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

Mass Spectrometry in Structural and Stereochemical Problems. XLVIII.[1] A Study of the Hydrogen Transfer Reactions Accompanying Fragmentation Processes of 1-Keto Steroids. Synthesis of Deuterated 5α-Androstan-1-ones[2]

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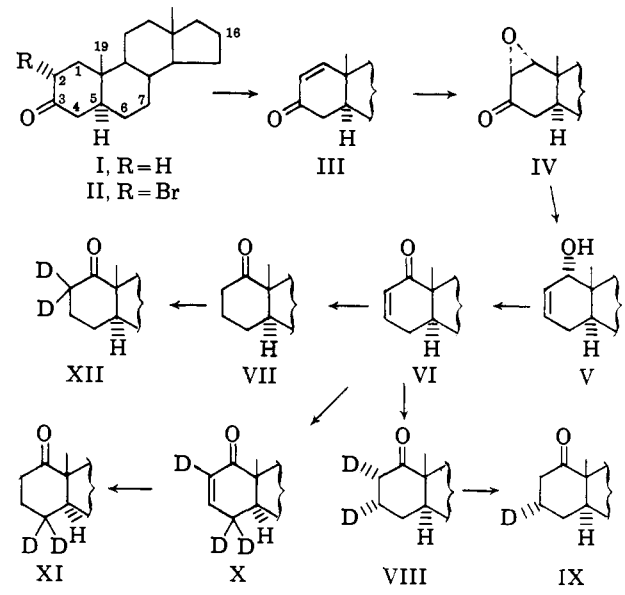
The mass spectra of 5α-androstan-1-one and derivatives labeled with deuterium at positions 2, 3, 4, 5, 6, 7, 16, and 19 have been measured. As a result, it has been possible to determine the major fragmentation modes and to gain insight into the mechanisms by which these processes occur. Similar labeled derivatives of Δ^2-5α-androsten-1-one have permitted an analysis of the mass spectrum of this α,β-unsaturated ketone.

Introduction

While it is now realized[3,4] that extensive correlations between the mass spectral fragmentation patterns of steroidal ketones and structure are not very feasible, the extensive deuterium labeling performed in this class[5-8] has given great insight into the mechanism of dissociative and rearrangement processes upon electron impact. Furthermore, the appreciation of the fact that the carbonyl group is not able to direct fragmentation (in the presence of a dominant hydrocarbon environment) in a consistent and predictable fashion has led to studies on other functional derivatives, such as the corresponding ethylene ketal[3,9] and dimethylamine.[10] The strong directing influence of these last two groups, resulting from the marked ability of the hetero atoms to stabilize a positive charge, leads to straightforward fragmentation modes, unaffected by other functional groups. However, the results now reported for 5α-androstan-1-one confirm the previous findings[5-8] for steroidal ketones, namely that a number of ions of comparable abundance are formed and that rearrangement processes, often complex in nature, are prevalent.

Synthesis of Deuterated 5α-Androstan-1-ones.—In our laboratory, experiments have been undertaken[7] to label 5α-androstan-3-one (I) with deuterium in many positions, including C-5, C-6, C-7, C-16, and C-19.[11] In view of the fact that a convenient synthetic route is available for the conversion of 3-ketones to the corresponding 1-ketones,[12] it was a simple matter to label 5α-androstan-1-one with the heavy hydrogen isotope in the above-mentioned positions. The procedure, now performed on 5α-androstan-3-one (I) for the first time, is outlined below. Bromination of the 3-ketone I with bromine in acetic acid yielded the 2α-bromo derivative II which, on dehydrobromination with calcium carbonate in dimethylacetamide, furnished the Δ^1-3-ketone III. Epoxidation of the double bond, most conveniently accomplished by hydrogen peroxide in refluxing ethanol under basic conditions, led to IV, which can be converted into the Δ^2-1α-ol V by heating with hydrazine hydrate. Oxidation of the allylic alcohol V with chromium trioxide in acetone solution gave the α,β-unsaturated ketone VI, which on catalytic hydrogenation, employing 10% palladium-on-charcoal as catalyst, yielded 5α-androstan-1-one (VII). Using the appropriately labeled 3-keto steroid,[7,11] deuterium was thus introduced into positions 5, 6, 7, 16, and 19 of VII.



Further isotopic labeling in ring A of the 1-ketone VII could be accomplished by standard chemical transformations of the unsaturated precursor VI. Thus, catalytic deuteration of VI gave 2α,3α-d_2-5α-androstan-1-one (VIII) which, after treatment with aqueous methanolic base, yielded 3α-d_1-5α-androstan-1-one (IX). Alternatively, base-catalyzed enolization of VI in deuterium oxide–deuteriomethanol gave the 2,4,4-d_3-derivative X, which, after catalytic hydrogenation and back exchange of deuterium from C-2, furnished 4,4-d_2-5α-androstan-1-one (XI). Finally, direct exchange in basic deuterated solvents of the ketone VII gave the 2,2-d_2- analog XII.

Discussion of Mass Spectral Fragmentation Processes

With 5α-androstan-1-one labeled with deuterium at positions 2, 3, 4, 5, 6, 7, 16, and 19 now available, we are

(1) Paper XLVII: N. Finch, I. H.-C. Hsu, W. I. Taylor, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **86**, 2620 (1964).

(2) We are indebted to the National Institutes of Health of the U. S. Public Health Service for financial support (Grants No. AM-04257 and CA-07195).

(3) G. von Mutzenbecher, Z. Pelah, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *Steroids*, **2**, 475 (1963).

(4) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, San Francisco, Calif., 1964, p. 140, *et seq.*

(5) D. H. Williams, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 2091 (1963).

(6) C. Beard, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *ibid.*, **86**, 269 (1964).

(7) R. H. Shapiro, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, in press.

(8) G. von Mutzenbecher, unpublished results on 15-keto steroids.

(9) H. Audier, A. Diara, M. de J. Durazo, M. Fetizon, P. Foy, and W. Vetter, *Bull. soc. chim. France*, 2827 (1963).

(10) Reference 4, pp. 74–80.

(11) C. Djerassi and M. A. Kielczewski, *Steroids*, **2**, 125 (1963).

(12) C. Djerassi, D. H. Williams, and B. Berkoz, *J. Org. Chem.*, **27**, 2205 (1962). The general method of P. S. Wharton (*ibid.*, **26**, 3615, 4781 (1961)) was employed.

TABLE I[a]

Compound	Isotopic purity, %	M+	m/e values						
d_0	100	274	259	256	241	231	203	124	111
2,2-d_2-VII	93	276	261	258	243	233	203	126	113
3α-d_1-VII	80	275	260	257	242	232	203	125	112
4,4-d_2-VII	82	276	261	258	243	233	203, 55% 204, 15% 205, 30%	125, 45% 126, 55%	113
5α-d_1-VII	55	275	260	257	242	232	203, 40% 204, 60%	124	112
6,6-d_2-VII	87	276	261	258, 85% 257, 15%	243	233	205	126	111
7ξ-d_1-VII	78	275	260	257	242	232	204	124	111
19-d_1-VII	83	275	259, 50% 260, 50%	257	241, 50% 242, 50%	231, 50% 232, 50%	204	125	112
16-d_1-VII	53	275	260	257	242	232	204	124	111

[a] Splittings of peaks are recorded in the table only in those cases where the minor portion can be established to be 10% or greater of the total intensity of the peak.

able to attempt a detailed interpretation of its mass spectrum. Also, in view of the interesting behavior of other α,β-unsaturated ketones,[4,7] an analysis of the spectrum of the precursor Δ^2-5α-androsten-1-one (VI) through labels at positions 2, 3, 4, 5, 6, 7, and 16 was undertaken and our conclusions are listed below.

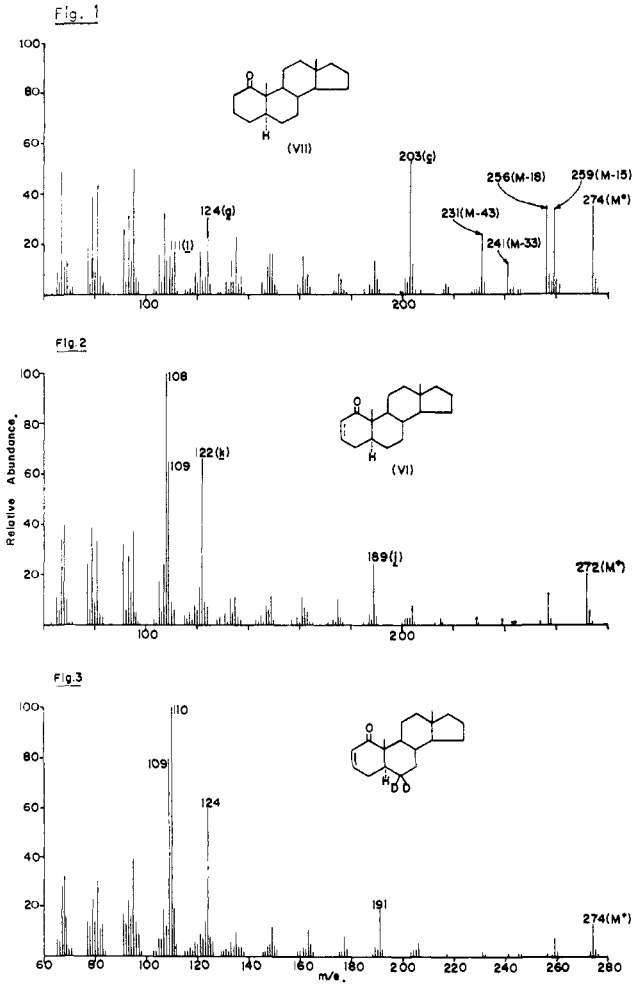
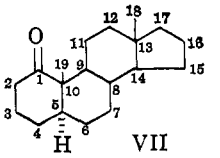


Fig. 1.—Mass spectrum of 5α-androstan-1-one (plotted with m/e 41 as base peak.
Fig. 2.—Mass spectrum of Δ^2-5α-androsten-1-one.
Fig. 3.—Mass spectrum of 6,6-d_2-Δ^2-5α-androsten-1-one.

5α-Androstan-1-one.—The mass spectrum of 5α-androstan-1-one (VII) is shown in Fig. 1. It can be seen that below the molecular ion (m/e 274), there are



prominent peaks due to the loss of methyl (m/e 259, M − 15) and the loss of water (m/e 256, M − 18). Not surprisingly, a peak corresponding to the combined loss of these two moieties is also evident (m/e 241, M − 33). Other significant ions are displayed at m/e 231, 203, and 124, while a fourth, less intense ion (m/e 111), whose shifts can readily be followed in the deuterated analogs, also merits consideration. The shifts of the major portions of these ions in the isotopically labeled derivatives are summarized in Table I. The isotopic purities of the derivatives are recorded in Table II. Where partial shifts are given in Table I, these are the values obtained after correcting for isotopic contaminants.

TABLE II
DETAILED ISOTOPIC PURITIES

	d_0, %	d_1, %	d_2, %	d_3, %
2,2-d_2-VII	..	3	93	4
3α-d_1-VII	8	80	12	..
4,4-d_2-VII	7	7	82	4
5α-d_1-VII	39	55	6	..
6,6-d_2-VII	2	9	87	2
7ξ-d_1-VII	22	78[a]	..	..
19-d_1-VII	13	83	4	..
16-d_1-VII	14	53	31	2

[a] $LiAlD_4$ of 80% isotopic purity was employed in the introduction of this label.

The first interesting point which is evident from Table I is that about 50% of the M − CH_3 peak is due to the loss of the C-19 methyl group from the molecular ion (see split of M − 15 peak between m/e 259 and 260 in 19-d_1-VII). It has been suggested by Biemann[13] that simple rupture of a carbon–carbon bond, one carbon atom removed from a carbonyl group with charge retention by the oxygen-containing fragment, is a very unfavorable process. Supporting evidence was provided in that loss of a methyl group from diisopropyl ketone XIII was very small compared with the same loss from 2,4-dimethylpentane (XIV).

(13) K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p. 133.

$$(CH_3)_2CHCCH(CH_3)_2 \longrightarrow (CH_3)_2CHC{\overset{+}{C}}HCH_3$$
XIII 0.12% Σ_{27}

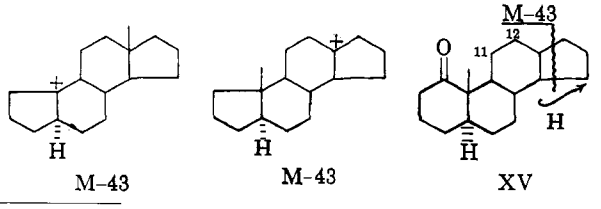
$$(CH_3)_2CHCH_2CH(CH_3)_2 \longrightarrow (CH_3)_2CHCH_2\overset{+}{C}HCH_3$$
XIV 4.36% Σ_{12}

However, it appears that no statement as to the relative preference for expulsion of the C-18 and C-19 methyl groups is warranted in the present case, because it cannot be ascertained to what extent each M − 15 species undergoes further decomposition before reaching the recorder; *i.e.*, the observed intensities of M − CH_2D and M − CH_3 ions in the spectrum of 19-d_1-VII are not necessarily a measure of the prevalence of expulsion of C-19 and C-18 methyl groups, respectively. For example, it will be seen (*vide infra*) that M − 43 ions are partly formed with loss of the C-19 methyl group. Therefore, the conclusion of importance is that although quantitative measurements are not possible, it can be stated definitely that cleavage of the 10–19 bond is not very unfavorable in the cyclic ketone VII.

By reference to Table I it can be seen that in no case is there an appreciable shift of the M − H_2O peak to M − DHO. Accurate analyses of the spectra of the labeled analogs indicate that there is no loss of DHO from the 2,2-d_2-, 7ξ-d_1-, and 19-d_1- derivatives. For 3α-d_1- ($\lesssim$8%), 4,4-d_2-, ($\lesssim$7%), 5α-d_1- ($\lesssim$5%), 6,6-d_2- ($\ll$15%) lower limits are not recorded in view of the large uncertainties which are involved in computing such small shifts in the presence of isotopic contaminants and unpredictable movements of weak neighboring ions. The process is obviously partially random, as ascertained for other cyclic ketones.[14-16] At a maximum, we have accounted for the loss of only 0.4 atom of deuterium. Unless an unprecedently large isotope effect is operating, this implies that hydrogen atoms from other parts of the molecule must be involved in this process. In view of the proximity of the oxygen function to C-11, hydrogen attached to this carbon atom would be an obvious candidate.

The *m/e* values of the M − (CH_3 + H_2O) peak (see Table I, *m/e* 241) in the 19-d_1-derivative are consistent with its formation by a combination of the two processes just described.

The labeling experiments show that carbon atoms 1–7 are retained in the *m/e* 231 (M − 43) fragment(s), but that the loss of the C-19 methyl group occurs in the formation of 50% of the peak. It is therefore possible that since none of the other labeled carbon atoms are lost in the formation of this peak, the *m/e* 231 ion arises through the associated expulsion of carbon monoxide and one of the C-19 or C-18 angular methyl groups to yield the products indicated below.



M-43 M-43 XV

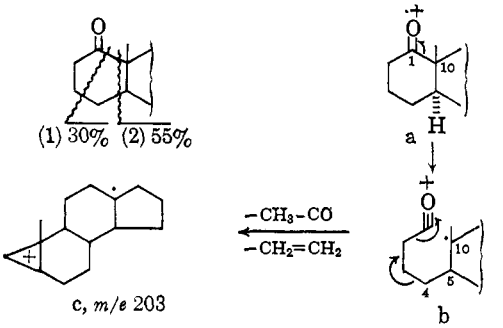
(14) E. Lund, H. Budzikiewicz, J. J. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 941 (1963).

(15) E. Lund, H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *ibid.*, **85**, 1528 (1963).

(16) D. H. Williams, H. Budzikiewicz, Z. Pelah, and C. Djerassi, *Monatsh.*, **95**, 166 (1964).

Rather surprisingly, none of the M − 43 ion arises by loss of C_3H_7 from ring D (see XV), a process prevalent in the fragmentation of pregnanes and cholestanes.[17] However, in the absence of high resolution measurements, the elision of a hydrocarbon fragment (C_3H_7), comprising the C-19 methyl group and ethylene derived from the unlabeled 11- and 12-methylene groups (see XV), presents another alternative leading to an M − 43 species.

Peak *m/e* 203.—Double-focusing measurements[18] have established that this ion is due to a hydrocarbon fragment $C_{15}H_{23}$ (98%), oxygen-containing fragments (2%) being negligible. Deuterium labeling, however, illustrates that the peak is a composite one, approximately 30% retaining C-4 and approximately 55% losing this carbon atom (see Table I). This observation illustrates the importance of isotopic labeling in mass spectrometric studies, even when high resolution spectra are available, as has been emphasized previously.[19] Since C-2 and C-3 are lost in all cases, cleavages (1) and (2) must be the major processes operating, associated with additional losses of methyl and hydrogen, respectively.



It is very likely that the first rupture occurring in ring A for both cleavages is of the 1–10 bond yielding b.[20] This corresponds to the prevalent α-cleavage observed in aliphatic ketones and initiated when ionization takes place by removal of a lone pair electron from oxygen (a).[21] Loss of ethylene and carbon monoxide from a, coupled with elimination of a methyl group, can give rise to *m/e* 203. It will be noted that since a radical is already present at C-10 in b, the loss of methyl would not be expected to occur from C-19, which is consistent with the observed facts (see Table I). Alternatively, if explusion of the methyl group precedes fragmentation in ring A, only those species which did not lose C-19 would be anticipated to undergo this fragmentation. A possible representation for this fragment is c.

The hydrogen loss from the charged fragment associated with cleavage (2) occurs partially (40%) from

(17) Reference 13, p. 339.

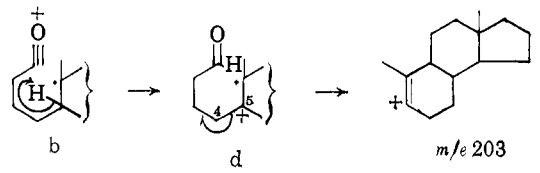
(18) We wish to thank Dr. R. M. Elliot of Associated Electrical Industries for the high resolution measurements which were performed with an MS-9 instrument.

(19) Z. Pelah, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 2470 (1963). An alternative explanation of the labeling data is that C-4 is retained in the whole of the *m/e* 203 ion and that in 55% of the species both hydrogen atoms originally attached to C-4 are lost. However, this interpretation must be regarded as extremely unlikely since cleavage of the 3–4 bond would be required in all cases (*vide supra*), thus necessitating the fission of three bonds of the same carbon atom (C-4).

(20) In this and all subequent publications from this laboratory, a fish-hook is employed to indicate a one electron shift, while an arrow implies a two-electron movement (see ref. 4, pp. xi–xiii).

(21) Reference 4, Chapter 1.

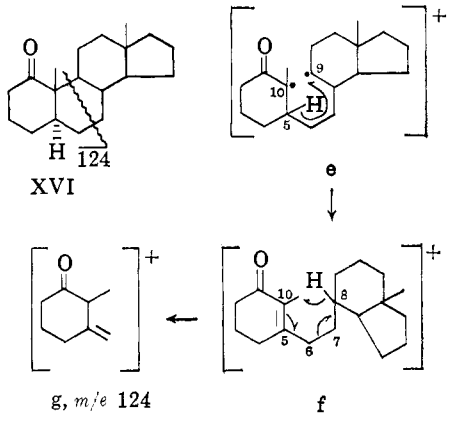
C-5 (see Table I) and a plausible mechanism accommodating this feature from b is illustrated.



b d *m/e* 203

This mechanism closely parallels one recently established to operate in the fragmentation of 5α-androstan-3-one.[7] The migration of hydrogen in a six-membered transition state is a well-established process in mass spectrometry, the driving force in the present case being the formation of an ionized olefinic linkage (d). Since the ionization potentials of olefins are less than those of hydrocarbons,[22] d is quite a favorable species. The rationale for the cleavage of the 4–5 bond in d is less obvious. Cleavage of a vinyl bond is normally regarded as unfavorable, but the recent observations on the mass spectra of steroids[7] suggests that a reappraisal of this view may be necessary in certain cases.

Peak *m/e* 124.—From Table I it can be seen that ring A and C-6 are retained by the charged species. This ion therefore formally corresponds to a fragment obtainable by cleavage of the 9–10 and 6–7 bonds in ring B with no net hydrogen transfer (see XVI). However, since hydrogen attached to C-4 (45%) and C-5 (100%) is lost during the fragmentation process (see Table I), approximately 55% of the cleavage must be occurring with transfer of one hydrogen atom in each direction and the remaining 45% with *transfer of two hydrogen atoms in each direction.* In the light of our knowledge that six-membered transition states and the formation of double bonds are favorable processes, the transfer of one hydrogen in each direction is not too surprising.



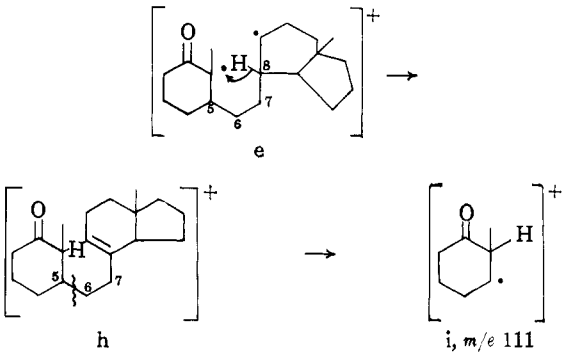
XVI e

g, *m/e* 124 f

Thus, if homolysis of the highly substituted 9–10 bond occurs (e), hydrogen radical transfer from C-5 can furnish f, the driving force being formation of the 5–10 double bond. Back-transfer from C-8 with concomitant fission of the 6–7 linkage then yields g, *m/e* 124. The process involving the transfer of two hydrogens in each direction cannot be explained with our present knowledge. It is pertinent to note that such complex rearrangements do not plague the rationalization of the spectra of molecules in which charge localization is specific.[3,9,10]

(22) F. H. Field and J. L. Franklin, "Electron Impact and the Properties of Gaseous Ions," Academic Press, Inc., New York, N. Y., 1957, p. 239.

Peak *m/e* 111.—The data given in Table I indicate that this ion too contains ring A, but does not retain C-6. Its genesis must involve hydrogen migration to the charged moiety and this can be accommodated if e, instead of going to f, undergoes the alternative hydrogen transfer from C-8 to h.

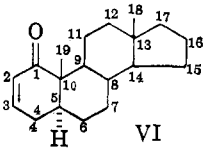


e

h i, *m/e* 111

Homolysis of the 5–6 bond then gives i, *m/e* 111. As to why 5–6 cleavage is favored over allylic 6–7 rupture is not apparent. Obviously, hydrogen radical transfer from C-7 in e, leaving a radical at C-7, would give a driving force for the subsequent 5–6 rupture. However, such a migration from C-7 suffers the disadvantage of having no obvious driving force and is in fact excluded by the labeling experiments (Table I).

Δ²-5α-Androsten-1-one (VI).—The compounds available to permit an analysis of the mass spectrum of



VI

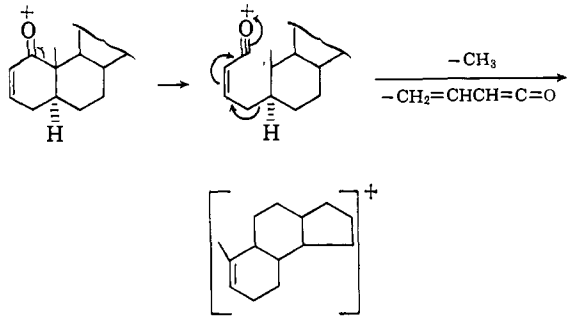
Δ2-5α-androsten-1-one (VI) are summarized in Table III.

TABLE III

Compound	M⁺	*m/e* values			
d_0-VI	272	108	109	122	189
2,4,4-d_3-VI	275	110, 70% 111, 30%	112	124, 50% 125, 50%	189
5α-d_1-VI	273	108, 40% 109, 60%	110	122	190
6,6-d_2-VI	274	110	109	124	191
7ξ-d_1-VI	273	108	109	122	190
16-d_1-VI	273	108	109	122	190

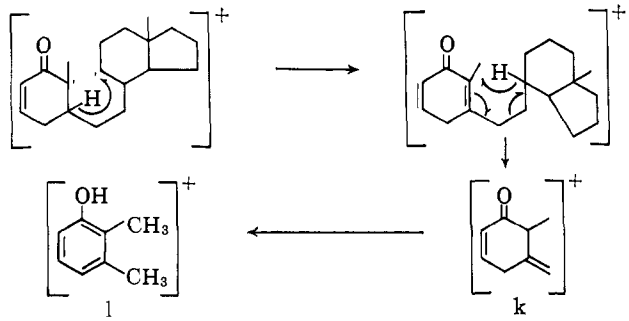
The isotopic purities of the 5α-d_1-, 6,6-d_2-, 7ξ-d_1-, and 16-d_1- derivatives can be read from the corresponding analogs in Table I since these enones were in fact the precursors of the saturated analogs. The isotopic purity of the 2,2,4-d_3- compound was d_2, 4%; d_3, 85%; d_4, 9%; d_5, 2%. The outstanding ions in the mass spectrum (Fig. 2) of Δ²-5α-androsten-1-one (VI) occur at *m/e* 108, 109, 122, and 189; their shifts in the various labeled compounds can be followed and are summarized in Table III.

Peak *m/e* 189 (M − 83).—The shifts of this ion in the deuterated analogs (Table III) indicate that C-5, C-6 (see Fig. 3), and C-7 are retained, while C-2 and C-4 are expelled. It therefore arises from loss of ring A (M − 68) and a methyl group (M − 15). By analogy to the *m/e* 203 ion in the spectrum of the saturated 1-ketone VII, the C-18 methyl group is probably lost as indicated in representation j.



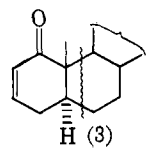
j, *m/e* 189

Peak *m/e* 122.—By reference to Table III, it can be seen that this charged fragment encompasses ring A and C-6. Since it occurs two mass units below g (*m/e* 124), it is probably the Δ^2-analog of this ion. Indeed, the complete loss of deuterium from C-5 and partial loss from C-4 (see Table III) indicate that the same mechanism (single and double hydrogen transfer in *each* direction) is operative in its formation. The single hydrogen transfer in each direction leading to k is indicated below. The greater importance of this type of fragmentation in the Δ^2-analog VI, when compared with the saturated ketone VII, may be due to the fact that k can derive further stabilization by isomerization to the phenol l.

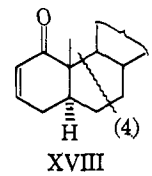


l k

Peaks *m/e* 108 and *m/e* 109.—Examination of the results presented in Table III indicates that both of these ions retain ring A. However, it should be pointed out that the shifts observed in the spectrum (Fig. 3) of the 6,6-d_2- derivative cannot be interpreted entirely unambiguously. One possibility is that *m/e* 108 is shifted mainly to *m/e* 109 and to a smaller extent to *m/e* 110 (by migration of one and two deuterium atoms, respectively, to the charged fragment), while *m/e* 109 moves to *m/e* 110 (gain of one deuterium atom). If this interpretation were correct, then both ions would be formed by cleavage (3) (see XVII) with, as a minimum, migration of one or two hydrogens in each direction (*m/e* 108) and migration of one hydrogen to the charged fragment (*m/e* 109).

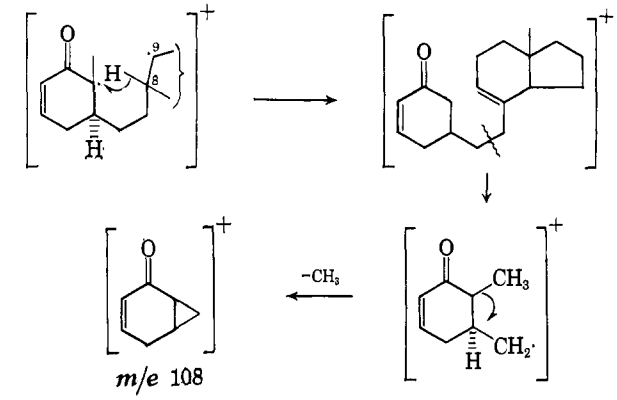


XVII



XVIII

The probability of such an interpretation being correct is very small since all mechanisms would require breaking two or three bonds connected to C-6. A much more reasonable assumption is that the *m/e* 108 ion encompasses C-6 and therefore moves to *m/e* 110 in the spectrum of 6,6-d_2-VI while *m/e* 109 is unmoved. This gives rise to a situation in which *m/e* 109 must be formed *via* cleavage (3) with gain of two hydrogens and loss of one hydrogen (note the quantitative loss of one deuterium atom from either C-2 or C-4 in its genesis —see Table III) while *m/e* 108 is furnished by cleavage (4) (see XVIII) with transfer of one hydrogen atom to the charged entity and associated loss of a methyl group. A speculative mechanism for the latter process is given below, but it must be admitted that at present the complex rearrangements involved in the formation of *m/e* 109 remain an enigma.



m/e 108

Experimental[23]

1α,2α-Oxido-5α-androstan-3-one (IV).—Δ^1-5α-Androsten-3-one (1.3 g.),[24] 25 cc. of ethanol, a solution of 10 cc. of 30% hydrogen peroxide, and 4 drops of 40% sodium hydroxide were heated at reflux temperature for 10 min. The resulting solution was diluted with ether, thoroughly washed with water, and dried over magnesium sulfate. Evaporation of the ether solution gave a crystalline mass (one spot on a thin layer chromatogram developed with hexane containing 15% ether). Recrystallization of this material from aqueous ethanol gave the oxidoketone (IV, 1.01 g.), m.p. 105–106°, $[\alpha]_D$ +113° (*c* 1.6); λ_{max}^{KBr} 5.9, 115, and 12.7 μ.

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.12; H, 9.79. Found: C, 78.88; H, 10.00.

Δ^2-5α-Androsten-1α-ol (V).—A mixture of 750 mg. of 1α,2α-oxido-5α-androstan-3-one (IV) and 12 cc. of 100% hydrazine hydrate was heated at reflux temperature for 15 min. After dilution of the cooled mixture with ether, the organic layer was washed with water and dried. The yellow gum which remained after evaporation of the ether was chromatographed on neutral alumina (Activity II) and elution with benzene gave 536 mg. of crystalline material, m.p. 103°. Recrystallization from aqueous methanol gave the allylic alcohol V as white needles, m.p. 103°, $[\alpha]_D$ +133° (*c* 1.5), no carbonyl absorption in the infrared.

Anal. Calcd. for $C_{19}H_{30}O$: C, 83.15; H, 11.02. Found: C, 82.52; H, 10.91.

Δ^2-5α-Androsten-1-one (VI).—Jones reagent was added dropwise to a chilled solution of Δ^2-5α-androsten-1α-ol (256 mg.) in 10 cc. of acetone with constant stirring at the rate of decolorization. The mixture was diluted with ether, washed with water, dried, and evaporated, yielding a crystalline product which was recrystallized from aqueous methanol to give the unsaturated ketone VI (157 mg.), m.p. 75–75.5°, $[\alpha]_D$ +134°, λ_{max} 225 mμ (ε 7600), $\lambda_{max}^{CHCl_3}$ 5.9 and 6.0 μ.

(23) All melting points are corrected and were determined on a Kofler block. Rotations were measured in chloroform and ultraviolet absorption spectra in 95% ethanol solution. Thin layer chromatography (t.l.c.) was performed on silica gel G (E. Merck, A.G., Darmstadt), the spots being developed by spraying with a 2% solution of ceric sulfate in 2 *N* sulfuric acid and subsequent heating. All 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 maintained at 270°. The ionizing energy was kept at 70 e.v. and the ionizing current at 50 μa. The microanalyses are due to Messrs. E. Meier and J. Consul. The isotopic purities of deuterated ketones are given in Table II.

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

Anal. Calcd. for $C_{19}H_{28}O$: C, 83.77; H, 10.36. Found: C, 83.40; H, 10.39; mol. wt., 272 (mass spec.).

5α-Androstan-1-one (VII).—Δ^2-5α-Androsten-1-one (38 mg.) was stirred under an atmosphere of hydrogen for 15 min. in cyclohexane (3 cc.) over palladium-on-charcoal (10%) at room temperature. The catalyst was removed by filtration and the filtrate evaporated to dryness. Recrystallization of the residue from aqueous methanol gave the ketone VII as white platelets (25 mg.), m.p. 71°, $[\alpha]D$ +99°, λ_{max}^{KBr} 5.9 μ.

Anal. Calcd. for $C_{19}H_{30}O$: C, 83.15; H, 11.02. Found: C, 82.85; H, 11.05; mol. wt., 274 (mass spec.).

3α-d_1-5α-Androstan-1-one (IX).—A solution of the Δ^2-1-one VI (18 mg.) in cyclohexane (2 cc.) was stirred under an atmosphere of deuterium in the presence of 10% palladium-on-charcoal for 15 min. The isolated reduction product (16 mg.), in acetone solution, was treated with Jones reagent to reoxidize any alcohol produced by overreduction. The material was reisolated and then heated at reflux temperature in a solution of 80% aqueous methanol (3 cc.) containing sodium hydroxide (100 mg.) for 3 hr. The usual work-up yielded, after one crystallization from aqueous methanol, 3α-d_1-5α-androstan-1-one (IX, 10 mg.), m.p. 69–70°.

2,4,4-d^2-Δ^2-5α-Androsten-1-one (X).—Δ^2-5α-Androsten-1-one (60 mg.), 4.5 cc. of deuteriomethanol, and 1 cc. of deuterium oxide containing 90 mg. of dissolved sodium were heated at reflux temperature for 1 hr. The reaction mixture was evaporated to dryness and the residue extracted with ether. The ether solution was washed, dried, and evaporated to dryness yielding a crystalline mass which was recrystallized from deuteriomethanol and deuterium oxide. The deuterated enone X (40 mg.) so obtained had m.p. 74–75°.

4,4-d_2-5α-Androstan-1-one (XI).—The above polydeuterated ketone X (20 mg.) was hydrogenated in cyclohexane over palladium-on-charcoal at room temperature and atmospheric pressure. The usual work-up gave an oil which was treated with a 5% solution of sodium hydroxide in 80% aqueous methanol (15 cc.) at reflux temperature for 20 min. The residue obtained by removal of the solvents was extracted with ether. The extract was washed with water, dried, and evaporated, and the residue was recrystallized from aqueous methanol furnishing XI (14 mg.), m.p. 70–71°.

2,2-d_2-5α-Androstan-1-one (XII).—A solution of 11 mg. of 5α-androstan-1-one in 3 cc. of deuteriomethanol and 0.5 cc. of deuterium oxide containing dissolved sodium (200 mg.) was heated at reflux temperature for 1 hr. After evaporation of the solvents, the residue was extracted with ether and the ether extract washed with deuterium oxide, dried, and evaporated to dryness. Recrystallization of the residue from deuteriomethanol and deuterium oxide gave the dideuterated ketone XII (8 mg.), m.p. 70–71°.

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Nuclear Magnetic Resonance Spectroscopy. The Effect of Structure on Magnetic Nonequivalence Due to Molecular Asymmetry[1]

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The difference in chemical shift between the methylene protons of a number of compounds related structurally to 1-phenylethyl benzyl ether has been measured. Correlations between structural features of these compounds and the magnitude of the magnetic nonequivalence induced by the center of molecular asymmetry provide a basis for qualitative identification of the most important factors contributing to the chemical-shift difference. Although it is clear that no single factor completely determines the magnitude of the methylene proton nonequivalence in these ethers, it is suggested that the proximity of the asymmetric center to the benzyl group results in a preferred conformation of the phenyl ring with respect to the methylene protons, and that the principal contribution to the nonequivalence originates in the magnetic anisotropy of the phenyl group.

Introduction

The protons of a methylene group or isopropyl group removed by one or more bonds from a center of molecular asymmetry may be magnetically nonequivalent and display AB-type nuclear magnetic resonance spectra.[2] The origin of this magnetic nonequivalence has been a subject of several investigations.[2-4] Although the existence of preferred conformations of the methylene group or the isopropyl group with respect to the asymmetric center[5] has generally been considered necessary for magnetic nonequivalence, several workers have pointed out that such preferred conformations are not a theoretical prerequisite for observable asymmetry.[6] The problem of the possible importance of small contributions to magnetic nonequivalence arising from an "intrinsic" asymmetry, independent of rotational conformer populations, has not yet been completely resolved; however, no convincing experiment has so far been reported which has demonstrated significant contributions from intrinsic asymmetry to an observed chemical shift. However, it should be emphasized that in discussing the relative importance of conformational preference and intrinsic asymmetry, the question at issue is not whether the former or the latter is *alone* responsible for magnetic nonequivalence, but rather how much, if any, of an observed nonequivalence should be ascribed to intrinsic asymmetry. There seems little doubt that conformational preference with respect to the asymmetric center must in general be responsible for the major contributions to the magnetic nonequivalence.

The work reported in this paper is concerned with an empirical study of the effect of structure on the magnitude of the magnetic nonequivalence of methylene and isopropyl groups close to the center of molecular asymmetry in relatively simple model compounds. As such, its immediate purpose was to determine those types of structural features which could be associated with large values of magnetic nonequivalence. However, attempts to reconcile the results obtained with an explanation of the manner in which the asymmetric

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(2) For a compilation of references to pertinent examples, see E. I. Snyder, *J. Am. Chem. Soc.* **85**, 2624 (1963).

(3) G. M. Whitesides, F. Kaplan, K. Nagarajan, and J. D. Roberts, *Proc. Natl. Acad. Sci. U. S.*, **48**, 1113 (1962), and references therein.

(4) H. S. Gutowsky, *J. Chem. Phys.*, **37**, 2196 (1962); H. S. Gutowsky, G. G. Belford, and P. E. McMahon, *ibid.*, **36**, 3353 (1962).

(5) It should be noted that the discussion in this paper is equally applicable to methylene groups adjacent to a dissymmetric center; see, for example, W. L. Meyer and R. B. Meyer, *J. Am. Chem. Soc.*, **85**, 2170 (1963).

(6) J. S. Waugh and F. A. Cotton, *J. Phys. Chem.*, **65**, 562 (1961); J. A. Pople, *Mol. Phys.*, **1**, 3 (1958).