 10, 2 Hz), 6.06 (bs, 1 H, C-4), 1.22 (s, 3 H, C-19, calcd 1.24), 0.64 (s, 3 H, C-18, calcd 0.65); MS *m/z* 298.2302 ($C_{21}H_{30}O$, M^+).

***cis*- and *trans*-5,17-Pregnadiene-3β,16α-diol 3-Acetate (16b).** A solution of 6.08 g of 16α,17α-epoxypregnenolone acetate[70] (**15**), 3 mL of acetic acid, and 30 mL of 85% hydrazine hydrate in 500 mL of isopropyl alcohol was refluxed on a steam bath for 15 min and immediately cooled in ice. The yellow solution was poured into 2 L of ice-water, and the resulting precipitate was taken up in chloroform. The organic layer was washed with water, dried, and evaporated. The yellow solid so obtained was chromatographed on silica gel to give 2.23 g (38%) of *cis*- and *trans*-**16b.** These isomers could be separated by more careful chromatography, the trans compound eluting from silica gel prior to the cis compound. *trans*-**16b** was obtained as stout needles from methanol: mp 186-192 °C (lit.[21] 193-194 °C); NMR δ 5.35 (m, 2 H, vinyl), 4.79 (m, 1 H, C-16), 4.57 (m, 1 H, C-3 axial), 2.02 (s, 3 H, acetate), 1.78 (d, 3 H, C-21, *J* = 7 Hz), 1.03 (s, 3 H, C-19), 0.76

(s, 3 H, C-18); MS *m/z* 298 (M − AcOH).

cis-**16b** was obtained as large prisms from methanol: mp 143–145 °C (lit.[21] 145–146 °C); NMR δ 5.56 (quart, 1 H, C-20, *J* = 7 Hz), 5.35 (m, 1 H, C-6), 4.58 (m, 1 H, C-3 axial), 4.40 (m, 1 H, C-16), 2.01 (s, 3 H, acetate), 1.73 (d, 3 H, C-21, *J* = 7 Hz), 1.03 (s, 3 H, C-19), 0.88 (s, 3 H, C-18); MS *m/z* 298 (M − AcOH).

3β-Acetoxy-5-pregnen-16-one (17). Hydrogenation of 1.71 g of the cis,trans mixture **16b** with 5% Pd/C in 200 mL of absolute ethanol[26] provided 5-pregnene-3β,16α-diol 3-acetate: yield, 1.6 g, 93%; mp 159–161 °C (methanol) (lit.[21] 160–161 °C); NMR δ 5.36 (m, 1 H, vinyl), 4.58 (m, 1 H, C-3 axial), 3.96 (m, 1 H, C-16), 2.32 (d, 2 H, C-4), 2.01 (s, 3 H, acetate), 1.02 (s, 3 H, C-19), 0.62 (s, 3 H, C-18); MS *m/z* 300 (M − AcOH).

A methylene chloride solution of 1.08 g of this acetate was added dropwise to a stirred mixture of 2.0 g of chromium trioxide and 3.3 mL of dry pyridine in 75 mL of methylene chloride. After 1 h the reaction mixture was diluted with ether, filtered through Florisil, and evaporated. The residue was chromatographed on silica gel to give **17**: yield, 0.90 g, 86%; mp 148.5–150 °C (lit.[21] 150–153 °C); NMR δ 5.40 (m, 1 H, vinyl), 4.61 (m, 1 H, C-3 axial), 2.03 (s, 3 H, acetate), 1.07 (s, 3 H, C-19), 0.74 (s, 3 H, C-18); MS *m/z* 298 (M − AcOH).

5-Pregnen-3β-ol-*16,16-d*$_2$ (18). A solution of 1.01 g **17** in 70 mL of a 70/30 dioxane/10% D_2SO_4 electrolyte was placed in the cathode compartment of an electrochemical cell.[28] An identical solvent mixture was added to the anode compartment, lead electrodes were inserted, and a current of 100 mA was maintained for 24 h. The catholyte was neutralized with dilute potassium hydroxide and the dioxane was evaporated. The product was taken up in ether, washed with water, dried, evaporated, and chromatographed on silica gel to give 0.52 g (61%) of **18**, 0.10 g (10%) of the corresponding acetate, and 0.11 g (12%) of hydrolyzed **17**. Recrystallization of **18** from methanol gave translucent flakes:[63] mp 133–135 °C (lit.[68] 134–135 °C); isotopic composition 73% d_2, 17% d_3, 6% d_1, 4% d_0.

5-Pregnen-3β-ol-*15,15,17α-d*$_3$ (19). A solution of 1.0 g of ketone **17**, 1 g of sodium, and 20 mL of D_2O in 50 mL of deuteriomethanol was refluxed for 24 h. Upon cooling 0.69 g (77%) of 3β-hydroxy-5-pregnen-16-one-*15,15,17α-d*$_3$ crystallized out of the reaction mixture. A solution of this labeled ketone in a 70/30 dioxane/10% H_2SO_4 electrolyte was electrochemically reduced at 200 mA for 12 h as described for **18**. Recrystallization of the chromatographed product from methanol gave **19**[63] as shiny flakes: isotopic composition 96% d_3, 4% d_2.

5-Pregnen-3β-ol-*17α,21,21,21,-d*$_4$. Exchange of the acidic protons of pregnenolone with alkaline deuteriomethanol and subsequent electrochemical reduction of the ketone functionality as described for **19** gave 5-pregnen-3β-ol-*17α,21,21,21-d*$_4$:[63] isotopic composition 79% d_4, 16% d_3, 3% d_2.

4-Pregnen-3-ones: *-16,16-d*$_2$, *-15,15,17α-d*$_3$, *-17α,21,21,21-d*$_4$. These steroids[63,65] were prepared from the corresponding labeled alcohols by Oppenauer oxidation in exactly the same manner as the unlabeled analogue **2**.

1,4-Pregnadien-3-one-*15,15,17α-d*$_3$. This dienone[63,65] was obtained from 4-pregnen-3-one-*15,15,17α-d*$_3$ by selenium dioxide dehydrogenation as described for 1,4-cholestadien-3-one-*8β-d*$_1$.

1,4-Cholestadien-3-one-*2,4,6,6-d*$_4$. This compound[63,65] was obtained from **6** by exchanging the active protons in alkaline deuteriomethanol as described for **19**.

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^1H, ^2H, and ^{13}C ENDOR Studies of Labeled Bis(biphenylenyl)propenyl Type Radicals in Isotropic Solutions and in Liquid Crystals

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Abstract: Partially deuterated and ^{13}C-labeled bis(biphenylenyl)propenyl radicals have been studied by means of ESR and ENDOR spectroscopy. Isotropic and anisotropic hyperfine contributions could be determined from measurements in isotropic solutions and in nematic and smectic phases of liquid crystals. Assignments of hyperfine coupling constants and shifts to molecular positions were achieved. Conclusions concerning molecular structure, e.g., twist angles, could be drawn by relating the experimental data to quantum mechanical calculations. These results could be confirmed by taking account of the ^{13}C hyperfine shifts determined by ESR and ^{13}C ENDOR experiments. A marked isotope effect on the β-proton hyperfine coupling could be observed when replacing ^1H by ^2H in the biphenylenyl moieties. The essential feature of the ^2H ENDOR measurements in liquid crystals is the detection of deuterium quadrupole splittings. The relaxation behavior of the different magnetic nuclei is discussed. A novel *multinuclear ENDOR standard* for checking the engineering design of a liquid-phase ENDOR spectrometer is proposed.

Introduction

Koelsch's radical (**1a**) is known to be one of the most inert, stable organic free radicals. In this respect it has attracted much attention, in view of the history of organic free radicals, for the following fact: The original publication of Koelsch was rejected in 1932 by a referee, since he did not realize that the alleged radical did not react readily with oxygen, and it took 25 years of time until the paper was accepted for publication.[2]

Koelsch's radical is unassociated even in the solid state, whereas the sterically less hindered related radical bis(biphenylenyl)propenyl (**2a**) was found to be dimeric to a high extent.[3] In the early years of ESR spectroscopy, this very radical was studied in a pioneering work of Hausser who succeeded in resolving 400 ESR lines, which is about one-third of the maximum number of lines expected from symmetry considerations.[4]

The most interesting aspect revealed by the ESR spectrum of **2a** is the exceptionally large hyperfine coupling constant of the proton at the central carbon atom, which significantly contradicts the predictions of the McConnell relation based on spin populations from HMO/McLachlan calculations.[5] The anomalously large hyperfine coupling constant could be explained by introducing a twisted allyl model and by assuming a "through-space" (hyperconjugative) interaction of the two p_z orbitals on the adjacent centers with the 1s orbital of the respective proton.[6]

In the present paper we give the results of an intense ENDOR investigation of the bis(biphenylenyl)propenyl system. In order to obtain detailed information about the isotropic and anisotropic hyperfine interactions and thus about the spin density distributions and the geometries of the radicals, we have applied elaborate techniques such as ENDOR in liquid-crystalline solvents, nonproton ENDOR, and TRIPLE resonance. For the nonproton ENDOR experiments, some deuterated and ^{13}C-labeled radicals of the bis(biphenylenyl)propenyl type had to be synthesized (see Figure 1). Our investigation consists of three parts:

(i) The isotropic ^1H, ^2H, and ^{13}C hyperfine coupling constants were measured, and the relative signs of all couplings were determined by the TRIPLE technique.[7] Moreover, the significant temperature dependence of the β-proton coupling constant of **2** was reinvestigated.

(ii) Information about the anisotropic contributions of all ^1H, ^2H, and ^{13}C hyperfine interactions was obtained by using nematic and smectic mesophases of liquid crystals as solvents. In addition, we succeeded in observing deuterium quadrupole splittings. Such splittings were recently described in three papers dealing with liquid-crystal ENDOR studies of deuterated phenalenyls[8,9] and Coppinger's radical.[10]

(iii) Recently the feasibility of nonproton ENDOR experi-

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