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Mass Spectrometry in Structural and Stereochemical Problems. L.[1] Fragmentation and Hydrogen Migration Reactions of α,β-Unsaturated 3-Keto Steroids[2]

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RECEIVED FEBRUARY 7, 1964

The cleavages of Δ^1- and Δ^4-androsten-3-one, induced upon electron impact, have been determined in an investigation of the mass spectra of suitably labeled derivatives. The spectra of these two types of α,β-unsaturated ketones show many similarities. For example, the Δ^1-3-ketone spectrum contains principal peaks at m/e 230, 188, and 122, while the spectrum of the Δ^4- isomer displays its prominent ions at m/e 230, 187, 149, and 124. Except for the m/e 230 ion associated with the expulsion of ketene, all the other ions are generated with accompanying specific as well as nonspecific hydrogen atom migrations. In all the specific transfers, the 8β-hydrogen atom plays the leading role. The other important itinerant hydrogen atoms are 5α- in the Δ^4- series and 11ξ- in the Δ^4- series. The mechanisms leading to the formation of the fragment ions as well as the syntheses of the deuterated derivatives are discussed. As part of the synthetic sequence, base-catalyzed deuterium equilibration of a $\Delta^{4,6}$-dien-3-one has been shown to result in the incorporation of three rather than the expected five deuterium atoms. The n.m.r. spectrum of the exchanged dienone unequivocally proves that only the hydrogen atoms bonded to C-2 and C-4 are replaced.

Introduction

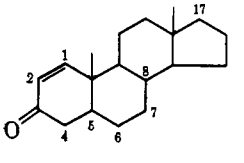
Since the initial measurements of the mass spectra of saturated steroidal ketones,[4] several intensive investigations[5] have been carried out on these systems, resulting in the realization that the fragmentation pathways are quite complex and do not lend themselves to a generally predictable behavior. It has now been concluded[6] that the fragmentation course of a polycyclic ketone is not largely controlled by the carbonyl group, especially in the presence of a predominantly hydrocarbon environment. However, even though saturated keto steroids do not exhibit a characteristic fragmentation pattern from one series to another (such as androstan-3-one *vs.* cholestan-3-one[4]), much has been learned about the mechanisms of the decompositions occurring in the mass spectrometer through the use of appropriately labeled derivatives within a given series.[5]

During our investigation of the mass spectral behavior of androstan-3-one[7] and related compounds, we had occasion to measure the mass spectra of both Δ^1- and Δ^4-androsten-3-one. In contrast to the class of saturated ketones, each of these α,β-unsaturated analogs exhibited a characteristic fragmentation pattern which eventually was shown to be of a general type, observable in the presence of many other substituents.[8]

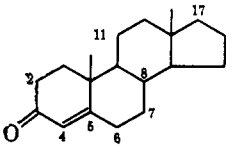
Therefore, α,β-unsaturated 3-ketones approach ethylene ketals[9] and steroidal amines[10] in their ability to control the fragmentation processes and hence yield largely predictable spectra.

The previous publications[11,12] have pointed out a few of the spectral features of α,β-unsaturated ketones, but the appropriately labeled derivatives were not available at that time to discuss all of the principal cleavages, especially those which were accompanied by hydrogen atom rearrangement. The prominent peaks in the high mass range have now been established through the use of deuterated derivatives, as well as of analogs containing a substituent label

Δ^1-5α-Androsten-3-one (I)[11] gives rise to a spectrum which contains three important (>30% of base peak) fragment ions occurring at m/e 230, 188, and 122 (Fig. 1). The peaks in the low mass range ($<m/e$ 122) appear to be the products of an indiscriminate and hence nondiagnostic breakdown of the steroid nucleus and will not be discussed. The m/e 230 ion results from the loss of ketene from ring A,[11] while the peak appearing at m/e 122 arises as a consequence of fissions of the 6–7 and 9–10 bonds with no *apparent* hydrogen migration. The m/e 188 ion is now shown to be $C_{14}H_{20}^+$, and not $C_{13}H_{16}O^+$, by examination of the spectrum of the 2,4,4-d_3- derivative,[11] and, therefore, this ion arises from a decomposition (see Fig. 1) which involves the cleavage of at least three carbon–carbon bonds.



I



II

The spectrum (Fig. 2) of Δ^4-androsten-3-one (II)[13] shows many similarities to that of its Δ^1- isomer. The principal peaks in the high mass range occur at m/e 230, 187, 149, and 124 and, as in the case of the Δ^1-analog, the peaks below m/e 124 appear to be the result of a random dissociation. The m/e 230 ion is due to the

(1) Paper XLIX: D. H. Williams and C. Djerassi, *Steroids*, **3**, 259 (1964).

(2) Supported in part by Grant No. CA-07195 from the National Institutes of Health, U. S. Public Health Service.

(3) National Science Foundation Cooperative Predoctoral Fellow 1963–1964.

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

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

(6) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, Inc., San Francisco, Calif., 1964, Chapter 8.

(7) R. H. Shapiro, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **86**, 2837 (1964).

(8) Various methyl groups and C-17 substituents have little or no effect on the general picture (see ref. 12). However, Δ^1- and Δ^4-3,11-diones show anomalous behavior which is discussed in the sequel. See also R. Tschesche, I. Mörner, and G. Snatzke, *Ann.*, **670**, 103 (1963).

(9) (a) G. von Mutzenbecher, Z. Pelah, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *Steroids*, **1**, 475 (1963); (b) H. Audier, A. Diara, M. J. Durazo, M. Fetizon, P. Foy, and W. Vetter, *Bull. soc. chim. France*, 2827 (1963).

(10) (a) W. Vetter, P. Longevialle, F. Khuong-Huu-Laine, Q. Khuong-Huu, and R. Goutarel, *ibid.*, 1324 (1963); (b) L. Dolejs, V. Hanus, V. Cerny, and F. Sorm, *Collection Czech. Chem. Commun.*, **28**, 1584 (1963); (c) Z. Pelah, M. A. Kielczewski, J. M. Wilson, M. Ohashi, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 2470 (1963).

(11) R. H. Shapiro, J. M. Wilson, and C. Djerassi, *Steroids*, **1**, 1 (1963).

(12) L. Peterson, *Anal. Chem.*, **34**, 1781 (1962).

(13) A. Butenandt, L. Karlson-Poschmann, G. Failer, U. Schiedt, and E. Bickert, *Ann.*, **575**, 123 (1952)

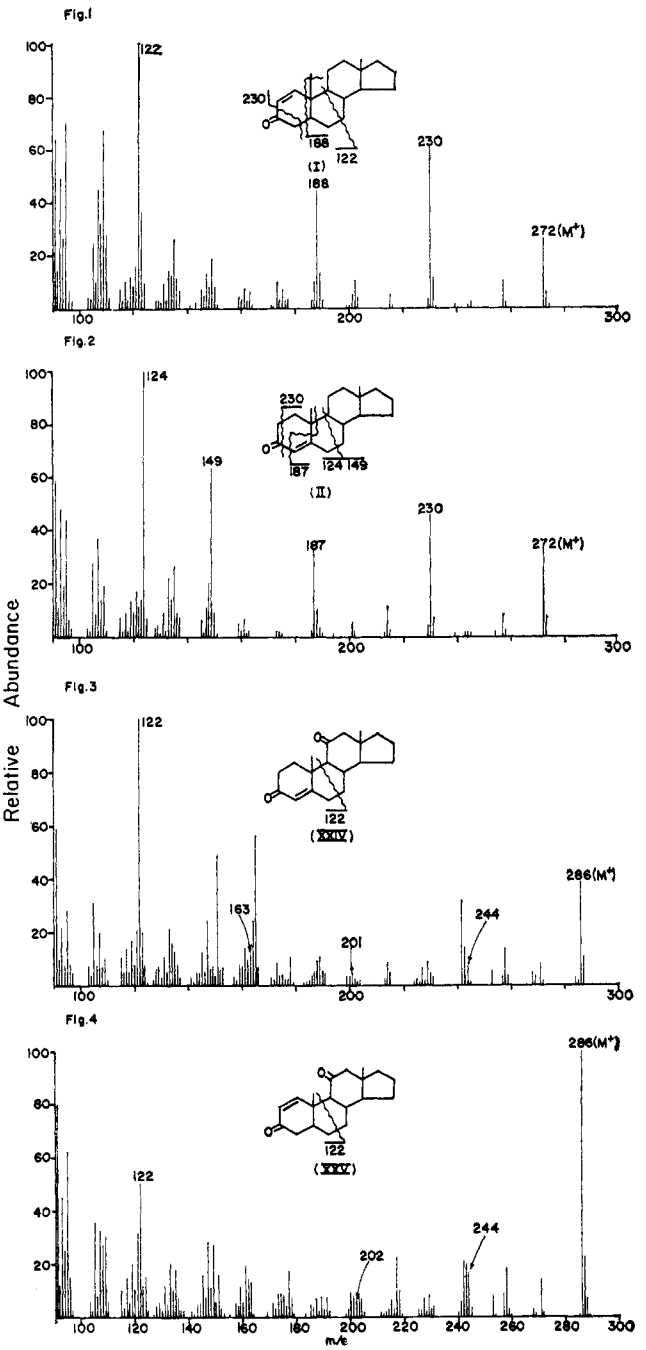


Fig. 1.—Mass spectrum of Δ^1-5α-androsten-3-one.
Fig. 2.—Mass spectrum of Δ^4-androsten-3-one.
Fig. 3.—Mass spectrum of Δ^4-androstene-3,11-dione.
Fig. 4.—Mass spectrum of Δ^1-5α-androstene-3,11-dione.

loss of the elements of ketene from ring A and both the *m/e* 149 and 122 ions are generated by fission of the 6–7 and 9–10 bonds, but the formation of the last two ions is accompanied by hydrogen atom migration. Although the *m/e* 187 ion is shown to be $C_{14}H_{19}^+$, different sites of cleavage are observed than for the Δ^1-3-ketone I.

The similarities between the spectra of I and II are viewed as being more than coincidental. It is evident that both enones behave in a related manner during the electron bombardment and the small difference in structure gives rise to a slightly varied decomposition. In point of fact, for both enones the 8β-hydrogen was found to migrate in all processes for which a hydrogen atom rearrangement was observed.

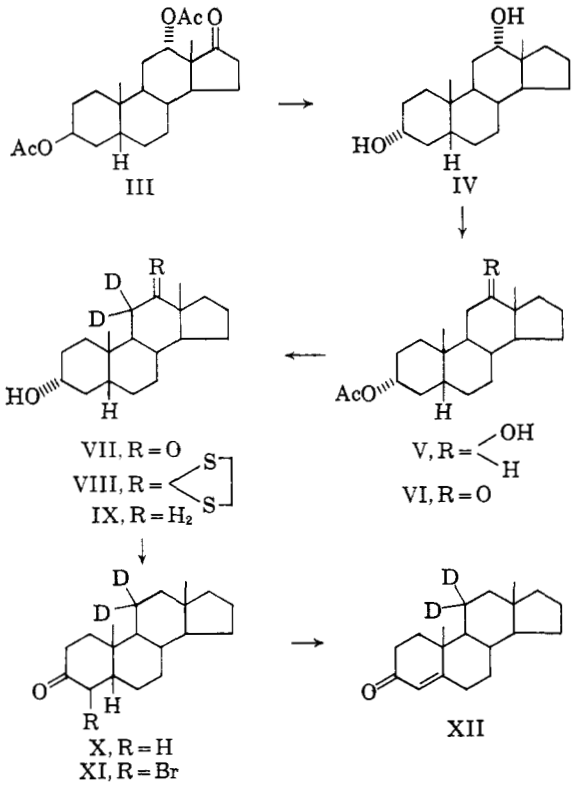
Results and Discussion

Syntheses of Deuterated Δ^1-3-Ketones.—Many of the labeled compounds in this series were prepared directly from the labeled saturated 3-ketone,[7] as was the parent enone I from 5α-androstan-3-one,[11] by bromination with pyridine hydrobromide perbromide[14] in acetic acid followed by dehydrobromination with calcium carbonate in boiling dimethylacetamide.[15] This technique was used to prepare the 19-d_1-,[16] 5α-d_1-, 6,6-d_2-, 7ξ-d_1-, and 8β-d_1-Δ^1-3-ketones, and in the first case, where larger quantities were used, the 19-d_1-Δ^4-3-ketone was also isolated and employed in the study of II. The other labeled Δ^1-3-ketones were 2,4,4-d_3-Δ^1-5α-androsten-3-one,[11] 1-methyl-Δ^1-androsten-3-one-17β-ol,[17] and Δ^1-cholesten-3-one.[18]

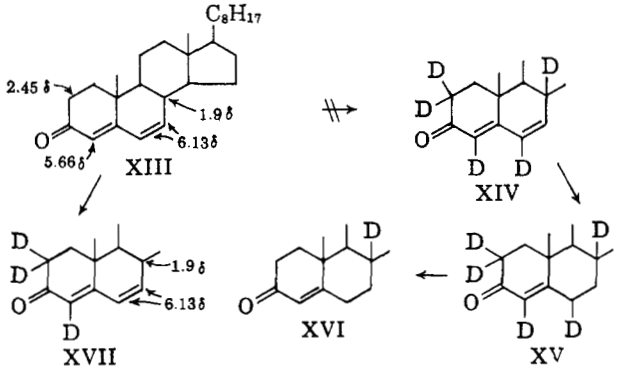
Syntheses of Deuterated Δ^4-3-Ketones.—Recently a French group[19] suggested that hydrogen atoms attached to C-8 and C-11 were implicated in the double transfer to ring A, resulting in the formation of the *m/e* 124 ion. Since this mechanism appeared to be extremely plausible, a synthetic program was initiated to prepare both the 8β-d_1- and 11,11-d_2- derivatives. The starting material was 5β-androstane-3α,12α-diol-17-one diacetate (III),[20] which not only contained an appropriate functional group in ring C, but also had the A/B *cis* configuration, suitable for eventual conversion to a Δ^4-3-ketone. A modified Huang-Minlon[21] reduction of the starting material III gave the 3,12-diol IV[22] which was partially acetylated to the 3α-monoacetate V and then oxidized to the 3α-acetoxy-12-ketone VI[22] with 8 *N* chromic acid in acetone.[23] This intermediate served as starting material for the preparation of both the C-11 and C-8 labeled Δ^4-3-ketones, although an alternate shorter procedure was also attempted for the latter case (see below).

The deuterium atoms were incorporated at C-11 by heating the keto acetate VI in basic deuteriomethanol containing heavy water. The resulting labeled keto alcohol VII was converted directly to the 12-thioketal VIII with ethanedithiol and boron trifluoride etherate. Overnight treatment of the thioketal VIII with freshly prepared W-7 Raney nickel[24] gave an excellent yield of the labeled 11,11-d_2-5β-androstan-3α-ol (IX)[25] which was transformed to the corresponding ketone X[25] by the Jones procedure.[23] Bromination[26] with pyridine hydrobromide perbromide[14] in acetic acid gave the 4β-bromo-3-ketone XI which was dehydrobrominated by way of its semicarbazone[27] to 11,11-d_2-Δ^4-androsten-3-one (XII).

(14) C. Djerassi and C. R. Scholz, *J. Am. Chem. Soc.*, **70**, 417 (1948).
(15) G. F. H. Green and A. G. Long, *J. Chem. Soc.*, 2532 (1961).
(16) C. Djerassi and M. A. Kielczewski, *Steroids*, **2**, 125 (1963).
(17) We wish to thank Dr. G. Raspé (Schering A.G., Berlin) for a gift of this substance.
(18) H. J. M. Fitches in "Advances in Mass Spectrometry," Vol. 2, edited by R. M. Elliott, Pergamon Press, Ltd., Oxford, 1963, p. 428.
(19) See M. J. Durazo, Ph.D. Thesis, University of Paris, 1963; H. Audier, M. Fétizon, and W. Vetter, *Bull. soc. chim. France*, 415 (1964).
(20) H. Reich and T. Reichstein, *Helv. Chim. Acta*, **26**, 2102 (1943).
(21) Huang-Minlon, *J. Am. Chem. Soc.*, **68**, 2487 (1946).
(22) H. Reich, *Helv. Chim. Acta*, **28**, 863 (1945).
(23) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. Weedon, *J. Chem. Soc.*, 39 (1946).
(24) H. R. Billica and H. Adkins, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 176.
(25) V. Prelog, L. Ruzicka, P. Meister, and P. Wieland, *Helv. Chim. Acta*, **28**, 618 (1945), describe the unlabeled compound.
(26) See P. A. Hart in "Steroid Reactions," edited by C. Djerassi, Holden-Day, Inc., San Francisco, Calif., 1963, Chapter 4.
(27) See T. H. Kritchevsky, D. L. Garmaise, and T. F. Gallagher, *J. Am. Chem. Soc.*, **74**, 483 (1952).



The first approach to a C-8 labeled enone involved the base-catalyzed equilibration of the enolizable hydrogen atoms in $\Delta^{4,6}$-cholestadien-3-one (XIII).[28] It was thought that if all five (2,2,4,6,8-) hydrogen atoms were replaced by deuterium (XIV), a selective reduction of the 6–7 double bond with lithium in liquid ammonia[29] (XV) followed by a back-exchange of the now superfluous deuterium atoms bonded to C-2, C-4, and C-6 would be the most direct route to the desired analog XVI. However, the exchange reaction led to the incorporation of only three deuterium atoms as shown by mass spectrometry. This fact is interpreted to mean that dienone XIII cannot enolize toward the π-system

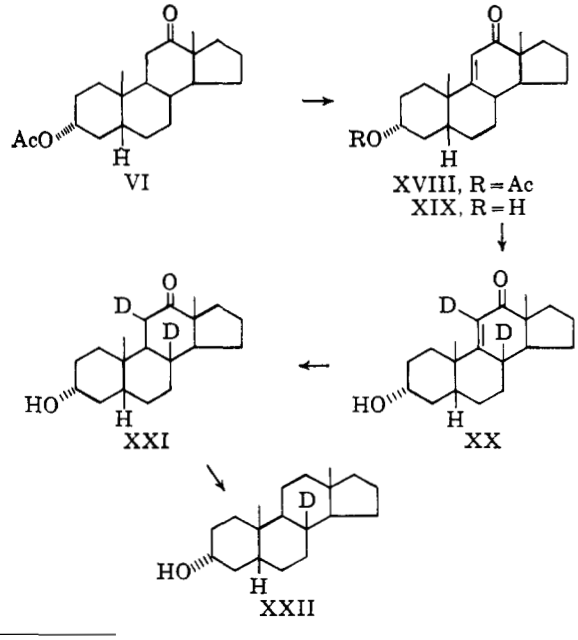


because the base is too bulky to approach and abstract the proton bonded to C-8. The hydrogen atoms attached to C-2 are exchanged by the normal enolization procedure, while the "extra" deuterium is introduced by the addition of deuteriomethanol to either the 4–5 or 6–7 double bond followed by the β- or vinylogous β-elimination of methanol. This phenomenon was also noted in the case of the Δ^1-3-ketone,[11] which exchanges, in addition to the C-4 hydrogens, the hydrogen bonded to C-2, even though the angular methyl group blocks enolization toward C-2.

The position of the third deuterium atom was proved by nuclear magnetic resonance spectrometry. The n.m.r. spectrum of $\Delta^{4,6}$-cholestadien-3-one (XIII) shows a sharp two-proton singlet at 6.13 p.p.m., a one-proton singlet at 5.66 p.p.m., and a two-proton multiplet centered at 2.45 p.p.m. In addition, the allylic hydrogen bonded to C-8 appears as a diffuse multiplet at about 1.9 p.p.m. The two-proton singlet arises from two equivalent hydrogens attached to doubly bonded carbon atoms, while the higher field one-proton signal is generated from the remaining olefinic hydrogen. By analogy with Δ^1-5α-androsten-3-one (I), in which the α-olefinic proton was found to resonate at higher field than the β-olefinic proton, the signal at 5.66 p.p.m. is assigned to the α-proton attached to C-4. Therefore, the protons attached to C-6 and C-7 are equivalent in this system and the hydrogen atom bonded to C-8 has a dihedral angle of *ca.* 90° with the C-7 hydrogen. Moreover, the C-6 and C-7 hydrogens must be equivalent or at least nearly so or else the usual AB splitting pattern would be observed. The signal at 2.45 p.p.m. is caused by the two saturated α-protons bonded to C-2.

The n.m.r. spectrum of the exchanged dienone XVII displayed a sharp two-proton singlet at 6.13 p.p.m. and the allylic signal at about 1.9 p.p.m. The one-proton singlet at 5.66 as well as the two-proton signal at 2.45 p.p.m. were completely absent, thus proving that the protons bonded to C-4 and C-2 were no longer present and the structure of the exchanged dienone is unambiguously represented by structure XVII.

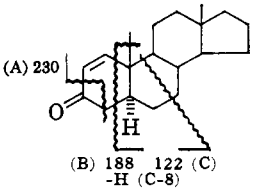
Since the exchange of the $\Delta^{4,6}$-dienone XIII could not be used as a method for the incorporation of deuterium at C-8, another route had to be chosen. For this purpose $\Delta^{9(11)}$-5β-androsten-3α-ol-12-one (XIX) was prepared in two steps from the saturated analog VI by treatment with selenium dioxide in acetic acid containing a trace of hydrochloric acid[30] followed by hydrolysis of the acetate XVIII. After base-catalyzed



(28) A. L. Wilds and C. Djerassi, *J. Am. Chem. Soc.*, **68**, 1712 (1946).
(29) J. E. Starr, ref. 6, Chapter 7.
(30) C. R. Engel, S. Rakhit, and W. W. Huculak, *Can. J. Chem.*, **40**, 921 (1962).

equilibration in deuteriomethanol, the exchanged product XX was immediately hydrogenated and the product XXI back-exchanged with methanolic sodium hydroxide solution in order to remove the deuterium atom attached to C-11. The carbonyl group was reduced by the modified Huang-Minlon reduction[21] to 8β-d_1-5β-androstan-3α-ol (XXII), which was then transformed into 8β-d_1-Δ^4-androsten-3-one (isotopic purity 65% d_1- species) by the same procedures used for the 11,11-d_2- derivative XII.

Mass Spectrum of Δ^1-5α-Androsten-3-one (I).—The mass spectrum of Δ^1-5α-androsten-3-one (I) is reproduced in Fig. 1. It can be seen that the principal peaks in the high mass range occur at *m/e* 230, 188, and 122. These ions result from the bond cleavages shown below.



Using suitably labeled derivatives the fragment ions were identified by observing the increase in *m/e* values for each peak as summarized in Table I.

TABLE I

SHIFTS IN PRINCIPAL PEAKS[a] FOR LABELED Δ^1-5α-ANDROSTEN-3-ONES

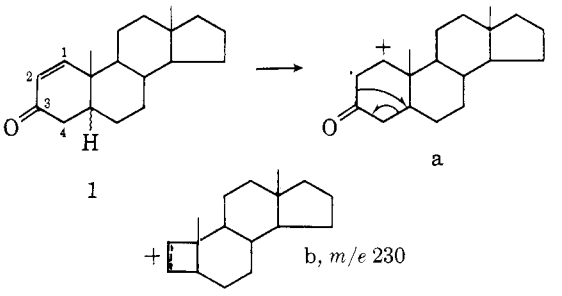
Δ^1-Androsten-3-one (I)	Isotopic purity, %	Isotopic impurities	*m/e* values for cleavage (A)	(B)	(C)
d_0-	..	...	230	188	122
2,4,4-d_3-	92	8% d_2-	231	188	125 (80%)
					124 (20%)
5α-d_1-	61	39% d_0-	231	189	123 (30%)
					122 (70%)
6,6-d_2-	93	7% d_1-	232	190	124
7ξ-d_1-	78	22% d_0-	231	189	122
8β-d_1-	92	8% d_0-	231	188 (100%)	122 (20%)
					123 (80%)
19-d_1-	88	12% d_0-	231	188	123
1-Me-17β-OH	..	...	260[b]	204[b]	136[c]
17-C_8H_{17}	..	...	342[d]	300[d]	122

[a] Peak intensities have been corrected for ^{13}C, isotopic impurities, and overlapping peaks. [b] Increased 16 mass units by 17β-OH. [c] Increased 14 mass units by 1-methyl. [d] Increased 112 mass units by 17-C_8H_{17}.

(A) M − 42 Ion (*m/e* 230).—This ion was previously[11] proved to be $C_{17}H_{26}^+$ by examination of the spectrum of 2,4,4-d_3-Δ^1-5α-androsten-3-one, which contained the corresponding ion at *m/e* 231 ($C_{17}H_{25}D^+$). Since 44 mass units (C_2D_2O) are lost from the molecular ion (*m/e* 275) of the d_3- derivative, either both heavy isotopes are lost with C-4 or some complex rearrangement process is operating in which a deuterium atom is transferred from C-4 to C-2 with concomitant or subsequent cleavage of the 1–2 and 3–4 bonds. The former process, in which C-2 remains with the charged species, is preferred since fragmentation of the 2–3 and 4–5 bonds with no accompanying hydrogen migration more closely resembles an "ordinary" chemical reaction.[31]

(31) H. M. Rosenstock and M. Krauss in "Mass Spectrometry of Organic Ions," edited by F. W. McLafferty, Academic Press, Inc., New York, N. Y., 1963, Chapter 1.

The process[32] may be visualized to proceed through a molecular ion of type a to give, after cyclization, the ionized olefin[33] b.

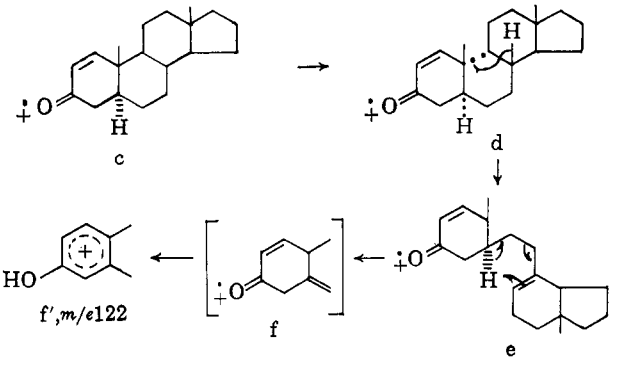


(B) M − 84 Ion (*m/e* 188).—The data in Table I show that the *m/e* 188 ion corresponds to $C_{14}H_{20}^+$ and that the labeled carbon atoms 1, 2, 4 (and hence 3), and 19 are expelled, while C-5, C-6, C-7, and C-17 are retained in the charged fragment. It will also be noticed that the 8β-hydrogen atom is transferred quantitatively to the neutral fragment.

This cleavage is represented formally by (B) in structure I, but we are unable to present a plausible mechanism, especially since no metastable ions were detected.

(C) *m/e* 122 Ion.—The base peak in the spectrum (Fig. 1) of Δ^1-5α-androsten-3-one (I) seems to be generated by a simple fission of the 6–7 and 9–10 bonds, *i.e.* a fragmentation process which is not accompanied or triggered by a hydrogen atom migration. However, after measurement of the spectrum of the 5α-d_1-Δ^1-3-ketone in which an *m/e* 123 peak was anticipated, it was found that the preponderant ion remained largely (70%) at *m/e* 122. The *m/e* values in Table I also show that another deuterium atom from either C-2 or C-4 is transferred to a small extent (20%). In order to harmonize this result, a hydrogen atom from another portion of the molecule must migrate toward ring A effecting the formation of the observed *m/e* 122 ion. The spectrum of 8β-d_1-Δ^1-5α-androsten-3-one shows that the heavy isotope is transferred away from the incipient fragment ion, thereby accounting for the reciprocal migration process.

An attractive mechanism for the formation of the base peak begins with the molecular ion c and homolysis of the 9–10 bond (d). The transformation of ion d into ion e is favorable, since the hydrogen atom migrates by way of a six-membered cyclic transition

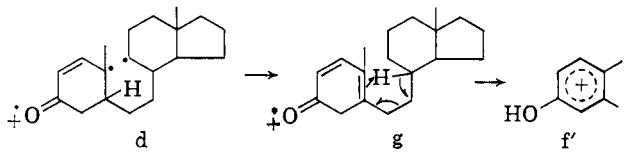


(32) A fishhook (↷) is used to designate the movement of a single electron and an arrow (⇝) signifies the shift of an electron pair; see ref. 6, pp. xi–xiii.

(33) See J. S. Shannon, *Australian J. Chem.*, **16**, 683 (1963), as well as ref. 6, p. xiii.

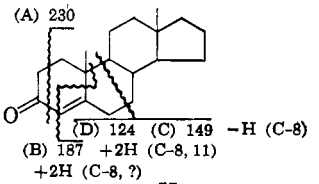
state during the formation of a Δ^8-double bond. This migration is exactly analogous to one operating in the fragmentation of 1-ketones.[5c] Likewise, the decomposition of e proceeds through a six-membered cyclic transition state, with the eventual formation of the aromatic system (f′) acting as the driving force.

Alternatively, the order of transfer can be reversed as shown below. This optional route may actually be preferred since the formation of ion g may be even more favorable than e.



d g f′

Mass Spectrum of Δ^4-Androsten-3-one (II).—The spectrum of Δ^4-androsten-3-one (II) is reproduced in Fig. 2. Aside from the high intensity peaks (*m/e* 230, 187, and 124) which have counterparts in Fig. 1, the *m/e* 149 ion is extremely abundant. The four principal cleavages are



II

The results of the investigation of the spectra of labeled derivatives of the parent enone II are listed in Table II.

TABLE II

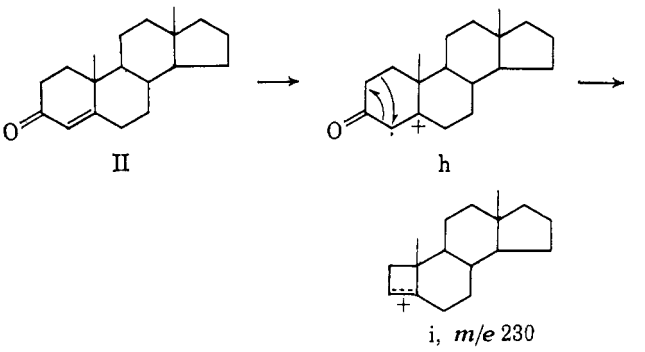
SHIFTS IN PRINCIPAL PEAKS[a] FOR LABELED Δ^4-ANDROSTEN-3-ONES

Δ^4-Androsten-3-one (II)	Isotopic purity, %	Isotopic impurities	*m/e* value for cleavage A	B	C	D
d_6–	..	...	230	187	149	124
10β-H[b]	..	...	216	187	149	110
2,2,4,6,6-d_5–	71	29% d_4	233	190	149	129
7β-d_1-17-one[c]	90	10% d_0	245	202	164	124
8β-d_1–	65	20% d_2[d]	231	187 (70%)	149 (50%)	124 (15%)
		15% d_0		188 (30%)	150 (50%)	125 (85%)
11,11-d_2–	83	14% d_1	232	189	150 (10%)	124 (40%)
		3% d_0			151 (90%)	125 (60%)
19-d_1–	88	12% d_0	231	187	149	125
17-OH	..	...	246	203	147[e]	124
17-C_8H_{17}	..	...	342	299	261	124

[a] Peak intensities have been corrected for ^{13}C, isotopic impurities, and overlapping peaks. [b] Unpublished results of P. A. Hart Stanford, University. [c] Unpublished results of G. von Mutzenbecher, Stanford University. [d] Assumed to be 8β,11-d_2. [e] *m/e* 165 − H_2O.

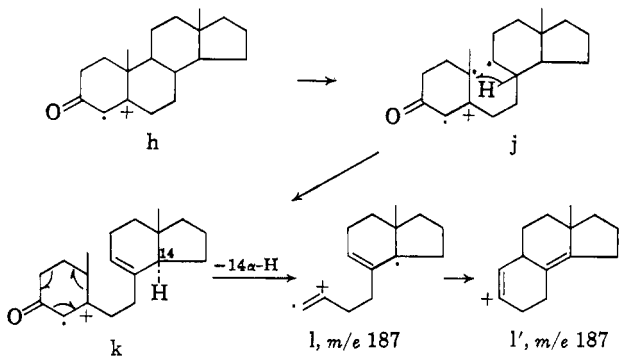
(A) M−42 Ion (*m/e* 230).—As in the case of the Δ^1- isomer, Δ^4-androsten-3-one (II), upon electron impact, loses the elements of ketene from ring A, a fact which is consistent with the observed shifts in Table II. This fragmentation mechanism may be viewed in an analogous manner to that postulated for the formation of the *m/e* 230 ion exhibited in the spectrum of I.

(B) M−85 Ion (*m/e* 187).—The *m/e* values for the B cleavage in Table II clearly demonstrate that C-2, C-3, and C-19 are lost, while C-4, C-6, C-7, C-11, and C-17 are retained in the charged fragment. Also evident in the table is the large transfer of hydrogen from



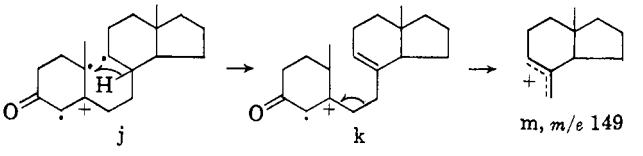
II h i, *m/e* 230

C-8 accounting for one of the two migrating atoms. These data lead to the reasonable assumption that C-1 and C-10 are also lost. The second itinerant hydrogen atom can only originate from C-12, C-18, or ring D and the 14α-hydrogen appears to be the most reasonable candidate although no labeled derivative is available to verify this assumption. We propose the following mechanism, which accommodates the required bond fissions, as well as the hydrogen loss from C-8 and C-14. Whether the *m/e* 187 ion should be represented by 1 or a cyclized variant 1′ is a moot question.



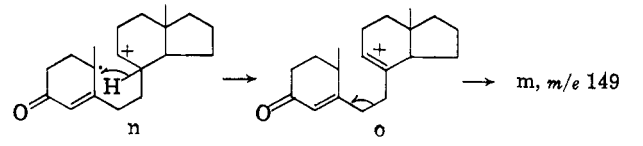
h j k l, *m/e* 187 1′, *m/e* 187

(C) *m/e* 149 Ion.—The *m/e* 149 ion results from the fission of the 6–7 and 9–10 bonds, with the positive charge remaining with the hydrocarbon moiety (rings C and D) accompanied by the transfer of one hydrogen atom to the neutral oxygen-bearing fragment. The *m/e* values in Table II show that *ca.* 50% of the migrating hydrogen atom originates from C-8, with a C-11 hydrogen making a small contribution. The major process leading to the formation of $C_{11}H_{17}^+$ may be represented as follows, the key step being fission of the allylically labilized 6–7 bond.



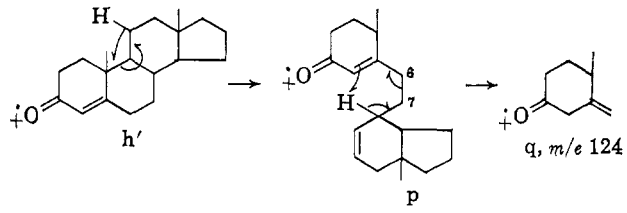
j k m, *m/e* 149

An alternative mechanism would involve the production of a different, yet highly stabilized, molecular ion n. The driving force for this fragmentation reaction is the formation of the ionized bond in o which fragments to give the observed ion m.

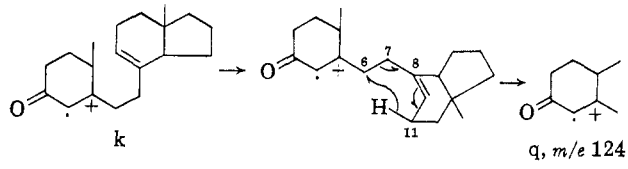


n o m, *m/e* 149

(D) *m/e* **124 Ion.**—The most abundant ion in the spectrum of Δ^4-androsten-3-one (II) is generated by a process similar to that which is involved in the formation of the *m/e* 149 ion. However, during the production of the *m/e* 124 species, two hydrogen atoms are transferred toward ring A and the charge remains with the oxygen-bearing fragment.[11] A suggested mechanism[19] for the production of this base peak, accompanied by the migration of hydrogen atoms from C-8 and C-11, is shown below.

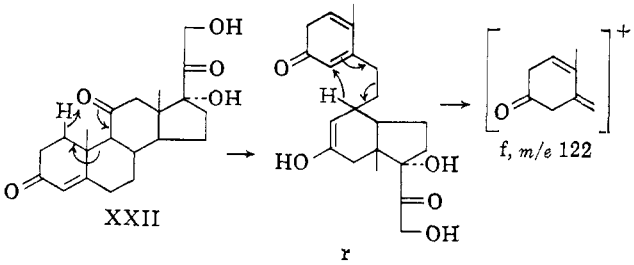


Alternatively, the migration of the C-8 hydrogen atom may precede that from C-11, in which case the sequence k → q′ may be invoked.



The plausibility of these processes was examined by measuring the spectra of the 8β-d_1- and 11,11-d_2-derivatives. It can be seen from the values in Table II that one or both of the suggested routes are correct.

Anomalous Systems.—The highly substituted steroid hormone cortisone (XXIII), fragments, under electron impact, in a manner which gives rise to only two major peaks in the high mass range.[18] The first of these ions occurs at *m/e* 300, while the base peak appears at *m/e* 122. Biemann[34] has rationalized the loss of 60 mass units (*m/e* 300) by a cleavage of the 17–20 bond with concomitant loss of an additional hydrogen atom, while the formation of the *m/e* 122 ions was represented by a sequence which involved a reciprocal hydrogen transfer from C-1 and C-8. The initiating 9–10 bond cleavage was postulated[34] to involve rearrangement of the C-1 hydrogen atom—a reaction which has recently been shown[5a] not to occur at all in saturated 11-ketones. Therefore, it seemed unlikely to us that such a transfer (XXIII → r) would be operative in this instance.

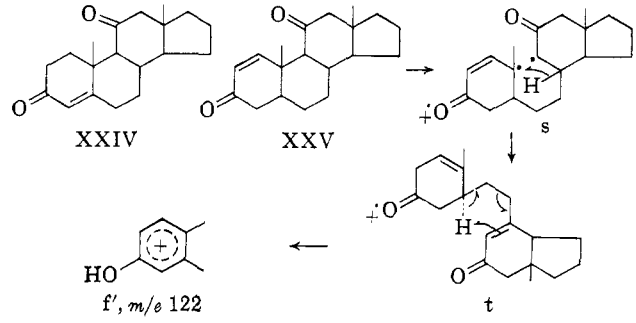


During an investigation of the mass spectra of 11-keto steroids,[5a] both Δ^4-androstene-3,11-dione (XXIV) and Δ^1-5α-androstene-3,11-dione (XXV) were prepared as intermediates in the syntheses of labeled derivatives. The spectra of both diones, as well as that of the fully exchanged (2,2,4,6,6,9,12,12-d_8-) Δ^4-3,11-dione, were measured and also displayed, as their major peaks, ions resulting from cleavages different from those noted in 11-deoxo analogs.

The spectrum (Fig. 3) of the Δ^4-3,11-dione XXIV exhibits many deviations from that of the Δ^4-3-ketone II. Only negligible corresponding ions appear at *m/e* 244 (M − 42), 201 (M − 85), 163 (149 + 14), and 124. It can be seen in Fig. 3 that the base peak occurs at *m/e* 122, as was observed in the spectrum of cortisone (XXIII),[18] indicating that the fragmentation reaction leading to the formation of this ion is a general process in Δ^4-3,11-diones. However, the mechanism postulated[34] for this process was shown to be inapplicable by the spectrum of the 2,2,4,6,6,9,12,12-d_8-Δ^4-3,11-dione, which showed a shift in the base peak to *m/e* 126. This fact clearly rules out the possibility of a hydrogen migration from C-1 to the C-11 carbonyl group and proves that one of the hydrogen atoms bonded to either C-2, C-4, or C-6 is lost from the charged fragment.

In actuality, the *m/e* 124 ion cannot be expected to be formed in the Δ^4-3,11-dione system (XXIII, XXIV), since it is now unequivocal that the transfer of a C-11 hydrogen atom is intimately associated with the production of this fragment. Therefore, it is felt that it is the absence of the C-11 hydrogen atoms, not the presence of the carbonyl group, which causes the observed variation in the base peak.

The spectrum of the Δ^1-3,11-dione XXV (Fig. 4) does not exhibit the M − 42 (*m/e* 244) or the M − 84 (*m/e* 202) ion as principal fragments, but the *m/e* 122 species is the most abundant one in the upper mass range. In contrast to the Δ^4- isomer XXIV, the presence of the 11-ketone should not interfere in the genesis of the *m/e* 122 ion, since the hydrogens attached to C-11 are not implicated. In fact, the reaction is predicted to be enhanced because of the formation of the conjugated system in ion t.



Conclusions

Although all of the ions appearing in the spectra of Δ^1- and Δ^4-androsten-3-one, such as the *m/e* 188 and 187 ions, respectively, are not as predictable as those exhibited in the spectra of steroidal ethylene ketals[9] or amines,[10] they are far more general and rational than the fragmentation patterns of saturated steroidal ketones.[5] It is not our intention to suggest that mass spectrometry supplant ultraviolet spectrophotometry in the recognition of the α,β-unsaturated carbonyl group; rather we emphasize the aid which a mass spectrum may offer in the determination of substituents contained in a fragment ion. In the many cases studied, the only substituent not applicable to this type of

(34) K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p. 343.

analysis is the 11-keto group. Moreover, the intriguing mechanistic implications suggested by the spectra of the labeled analogs have a great deal of intrinsic interest.

Experimental[35]

Δ^1-5α-Androsten-3-one (I).[11]—To a boiling suspension of calcium carbonate (0.8 g.) in N,N-dimethylacetamide (10 ml.)[15] was added crystalline 2α-bromoandrostan-3-one[36] (1.0 g.) and the mixture was heated under reflux for 15 min. After cooling to room temperature, the mixture was carefully poured into excess cold 2% hydrochloric acid and the precipitated solid was extracted with three 50-ml. portions of ether. The combined ethereal solutions were washed with water, dried over anhydrous magnesium sulfate, and evaporated to dryness at reduced pressure leaving a crystalline residue (583 mg.), m.p. 70–75°. A thin layer chromatogram developed with benzene–ethyl acetate (19:1) showed three spots with the least polar material predominating. The residue was recrystallized three times from petroleum ether giving large, chromatographically homogeneous prisms (240 mg., 33%), m.p. 102–103°, $[\alpha]^{30}D$ +43.5° (*c* 1.85), λ_{max} 229 mμ (log ϵ 4.04), $\nu_{max}^{CHCl_3}$ 1670 and 1605 cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{28}O$: C, 83.77; H, 10.36; mol. wt., 272.41. Found: C, 83.66; H, 10.28; mol. wt., 272 (mass spec.).

The Δ^1-3-ketones labeled at C-5, C-6, C-7, C-8, and C-19 were obtained by the same general procedure as above. However, the products were purified by preparative thin layer chromatography. The plates were developed with benzene–ethyl acetate (19:1) and the solvent was allowed to traverse about 15 cm. The material with the highest R_f value was the desired Δ^1-3-ketone, the intermediate band corresponded to the labeled androstan-3-one, while the most polar material (if present) was the Δ^4-3-ketone.

Δ^4-Androsten-3-one (II).[13]—A solution of Δ^5-androsten-3β-ol-17-one[37] (60 g.) in diethylene glycol (1 l.), *n*-butyl alcohol (300 ml.), and 95% anhydrous hydrazine (200 ml.) was heated under reflux (140–142°) in an atmosphere of oxygen-free nitrogen[38] for 2 hr. The external heating was discontinued while potassium hydroxide (80 g.) was added in about 5-g. increments during 15 min. The temperature was allowed to increase to 200–202° by codistillation of the solvents and allowed to remain at this temperature for 4 hr. After cooling to about 50°, the mixture was poured into 3 l. of ice–water and the product (57 g.) was isolated with ether. The crude Δ^5-androsten-3β-ol, m.p. 133–134° (reported[39] m.p. 131°), was used in the next step without purification.

A portion of the above alcohol (7 g.) was converted to the Δ^4-3-ketone by the Oppenhauer oxidation.[40] A yield of 5.6 g. (80%) of pure Δ^4-androsten-3-one (II) was obtained as long needles, m.p. 104–105°, $[\alpha]^{26}D$ +109° (*c* 1.02), λ_{max} 240 μ (log ϵ 4.23) and $\nu_{max}^{CHCl_3}$ 1652 cm.$^{-1}$ (reported[13] m.p. 104–105°, $[\alpha]D$ +110°, λ_{max}^{EtOH} 242 mμ (log ϵ 4.22)).

11,11-d_2-5β-Androstan-3α-ol-12-one 12-Ethylene Thioketal (VIII).—A solution of 5β-androstan-3α-ol-12-one acetate[22] (214 mg.) and sodium methoxide (200 mg.) in deuteriomethanol was heated to boiling, heavy water (*ca.* 1.5 ml.) was added to incipient turbidity, and the resulting solution was heated at reflux temperature for 9 hr. After cooling to room temperature, a small amount of heavy water was added and the product was extracted with three 15-ml. portions of dry ether. The organic solution was dried and evaporated to dryness at reduced pressure leaving a solid residue, which was immediately dissolved in ethanedithiol (1 ml.) and the resulting solution treated with boron trifluoride etherate (0.5 ml.). After standing 15 min. at room temperature, the product was precipitated by addition to aqueous methanol (1:1) and collected by filtration. One crystallization from methanol gave 207 mg. (88%) of thioketal VIII, m.p. 149–150°, which showed no carbonyl absorption in the infrared.

11,11-d_2-5β-Androstan-3α-ol (IX).—To a solution of the thioketal VIII (185 mg.) in ethanol (15 ml.) was added freshly prepared W-7 Raney nickel[24] (from 5 g. of alloy). The resulting suspension was heated under reflux for 13 hr., cooled to room temperature, and filtered through Celite. The colorless filtrate was evaporated to dryness and the residue was crystallized from acetone giving 11,11-d_2-5β-androstan-3α-ol (IX, 117 mg., 85%) as fine needles, m.p. 145–146° (reported[25] for the unlabeled compound, m.p. 146–146.5°).

11,11-d_2-5β-Androstan-3-one (X).—A solution of the alcohol IX (105 mg.) in reagent grade acetone (35 ml.), warmed to 40° and then cooled to room temperature, was treated dropwise with 8 *N* chromic acid solution[23] until a yellow color persisted for 5 min. The crude reaction mixture was concentrated at reduced pressure to about 5 ml. and the concentrate was added to cold water (15 ml.). The organic material was extracted with ether, and the ether phase was dried over magnesium sulfate and evaporated at reduced pressure. The resulting sirup (95 mg., 91%) crystallized upon standing and showed m.p. 46–49°. The infrared spectrum contained a carbonyl band at $\nu_{max}^{CHCl_3}$ 1704 cm.$^{-1}$. The melting point for the crude unlabeled 5β-3-ketone is reported[25] to be 45–50° and, after purification, 59–60°. The labeled material showed only one spot on a thin layer chromatogram (with the same R_f as authentic material) developed with benzene–ethyl acetate (19:1) and was not purified further.

11,11-d_2-Δ^4-Androsten-3-one (XII).—To a solution of 11,11-d_2-5β-androstan-3-one (X, 80 mg.) in acetic acid (1 ml.) was added pyridine hydrobromide perbromide[14] (93 mg.) in five portions, waiting after each addition for the solution to decolorize. When the addition was complete, water was added to the now yellow solution and the precipitate was collected by filtration and washed with water. A single crystallization from methanol gave material which showed m.p. 151–155° and only a trace on impurity by t.l.c.

The crude bromo ketone was dissolved in acetic acid (7 ml.) and semicarbazide hydrochloride (50 mg.) and sodium acetate (50 mg.) were added in one portion. By the use of a preheated oil bath, the reaction mixture was heated at 70 ± 2° for 2 hr. in an atmosphere of nitrogen. After this time, pyruvic acid (1 ml.) and water (2 ml.) were added and the mixture was heated at the same temperature for an additional 2 hr. After cooling to room temperature, the dark brown solution was diluted with ether (20 ml.) and the ethereal solution was washed with 5% sodium hydroxide solution until the washings were basic, followed by water washings until the latter were neutral. Drying and evaporating in the usual manner gave an oil which displayed several spots in a thin layer chromatogram, one of which had an R_f corresponding to the desired material. The oil was filtered through a column of neutral alumina (7 g., Activity II). The least polar materials were eluted as mixed fractions with hexane (50 ml.) and hexane–benzene (9:1, 50 ml. and 8:2, 50 ml.), while the desired material and one other contaminant were eluted with hexane–benzene (7:3, 50 ml. and 6:4, 50 ml.). The latter fractions were evaporated to dryness giving a semicrystalline solid (20 mg.), which was applied to a preparative chromatoplate and eluted with benzene–ethyl acetate (19:1). The silica containing the desired material (dark band under ultraviolet light) was scraped from the plate and eluted with dry ether. Evaporation of the solvent gave chromatographically homogeneous 11,11-d_2-Δ^4-androsten-3-one (14 mg.), m.p. 104–105° (undepressed when admixed with authentic enone II). The isotopic purity of the labeled material was 3% d_0-, 14% d_1-, and 83% d_2- as determined by mass spectrometry.

$\Delta^{9(11)}$-5β-Androsten-3α-ol-12-one Acetate (XVIII).—To a solution of 5β-androstan-3α-ol-12-one acetate[22] (VI, 400 mg.) in acetic acid (10 ml.) containing concentrated hydrochloric acid (1 drop) was added selenium dioxide (275 mg.) and the suspension was heated at reflux temperature for 20 hr. The mixture was cooled, diluted with ether (30 ml.), and filtered through anhydrous sodium sulfate. The filtrate was washed with water, saturated solution of sodium bicarbonate, and again with water; the ether

(35) The mass spectra were determined with a Consolidated Electrodynamics Corp. mass spectrometer No. 21-103C using an all-glass inlet system heated to 200°, while the isatron temperature was kept at 270°. The ionizing energy was maintained at 70 e.v. and the ionizing current at 50 μa. All melting points of analytical samples are corrected and were obtained on a Kofler hot stage, while routine melting points were taken in capillary tubes and are uncorrected. Optical rotations were measured in chloroform, while the ultraviolet absorption spectra were obtained on 95% ethanolic solutions.

Analytical chromatoplates had a thickness of 0.25 mm. of silica gel G, and the compounds were detected by spraying with a 2% ceric sulfate solution in 1 *N* sulfuric acid. Preparative chromatoplates had a thickness of 1 mm. of silica gel HF_{254} containing a trace of disodium 3,5-dihydroxypyrenedisulfonate (Bayer A.G., Leverkusen). The compounds were detected by the use of an appropriate ultraviolet lamp.

(36) C. Djerassi, *J. Org. Chem.*, **12**, 823 (1947).

(37) We wish to thank Syntex S.A., Mexico City, for a generous sample of this material.

(38) F. Aylward and M. Sawistowska, *Chem. Ind.* (London), 489 (1962).

(39) A. Butenandt and L. A. Suranyi, *Chem. Ber.*, **75**, 591 (1942).

(40) The exact conditions of J. F. Eastham and R. Teranishi, *Org. Syn.*, **35**, 39 (1955), were employed.

solution was dried over anhydrous sodium sulfate and evaporated to dryness at reduced pressure. The yellow oily residue was dissolved in petroleum ether and filtered through neutral alumina (3 g., Activity II). Evaporation of the solvent gave a colorless oil (320 mg.) which crystallized upon standing for 4 hr. Recrystallization from methanol–water gave glistening plates (300 mg., 75%), m.p. 130–131.5°. The analytical sample was recrystallized twice more from the same solvent and showed m.p. 132–133°, $[\alpha]^{26}D$ +88.1° (*c* 0.93), λ_{max} 240 mμ (log ε 4.11); ν_{max}^{KBr} 1737, 1675, and 1591 cm.$^{-1}$; and $\nu_{max}^{CHCl_3}$ 1720, 1661, and 1591 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.10; H, 9.19.

$\Delta^{9(11)}$-5β-Androsten-3α-ol-12-one (XIX).—To a solution of the above acetate XVIII (195 mg.) in methanol (14 ml.) was added 2.5 *N* sodium hydroxide solution (2.7 ml., 270 mg. NaOH) and the resulting solution was heated under reflux for 1.5 hr. After cooling the solution to room temperature and pouring it into water (50 ml.), the separated solid was isolated with ether in the usual manner. The product (168 mg.) was obtained as tiny needles, m.p. 146.5–147.5°. The analytical sample was thrice recrystallized from petroleum ether–ethyl acetate and showed m.p. 147–148°, $[\alpha]^{26}D$ +67.6° (*c* 1.04), λ_{max} 241 mμ (log ε 4.10); $\nu_{max}^{CHCl_3}$ 3590, 1660, and 1595 cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.12; H, 9.79. Found: C, 79.04; H, 9.79.

8β,11-d_2-$\Delta^{9(11)}$-5β-Androsten-3α-ol-12-one (XX).—A clean piece of sodium (*ca.* 100 mg.) was dissolved in deuteriomethanol (10 ml.) and to this solution was added the hydroxyenone XIX (150 mg.). After heating to boiling, deuterium oxide (*ca.* 6 ml.) was added and the solution was heated at reflux for an additional 36 hr. The solution was cooled to ambient temperature, added to deuterium oxide (5 ml.), and the resulting cloudy mixture was extracted with three 10-ml. portions of dry ether. The organic layer was washed with a little deuterium oxide, dried, and evaporated to dryness leaving a very slightly yellow crystalline residue (150 mg.), m.p. 145–147°. The mass spectrum showed this material to contain two deuterium atoms by the occurrence of the molecular ion at *m/e* 290.

8β-d_1-5β-Androstan-3α-ol (XXII).—The crude labeled enone XX (150 mg.) was dissolved in cyclohexane–ethyl acetate (1:1, 10 ml.), 10% palladized charcoal (100 mg.) was added, and the suspension was treated with hydrogen while stirring vigorously. Within 3 min., 13 ml. of hydrogen had been absorbed (theoretical uptake was 12.2 ml.) and no additional absorption was noted during the next 30 min. Removal of the catalyst and evaporation of the solvent to dryness led to a semisolid material which showed two spots on a chromatoplate developed with ether.

No attempt was made to separate the mixture into its components, but the crude residue was dissolved in methanol (5 ml.) and 2.5 *N* sodium hydroxide solution (1 ml.) was added and the solution was heated at the boiling point for 1 hr. After cooling the solution to room temperature, all of the solvent was removed under reduced pressure at steam bath temperature.

To the residue was added diethylene glycol (8 ml.) and 95% anhydrous hydrazine (2 ml.) and the solution was heated in an open flask until the temperature reached 200°. The flask was equipped with a reflux condenser and heating was continued at 200–205° for 4.5 hr. The cooled reaction mixture was poured into water (20 ml.) and the product was isolated with ether. A thin layer chromatogram developed with benzene–ethyl acetate (9:1) showed two materials, one of which had an identical R_f with that of unlabeled authentic 3α-ol, while the other, being very polar, was probably the 3α,12ξ-diol from over-hydrogenation. No attempt was made to isolate the contaminant.

The crude product was adsorbed on neutral alumina (15 g., Activity II) and eluted with hexane–benzene mixtures. The fractions eluted with a 1:2 mixture contained the desired material as determined by t.l.c. Evaporation of the solvent gave 8β-d_1-5β-androstan-3α-ol (XXII, 50 mg., 36% from unlabeled enone XIX), m.p. 145–146°.

This material was converted to the Δ^4-3-ketone by the same sequence used above for the 11,11-d_2 derivative. The final product had an isotopic purity of 65%, the contaminants being 20% d_2- (extra deuterium assumed to be at C-11) and 15% d_0-ketone.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STANFORD UNIVERSITY, STANFORD, CALIF.]

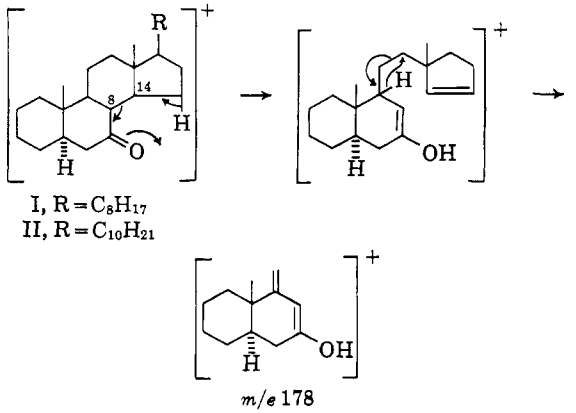
Mass Spectrometry in Structural and Stereochemical Problems. LI.[1] Mass Spectral and Enolization Studies on 7-Keto-5α-androstanes[2]

BY R. BEUGELMANS, R. H. SHAPIRO, LOIS J. DURHAM, D. H. WILLIAMS, H. BUDZIKIEWICZ, AND CARL DJERASSI

RECEIVED FEBRUARY 7, 1964

A mass spectrometric study of four 7-keto-5α-androstanes and a number of deuterated analogs has demonstrated that these compounds show two characteristic fragmentation modes. Mass spectrometry and nuclear magnetic resonance have been employed to illustrate that, in contrast to the enolization of the analogous steroidal 11-ketones, two deuterium atoms can be introduced at C-6 before introduction of a third deuterium atom at C-8. Moreover, under certain conditions, it has been shown that equatorial ketonization of the Δ^6-enol prevails over the alternative axial ketonization.

In the mass spectrum of cholestan-7-one (I)[3] or sitostan-7-one (II)[4] one of the most important fragmentation modes involves cleavage across ring C with charge retention by the ketonic fragment leading to ions *m/e* 178. It has been suggested[3,4] that the primary process occurring is cleavage of the 8–14 bond with concomitant transfer of hydrogen from C-15 to the carbonyl group in a six-membered transition state. Rupture of the 11–12 bond and loss of an allylic hydrogen radical could then furnish the *m/e* 178 ion. Although such a mechanism appears very



I, R = C_8H_{17}
II, R = $C_{10}H_{21}$

m/e 178

(1) Paper L: R. H. Shapiro and C. Djerassi, *J. Am. Chem. Soc.*, **86**, 2825 (1964).

(2) Financial support by the National Institutes of Health (Grants No. AM-04257 and CA-07195), National Science Foundation Cooperative Fellowship (to R. H. S.), and N.A.T.O., Paris (travel grant to R. B.) is gratefully acknowledged.

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

(4) (a) K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, Chapter 9; (b) C. Djerassi, *Pure Appl. Chem.*, in press.