2,2,4,4-d_4-Cholesteryl Trimethylsilyl Ether.—This procedure is identical with that used in the synthesis of the 3α-d_1-labeled trimethylsilyl ether with a few variations. *t*-Butyl alcohol was replaced with *t*-butyl alcohol-O-d_1 and acetic acid-O-d_1 was added to the reaction mixture instead of unlabeled acetic acid. In the reduction of the keto group, lithium aluminum hydride was used instead of lithium aluminum deuteride. The labeled cholesterol analog (IX) was converted to its trimethylsilyl ether by procedure B giving 45 mg of 2,2,4,4-d_4-cholesteryl trimethylsilyl ether, mp 115–118°. The mass spectrum indicated the following isotopic distribution: 3% d_0 (*m/e* 458), 3% d_1 (*m/e* 459), 8% d_2 (*m/e* 460), 29% d_3 (*m/e* 461), 54% d_4 (*m/e* 462), and 3% d_5 (*m/e* 463).

2,2,4-d_3-$\Delta^{3,5}$-Cholestadien-3-yl Acetate (XII).—In a nitrogen atmosphere was placed 200 mg of Δ^4-cholesten-3-one (III) and 500 mg of potassium *t*-butoxide. *t*-Butyl alcohol-O-d_1 (5 ml) was added and the yellow solution allowed to stir at room temperature for 2 hr. After the addition of 1 ml of acetic anhydride, the solution was stirred for another hour. The mixture was poured into cold water, extracted with ether, washed twice with cold water, twice with aqueous sodium bicarbonate, and twice more with cold water, and dried for 16 hr at 0° over anhydrous magnesium sulfate. The ether was evaporated and the fluorescent, oily product was recrystallized twice from ethanol and once from methanol giving 123 mg of white needles: mp 79–81°; $[\alpha]^{25}D$ −99.0° (*c* 1.003); λ_{max}^{EtOH} 235 mμ (log ϵ 4.25), λ_{max}^{Nujol} 5.7, 6.0, and 6.2 μ. The nmr spectrum exhibited one vinylic proton signal at δ 5.4.[35]

2,2,4-d_3-Cholesteryl Trimethylsilyl Ether.—A solution of 121 mg of sodium borohydride in absolute ethanol (4 ml) was slowly added over a period of 30 min to an ice-cold stirring solution of 61 mg of 2,2,4-$\Delta^{3,5}$-cholestadien-3-yl acetate (XII) in 11 ml of absolute ethanol. Stirring was continued at 0° for 42 hr, at which time the mixture was heated under reflux for 1 hr. Concentrated hydrochloric acid (1.8 ml) was added dropwise and refluxing was continued for another hour. After cooling to room temperature, the mixture was extracted twice with ether and the combined ethereal extracts were washed four times with water. The ethereal solution was then dried over anhydrous magnesium sulfate. The ether was evaporated, and the mixture of products was dissolved in a minimum amount of benzene. Chromatography on silica (Davison, Grade 950, 60–200 mesh) with benzene as eluent gave a white powder which was recrystallized from methanol to give 22 mg of 2,2,4-d_3-cholesterol (XI), mp 141–143°.

Utilizing procedure B this deuterated analog was converted to 7.1 mg of 2,2,4-d_3-Δ^5-cholesten-3β-yl trimethylsilyl ether, mp 114–116°. The mass spectrum showed the following isotopic distribution: 8% d_1 (*m/e* 459), 29% d_2 (*m/e* 460), 55% d_3 (*m/e* 461), 6% d_4 (*m/e* 462), and 2% d_5 (*m/e* 463).

Registry No.—II, 1856-05-9; XIV, 7604-81-1; XV, 7604-82-2; XIII, 7604-83-3; N-(triethylsilyl)acetamide, 7604-84-4; cholesterol 3β-triethylsilyl ether, 7604-85-5; X trimethylsilyl ether, 7604-86-6; 2,2,4,4-d_4-cholesterol trimethylsilyl ether, 7604-87-7; XII, 7604-88-8; 2,2,4-d_3-Δ^5-cholesterol trimethylsilyl ether, 7604-89-9; XI, 7604-90-2; 2,2,4,4-d_4-cholesterol trimethylsilyl ether, 7604-91-3.

Acknowledgment.—Grateful acknowledgment is made to Dr. Peter Brown for stimulating discussion in connection with this work.

(35) This spectrum was recorded by Mr. D. McMillan with a Varian A-60 spectrometer, employing deuteriochloroform as solvent and tetramethylsilane as internal reference.

Mass Spectrometry in Structural and Stereochemical Problems. CXXVI.[1] Synthesis and Fragmentation Behavior of Deuterium-Labeled 17-Keto Steroids[2]

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Deuterium labeling of 5α-androstan-17-one and high-resolution mass spectrometry establish that the diagnostically significant *m/e* 230 peak arises from loss of carbon atoms 17 and 16. The two hydrogen atoms which migrate from the charged portion of the molecule do not originate from the C-18 angular methyl group but come from at least three positions, C-7, C-8, and C-12. These results are interpreted in terms of a fragmentation sequence involving breaking of the 13,17 bond followed by a random migration of one hydrogen to the radical center (C-17) and subsequent site-specific McLafferty migration of the second hydrogen. The lack of deuterium removal from positions other than C-15 and C-16 suggests that the hydrogen migration occurring in the formation of *m/e* 217 may be a random process as well. The genesis of each of the significant peaks in the high mass range of the mass spectrum of 5α-androstan-17-one is discussed.

Our preliminary study[4] of the mass spectra of steroidal ketones revealed the dependency of characteristic features of fragmentation on the position of the keto function. The complexity of the fragmentation process soon came to be appreciated as the result of further study in our laboratory.[5] Subsequently, our main interest in the mass spectral behavior of steroidal ketones centered on the reaction mechanisms involving hydrogen transfer. Detailed studies[6] on a number of deuterated steroidal ketones demonstrated further the significance of hydrogen rearrangements in the fragmentation mechanisms and illustrated the necessary steric requirements for these rearrangements. In continuing these studies, we now have investigated in a detailed manner the electron-impact fragmentation and accompanying hydrogen transfers in the only two remaining steroidal ketone types, the 17- and 20-keto steroids. This paper describes the synthesis of the deuterated 17-keto steroids, the mass spectral results, and the derived conclusions. Such studies seemed particularly relevant because of the biological importance

(1) Paper CXXV: J. Dickman and C. Djerassi, *J. Org. Chem.*, **32**, 1005 (1967).

(2) Financial assistance by the National Institutes of Health (Grants No. AM-04257 and CA-07195) is gratefully acknowledged.

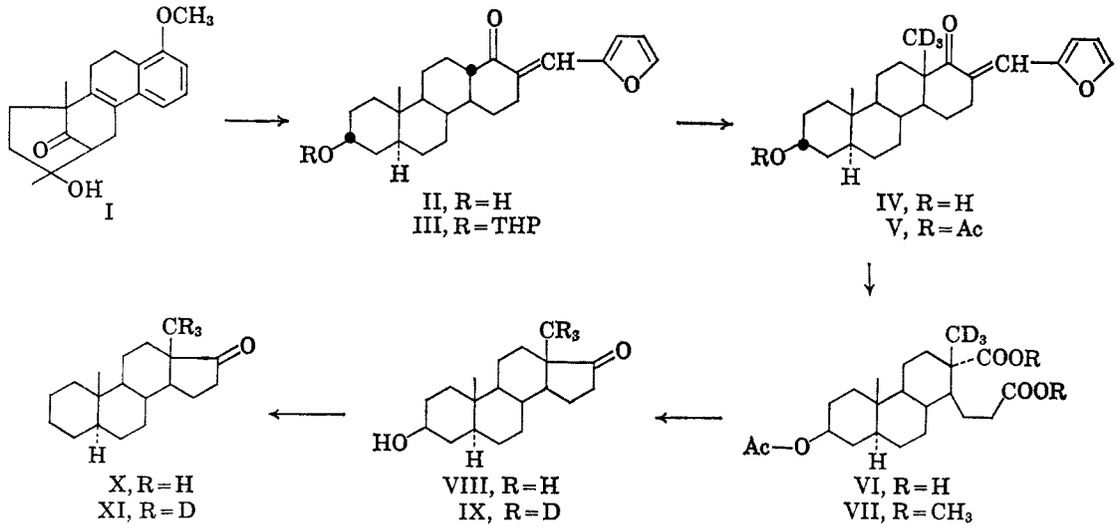
(3) Recipient of a National Institutes of Health Special Fellowship, 1965–1966, while on leave from the College of Forestry, Syracuse University.

(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); (d) R. Beugelmans, R. H. Shapiro, L. J. Durham, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *ibid.*, **86**, 2832 (1964).

(6) (a) R. H. Shapiro, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *ibid.*, **86**, 2837 (1964); (b) J. E. Gurst and C. Djerassi, *ibid.*, **86**, 5542 (1964); (c) C. Djerassi, G. von Mutzenbecher, J. Fajkos, D. H. Williams, and H. Budzikiewicz, *ibid.*, **87**, 817 (1965); (d) C. Djerassi, R. H. Shapiro, and M. Vandewalle, *ibid.*, **87**, 4892 (1965); (e) C. Djerassi and L. Tökés, *ibid.*, **88**, 536 (1966); (f) H. Gutzwiller and C. Djerassi, *Helv. Chim. Acta*, in press.

SCHEME I



of these two types. The subject of the 20-keto steroids is taken up in the sequel.[7]

Synthesis of 17-Keto Steroids

Inasmuch as the interconversion of 17-keto and 20-keto steroids is a well known and often traveled path in steroid chemistry[8] the major problem of labeling in a number of cases was resolved into one of preparing either the desired 17-keto steroid or the 20-keto steroid.[7] In fact the syntheses of the two types of keto steroids were closely associated with one another and the division of reporting their syntheses in this report and its sequel[7] is somewhat arbitrary.

Here, as in previous studies in this series,[5,6] it was necessary to synthesize the "naked" steroidal ketones and their appropriately labeled analogs since we wished to fix our attention on the fragmentation induced by a keto group in a specific position of a steroid without the complications caused by the presence of other functional groups.

The loss of ring D, either completely or partially, with single or double migration of hydrogen from the charge-retaining moiety had been proposed as a source of two of the more prominent ions appearing in the high mass region in the mass spectrum of 5α-androstan-17-one.[4] This proposal clearly called for the labeling of a number of nearby positions (C-18, C-12, C-8, C-7) in order to ascertain the point of origin of the migrating hydrogen. Positions on ring D (C-15, C-16) as well as a position (C-3) more distant from the locality of fragmentation also were labeled in order to establish firmly which carbon atoms were lost in the various fragmentation processes of interest.

18,18,18-d_3-5α-Androstan-17-one (XI) was prepared by way of the Johnson[9] total synthesis of steroids. Starting with the condensation product (I),[10] the tetrahydropyranyl ether (III) of the furfurylidene derivative (II) was prepared in five steps according to the published procedures[11] which were only slightly modified. The angular methylation with d_3-methyl iodide and subsequent hydrolysis of the tetrahydropyranyl ether according to the reported directions[11b] produced the C–D *trans*-trideuteriomethyl derivative (IV). Mass spectrometric analysis indicated that the isotopic purity of the d_3 product was greater than 96%. More precise values of 98% d_3 and 2% d_2 were obtained from the mass spectra of subsequently synthesized homologs which gave more intense molecular ion peaks. Since IV was employed for the synthesis of all the 18-d_3-labeled compounds, all must have the same isotopic purity. Conversion of alcohol IV to acetate V and ozonolysis followed by hydrogen peroxide treatment afforded dicarboxylic acid VI which in turn was converted to dimethyl ester VII. Dieckmann cyclization of diester VII appeared to be sensitive to the presence of oxygen but under properly controlled conditions gave the β-keto ester which was hydrolyzed and decarboxylated directly to give the labeled *dl*-18-d_3-epiandrosterone (IX) which was contaminated to a slight extent with olefinic material as evidenced by a small M − 2 ion in its mass spectrum. The infrared and mass spectra of the labeled *dl*-epiandrosterone were identical with those of authentic samples with the exception of the absorption and mass shifts owing to incorporation of deuterium atoms.

Conversion of the labeled *dl*-epiandrosterone (IX) to its tosylate followed by lithium aluminum hydride treatment and then Jones oxidation[12] produced *dl*-18-d_3-5α-androstan-17-one (XI) (see Scheme I). The transformation of the unlabeled *d*-epiandrosterone (VIII) to 5α-androstan-17-one (X) was effected in the same manner. With the exception of absorption and mass shifts owing to the incorporation of deuterium, both *d*- and *dl*-androstan-17-ones and their intermediates displayed identical thin layer chromatographic and spectral behavior. The olefinic contaminant present in the labeled epiandrosterone was carried through the synthesis but eventually was removed from the

(7) L. Tökés, R. T. LaLonde, and C. Djerassi, *J. Org. Chem.*, **32**, 1020 (1967).

(8) D. M. Feigl, "Steroid Reactions," C. Djerassi, Ed., Holden-Day, Inc., San Francisco, Calif., 1963, Chapter 10.

(9) W. S. Johnson, *J. Am. Chem. Soc.*, **78**, 6278 (1956), and subsequent papers.

(10) We gratefully acknowledge a gift of this material from Professor W. S. Johnson of this department.

(11) (a) W. S. Johnson, J. Szmuszkovicz, E. R. Rogier, H. I. Hadler, and H. Wynberg, *J. Am. Chem. Soc.*, **78**, 6285 (1956); (b) W. S. Johnson, B. Bannister, and R. Pappo, *ibid.*, **78**, 6331 (1956).

(12) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

final product by thin layer chromatography employing silver nitrate impregnated silica gel.

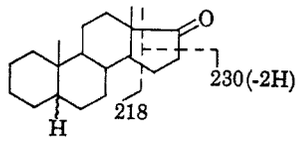
12,12-d_2-5α-Androstan-17-one (XIV) was obtained by the degradation of the C-17 acetyl side chain of 12,-12-d_2-5α-pregnan-20-one (XII).[7] This degradation was achieved by ozonolysis of the enol acetate derivative (XIII) according to the literature procedure.[13] Baeyer–Villiger oxidation[14,15] of the available 8β-d_1-5α-pregnan-20-one (XV)[7] followed by lithium aluminum hydride reduction of the resulting 17-acetoxyandrostane (XVI) and Jones oxidation[12] produced 8β-d_1-5α-androstan-17-one (XVII). 7β-d_1-5α-Androstan-17-one (XX) was obtained by a modified Wolff–Kishner[16] reduction of the C-17 monoketal of 7β-d_1-5α-androstane-3,17-dione (XVIII) followed by acid hydrolysis of the resulting C-17 ketal intermediate XIX.[6c]

Synthesis of 3α-d_1-5α-androstan-17-one (XXI) was achieved by treating the tosylate of 3β-hydroxy-5α-androstan-17-one (VIII) with lithium aluminium deuteride and then subjecting the resulting crude product to Jones oxidation. Base-catalyzed deuterium exchange converted 5α-androstan-17-one (X) to its 16,16-d_2 analog (XXII). 15α-d_1-5α-Androstan-17-one (XXV) was obtained by catalytic deuteration of Δ^{15}-5α-androstan-17-one (XXIII)[6c] followed by back exchange of the 16-deuterium atom in the intermediate XXIV (see Scheme II). The isotopic compositions of the above described, deuterium-labeled 17-keto steroids are given in Table I.

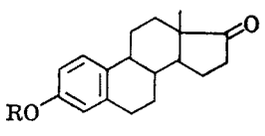
The hitherto unknown 5β-androstan-17-one (XXVI) was prepared by a modified Wolff–Kishner reduction[16] of 17β-acetoxy-5β-androstan-3-one (XXVIa)[17–19] followed by chromium trioxide oxidation of the intermediate 17β-alcohol (XXVIb).

Discussion of Mass Spectra

The electron impact induced fragmentation pattern of 5α-androstan-17-one (X) has been reported in connection with the initial survey of the mass spectrometric behavior of steroidal ketones,[4] but no detailed interpretation was attempted because of the absence of isotopically labeled substrates. The mass spectra of some substituted androstan-17-ones, without any interpretations, have been reported by Fitches.[20]



X, 5α
XXVI, 5β



XXVII

The applicability of mass spectrometry as a potential analytical tool in certain biochemical experiments

(13) C. W. Marshall, T. H. Kritchevsky, S. Lieberman, and T. F. Gallagher, *J. Am. Chem. Soc.*, **70**, 1837 (1948).
(14) V. Burckhart and T. Reichstein, *Helv. Chim. Acta*, **25**, 1434 (1942).
(15) P. deRuggieri, C. Ferrari, and C. Gandolfi, *Gazz. Chim. Ital.*, **91**, 686 (1961).
(16) Huang-Minlon, *J. Am. Chem. Soc.*, **68**, 2487 (1946).
(17) A. Butenandt, K. Tscherning, and H. Dannenberg, *Z. Physiol. Chem.*, **248**, 205 (1937).
(18) J. Fajkos, *Collection Czech. Chem. Commun.*, **23**, 1559 (1952).
(19) A generous specimen of this compound was supplied by Syntex, S. A., Mexico City.
(20) H. J. M. Fitches in "Advances in Mass Spectrometry," Vol. II, R. M. Elliott, Ed., The Macmillan Co., New York, N. Y., 1963, p 442.

SCHEME II

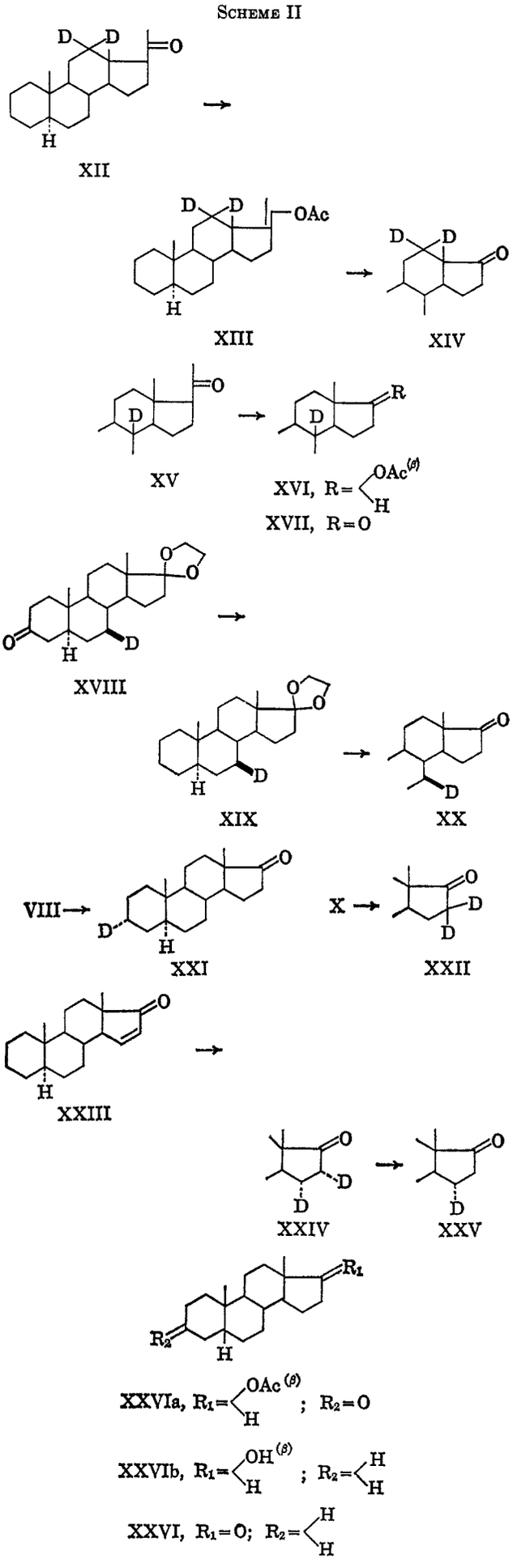


TABLE I

SHIFTS[a] OF THE PRINCIPAL PEAKS (*m/e*) IN THE MASS SPECTRA OF DEUTERATED 5α-ANDROSTAN-17-ONES

Compd	Isotope compn (%)	M+	M − 15 (%)	M − 18 (%)	M − 28 (%)	M − 33 (%)	M − 44 (%)	M − 56	M − 57 (%)	M − 59	M − 71	M − 99 (%)
d_0 (X)	...	274	259	256	246	241	230	218	217	215	203	175
3α-d_1 (XXI)	d_0 (2)											
	d_1 (98)	275	260	257	247	242	231	219	218	216	204	176
7β-d_1 (XX)	d_0 (5)			256 (25)		241 (20)	230 (15)			215 (30)		
	d_1 (95)	275	260	257 (75)	247	242 (80)	231 (85)	219	218	216 (70)	204	176
8β-d_1 (XVII)	d_0 (1)											
	d_1 (96)			256 (40)		241 (35)	230 (15)			215 (35)		175 (20)
	d_2 (3)	275	260	257 (60)	247[b]	242 (65)	231 (85)	219	218	216 (65)	204	176 (80)
15α-d_1 (XXV)	d_0 (8)											
	d_1 (90)				246 (~40)				217 (50)	215 (45)		
	d_2 (2)	275	260	257	247 (~60)	242	231	218	218 (50)	216 (55)	203	175
12,12-d_2 (XIV)	d_0 (2)											
	d_1 (21)											
	d_2 (68)						231 (5)					
	d_3 (9)	276	261	258	248[b]	243	232 (95)	220	219	217	205	175[b]
16,16-d_2 (XXII)	d_1 (11)				246 (~40)				217 (85)			
	d_2 (89)	276	261	258	248 (~60)	243	230	218	219 (15)	215	203	175
18,18,18-d_3 (XI)	d_2 (2)		259 (25)			241 (15)						
	d_3 (98)	277	262 (75)	259	249	244 (85)	233	221	220	218	206	178

[a] Reported shifts are corrected for isotopic impurity as well as ^{13}C contributions and are greater than 90% unless otherwise indicated. [b] Mainly at indicated *m/e* value, but exact calculation was impossible because of low intensity of peak or isotopic contaminants.

has been suggested[21] on the basis of observations in the estrogen series.[22] During these investigations[22] the fragmentation behavior of a number of estrone (XXVII) derivatives had been correlated, and it was found that the genesis of several ions in their mass spectra was affected by the presence of the 17-keto function. This was another reason why we wished to learn more about the fragmentation behavior of the "naked steroid," 5α-androstan-17-one (X).

It is apparent from the comparison of the mass spectra of 5α- (X) and 5β-androstan-17-one (XXVI) (Figures 1 and 2) that the configuration at C-5 has no major effect on the general fragmentation pattern, although definite differences are found in the relative intensities of some of the ions in the low mass range. These differences are probably indicative of a more intense hydrocarbon type fragmentation around the A/B ring junction in the more strained 5β (A/B *cis*) system.[23] The presence of a very intensive molecular ion in the mass spectra of steroidal monoketones is a common feature[4] and its occurrence in both Figures 1 and 2 is not surprising.

As noted in the description of the synthetic work, deuterium labeling was carried out only in the 5α series and the following interpretation of the characteristic features in Figure 1 is possible with the presently available data (Table I).

The expulsion of a methyl radical (*m/e* 259), a molecule of water (*m/e* 256), and the combination of these two (*m/e* 241) from the molecular ion is revealed in the mass spectrum of 5α-androstan-17-one as it is in the spectra of most steroidal ketones.[4,6,24] It is worth mentioning, however, that the mass spectrum of 18-d_3-5α-androstan-17-one (Table I) disclosed a substantial decrease in the loss of C-18 (25%) in the

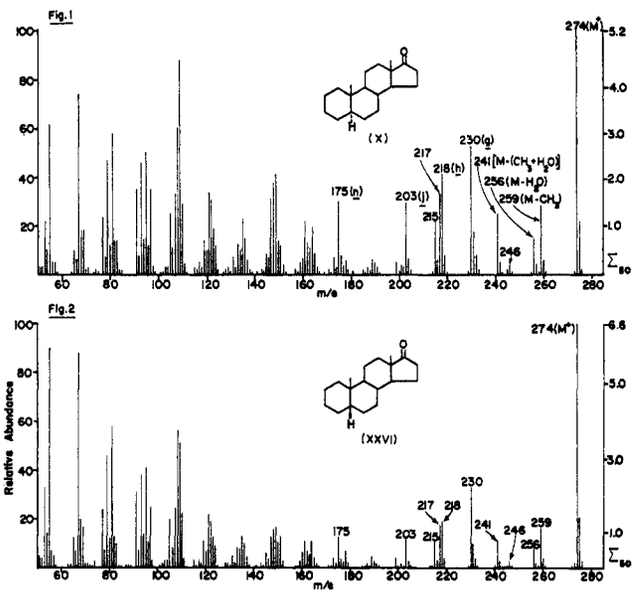


Figure 1.—Mass spectrum of 5α-androstan-17-one (X).
Figure 2.—Mass spectrum of 5β-androstan-17-one (XXVI).

M − CH_3 process compared with that (60%) in the corresponding hydrocarbon, 5α-androstane.[25] The reason for this observation apparently is associated with the presence of the C-17 carbonyl function which strongly enhances the homolysis of the 13,17 bond[26] at the expense of the required fission of the 13,18 bond. The same preference for the 13,17 bond cleavage, thereby reducing the C-18 contribution in the M − CH_3 process, was also observed in both 5α-pregnane[25] and 5α-pregnan-20-one.[7] Not only is the C-18 contribution to the *m/e* 259 ion (M − CH_3) small (Figure 1), but the loss of the C-18 methyl group in the combined loss of a methyl radical and water (*m/e* 241) is small (15%) as well. It is conceivable that the

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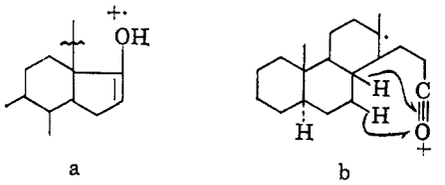
(22) C. Djerassi, J. M. Wilson, H. Budzikiewicz, and J. W. Chamberlin, *J. Am. Chem. Soc.*, **84**, 4544 (1962).
(23) P. Natalis, *Bull. Soc. Chim. Belges*, **72**, 264 (1963).
(24) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 2, Holden-Day, Inc., San Francisco, Calif., 1964, Chapter 20.
(25) L. Tökés, Ph.D. Thesis, Stanford University, 1965.
(26) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretations of Mass Spectra of Organic Compounds," Holden-Day, Inc., San Francisco, Calif., 1964, Chapters 1 and 8.

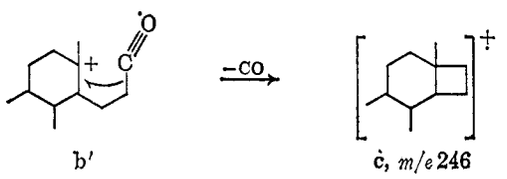
elimination of the 18-angular methyl group which does occur proceeds through the enolic molecular ion (a) in which C-18 is allylically activated.

The substantial loss of deuterium from the 7β (25%, Table I) and 8β positions (40%) in both the M − H_2O (*m/e* 256) and M − (H_2O + CH_3) (*m/e* 241) processes shows that the hydrogen transfers are preceded by a C–C bond rupture which facilitates the approach of the carbonyl oxygen to the C-8 and C-7 positions. Molecular ion b which is formed by the homolysis of the fully substituted α bond of the ionized C-17 carbonyl function,[26] is one of the probable intermediates for such hydrogen transfers.



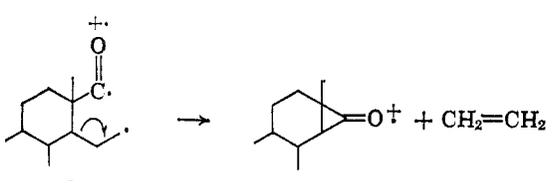
a b

According to high-resolution mass spectrometric analysis, the small M − 28 peak at *m/e* 246 represents at least two major ion components. One of these ions contains no oxygen atom and, therefore, is formed by the expulsion of carbon monoxide from the molecular ion. Cleavage of the 16,17 bond in molecular ion b′, yielding an ionized cyclobutane derivative (c, *m/e* 246), may be a possible explanation for this fragmentation.



b′ ċ, *m/e* 246

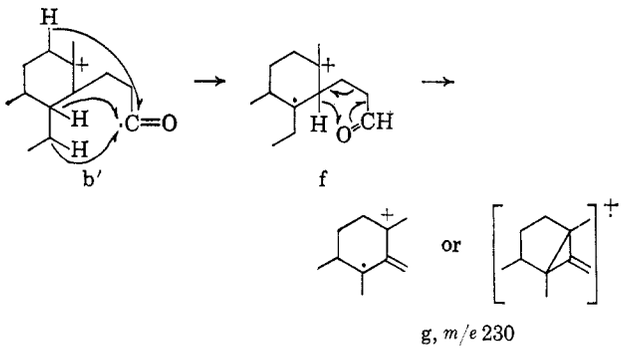
The deuterium-labeling results (Table I) suggest that the other major part of the M − 28 ion, in which the oxygen atom is retained, is formed by the loss of a neutral ethylene molecule, mainly from ring D (carbons 15 and 16). This process can be understood in terms of the homolysis of the activated 16,17 bond in molecular ion d (also an α-cleavage product of the ionized carbonyl group)[26] to produce a cyclopropanone derivative (e, *m/e* 246) and a neutral molecule of ethylene.



d e, *m/e* 246

The most characteristic fragmentation product (Figure 1) of androstan-17-one is the ion of mass 230 (M − 44) which arises by elimination of a neutral acetaldehyde molecule (see dotted line in formula X). This reaction involves the cleavage of the 15,16 and 13,17 bonds, in conjunction with the transfer of two extra hydrogen atoms from the charge-retaining moiety.[4] The deuterium-labeling data (Table I) clearly demonstrate the loss of C-16, a result confirmed by the recent report by Egger and Spiteller[27] that the label at C-16 in 16,16-d_2-3α-acetoxy-5α-androstan-17-one is lost. There remains only the determination of the origin of the two migrating hydrogen atoms.

On the basis of interatomic distance considerations, there are several sites (C-7, C-8, C-11, C-12, C-14, and C-18) in molecular ion b′, from which hydrogen transfer could occur. Egger and Spiteller[27] have proposed C-18, without labeling results, as the first of the two sites from which hydrogen is transferred in a two-step transfer mechanism starting with molecular ion b′. Interestingly, no significant contribution could be observed (Table I) from C-18 which is one of the three positions (C-12, C-14, and C-18) activated by the adjacent electron-deficient site on C-13. The observed transfer values from the implicated positions for which deuterium labeling is currently available (C-7, C-8, C-12, and C-18) represent the transfer of only about one-third of a hydrogen atom. This and the demonstration that at least three sites are involved (C-7, C-8, and C-12) indicates a somewhat random transfer process for at least one, and possibly both, of the two hydrogen atoms involved. The direct formation of the *m/e* 230 ion from the molecular ion has been substantiated by the presence of a metastable peak at *m/e* 193.1 ($230^2/274$ = 193.1) in an AEI MS-9 spectrum of 5α-androstan-17-one. One possible mechanism (b′ → g) consistent with the results is a two-step hydrogen transfer from b′, the first hydrogen transfer being a random one while the second is site specific occurring by way of the McLafferty rearrangement (f → g).

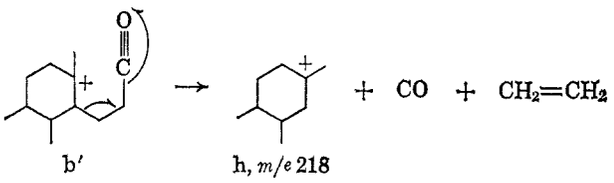


b′ f

g, *m/e* 230

The *m/e* 215–218 peaks in Figure 1 represent a rather complicated group of ions. Two of them, *m/e* 218 and 217, are formed by losing 56 and 57 mass units, respectively, from the molecular ion and in the mass spectra of the previously reported[22] estrone analogs these ions apparently are due to the loss of ring D (fission of the 14,15 and 13,17 bonds) with no, or a single, hydrogen transfer from the charge retaining side.[22] High-resolution analysis of these ions, however, showed that the *m/e* 217 peak represents not only C_{16}-H_{25}, but also 20% of an oxygen-containing fragment ($C_{15}H_{21}O$) while only traces of oxygen-containing species were found in the ion of mass 218. The deuterium labeling results (Table I) are in good agreement with this observation since all ring D labels were lost in the *m/e* 218 ion while some of the labels were retained on both carbons 15 and 16 in the *m/e* 217 ion. The partial retention of labels on C-15 and C-16 indicates that a fraction of *m/e* 217 is formed by losing a C_4H_9 radical from some other, undetermined part of the molecule. A plausible path for the genesis of the ion of mass 218

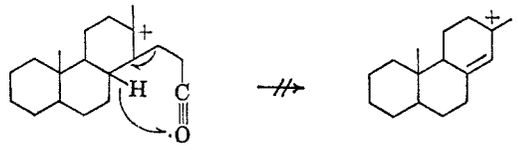
(27) H. Egger and G. Spiteller, *Monatsh. Chem.*, **97**, 579 (1966).



b′ h, *m/e* 218

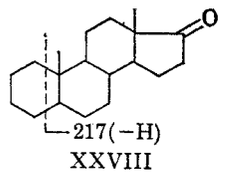
originates in molecular ion b′, and yields an ionized olefin h (*m/e* 218).

The fragmentation pattern of a major part (at least 50%) of the *m/e* 217 ion, which contains no oxygen atom and lost both C-15 and C-16 labels, is reminiscent also of the ring D cleavage of androstane.[25] In the present case, however, the required primary fission of the 13,17 bond should be enhanced by the presence of the carbonyl function on C-17. In the resulting intermediate (b′) the carbonyl group could abstract a hydrogen atom from several different positions (depicted below in terms of C-8) and subsequent fission of the 14,15 bond then would give the corresponding *m/e* 217 ion (i). Inspection of Table I reveals that none of the labeling in any of the positions other than C-15 and C-16 is removed. By analogy to the results in the hydrocarbon series[25] where virtually every carbon atom in the steroid skeleton had been labeled separately, it seems likely that a very strong isotope effect is operating so that deuterium labeling of individual sites will not shed much light on the origin of the itinerant hydrogen atom.



b′ i, *m/e* 217

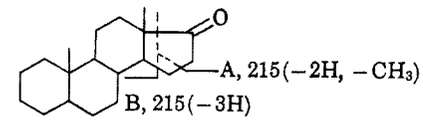
The minor, oxygen-containing part (~20%) of the *m/e* 217 peak (see XXVIII) could not be identified clearly, but probably a significant part of it is due to a ring A cleavage which was found[25] to be responsible for about 90% of the *m/e* 203 ion in the androstane spectrum. This fragmentation would account for about 15% of the rather high retention (~50%) of the 15α-d_1 label, but with the currently available data no definite explanation is possible for the remaining 35% of it.



XXVIII

The *m/e* 215 ion also represents a complicated case. The only possible empirical formula for the expelled fragment of 59 mass units is C_3H_7O and according to the deuterium labeling results (Table I) C-16 is lost entirely while labels at C-8, C-7, and C-15 are lost in part. The presence of metastable peaks at both *m/e* 201.0 ($215^2/230 = 201.0$) and *m/e* 168.7 ($215^2/274 = 168.7$) in the AEI MS-9 spectrum of 5α-androstan-17-one suggests that at least two different processes may be responsible for the formation of the *m/e* 215 ion; one process would involve the *m/e* 230 ion as intermediate, while the other would be its direct formation from the molecular ion.

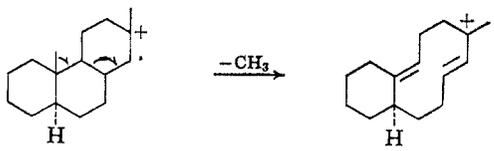
The 55% retention of the C-15 deuterium label could be understood best in terms of the two-step cleavage process (A in XXIX) by ejection of a methyl radical from the *m/e* 230 species in which C-15 with its protons is retained. Since participation of the C-18 function is excluded by the deuterium-labeling results (Table I), it is very likely that the 19 angular methyl group is involved in this fragmentation. However, for further mechanistic considerations with respect to this cleavage process, it would be necessary to know more about the identity of the *m/e* 230 ion (*vide supra*).



XXIX

There are two possible interpretations for the 45% loss of the 15α-d_1 label, but both of them are ambiguous. It could be indicative of either the loss, to the extent of 45%, of C-15 or of a stereospecific hydrogen transfer from this position. According to the first interpretation, if 45% of C-15 is lost, then this fragmentation (B in XXIX) must be accompanied by a triple hydrogen transfer from the charge-retaining side and, since the migration of less than one hydrogen is apparent from positions 7 and 8, it is not obvious from where the remaining hydrogens would originate. On the other hand, loss of a hydrogen atom from C-15 would also be surprising since the 15,16 bond has to be cleaved subsequently. It is likely, therefore, that this fragmentation step is accompanied by other rearrangements.

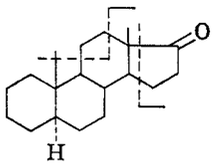
According to high-resolution analysis the *m/e* 203 ion in Figure 1 contains no oxygen atom. As the deuterium labeling results of Table I reveal, all the labels in ring D are lost, while they are retained on C-3, C-7, C-8, C-12, and C-18. On the basis of this result and the observed metastable peak at *m/e* 189.0 ($203^2/218 = 189.0$) but absence of a metastable peak at *m/e* 150.4 ($203^2/274 = 150.4$), it is assumed that the same fragmentation pattern is responsible for the *m/e* 203 ion in the mass spectrum[7] of both 5α-pregnan-20-one and 5α-androstan-17-one (Figure 1). In both cases the ion of mass 218 is implicated as an intermediate, which upon losing a methyl radical from C-19 (note from Table I that C-18 is retained entirely), would yield j (*m/e* 203) in which the tertiary carbonium ion is allylically stabilized.



h, *m/e* 218 j, *m/e* 203

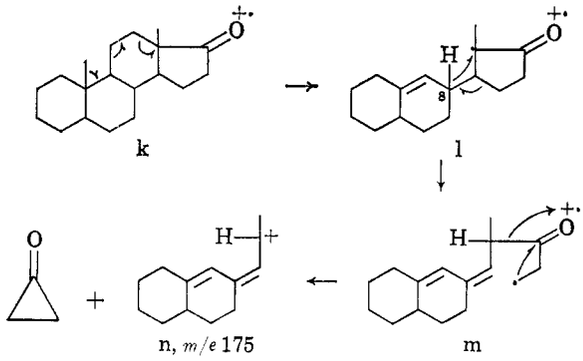
In the high mass range of Figure 1, there is one more peak, at *m/e* 175 ($C_{13}H_{19}$ by high resolution), where deuterium-labeling sheds some light on its nature. The deuterium-labeling results (Table I) reveal the elimination of ring D and most of C-12, and the retention of carbon atoms 3, 7, and 18 and most of C-8 in this ion. The loss of C-12 without C-11 would be

very unlikely and from a mechanistic point of view expulsion of C-19 is favored as the source of CH_3 needed to complete the production of this $C_{13}H_{19}$ ion (see XXX).



XXX

One plausible sequence (k–n) is depicted below and the reason for postulating the internal migration of the C-8 hydrogen atom (see l → m) is that fission of two bonds connected to one carbon atom (C-13 in the present case XXX) constitutes *prima facie* evidence of a radical transfer to that center in one of the intermediates.



k l n, *m/e* 175 m

In summary, it may be stated that the present deuterium labeling and high-resolution results demonstrate the unusual complexity of the fragmentation of 17-keto steroids and that unjustified "mechanistic" conclusions would probably have been drawn without the aid of these two powerful techniques. The fact that most of the fragment ions are hydrocarbons confirms the earlier conclusion[24] that the carbonyl group is not a very effective fragmentation-directing group.

Experimental Section[28]

18,18,18-d_3-*dl*-17-Furfurylidene-D-homoepiandrosterone (IV).—The condensation product I was converted to ***dl*-17-furfurylidine-18-nor-D-homoepiandrosterone tetrahydropyranyl ether** (III) according to the procedures published by Johnson, *et al.*[11] Introduction of the d_3-labeled angular methyl group and the subsequent hydrolysis of the tetrahydropyranyl ether function were carried out by using d_3-methyl iodide instead of methyl iodide according to the reported procedure.[11b] In two separate experiments the desired *trans*-trideuteriomethylated product (IV) was obtained in 13 and 16% yield (starting with 0.69 and 2.0 g of III, respectively), mp 220–225°. Recrystallization from ether–ethyl acetate mixture gave mp 225–228° (lit.[11b] 224–226°), λ_{max} 321 mμ (log ε 4.28–4.37) [lit.[11b] 323 mμ (log ε 4.34)]. This product exhibited better than 96% d_3 isotopic purity in its mass spectrum. No attempt was made to isolate the C–D *cis*-methylated product.

18,18,18-d_3-*dl*-17-Furfurylidene-D-homoepiandrosterone Acetate (V).—The pure *trans*-deuteriomethylated alcohol (IV, 300 mg, mp 223–227°, softening around 220°) was converted into its acetate derivative V by treatment with isopropenyl acetate according to the reported procedure.[11b] The resulting product (327 mg, 98% yield) showed mp 189–192° (lit.[11b] 192–192.5°), λ_{max} 319–320 mμ (lit.[11b] 322.5 mμ).

18,18,18-d_3-*dl*-3β-Acetoxyetioallohomobilianic Acid (VI).—The ozonolysis and the subsequent hydrogen peroxide treatment of the acetate derivative (V, 320 mg) were accomplished by following the reported procedure.[11b] The reaction mixture from the hydrogen peroxide treatment was worked up as follows. The volume of the solution was reduced by evaporating the acetic acid at 50° (15 mm), then it was diluted with ether and washed with ferrous sulfate solution containing a few drops of sulfuric acid. The ether phase was then extracted with sodium bicarbonate solution, and the collected bicarbonate solution was washed with ether, acidified with hydrochloric acid, and then extracted with ether. This extract was washed to neutrality and dried over magnesium sulfate. Evaporation of the ether gave 279 mg (94%) of colorless crystalline 18-d_3-*dl*-3β-acetoxyetioallohomobilianic acid (VI), mp 186–189° (lit.[11b] 185–188° or 237–239°).

18,18,18-d_3-*dl*-3β-Acetoxyetioallohomobilianic Acid Dimethyl ester (VII).—The crude acid VI was treated with an excess of diazomethane in ether.[11b] The resulting crude ester VII amounted to 292 mg (98%), mp 135–138° (lit.[11b] 136–137°).

18,18,18-d_3-*dl*-Epiandrosterone (IX).—The crude ester (VII, R = CH_3, 140 mg) was subjected to Dieckmann cyclization in the presence of potassium *t*-butoxide and following the procedure reported by Johnson, *et al.*[11b] The resulting acetoxy β-keto ester was decarboxylated and hydrolyzed without isolation. All of these reactions were carried out in an atmosphere of prepurified nitrogen and the reaction mixtures were degassed and rinsed with nitrogen. The resulting crude product [79 mg, 82%, mp 136–146° (154–157° in another experiment)], upon recrystallizations from methylcyclohexane and then from ether provided the pure 18-d_3-*dl*-epiandrosterone (IX) which exhibited mp 145–147° and some of it at 161–164° (lit.[11b] 161–162° with metamorphic change at 140–150°). This product showed identical infrared and mass spectra with those of an authentic epiandrosterone sample with the exception of mass shifts and absorptions owing to the deuterium atoms. The mass spectrum of this sample, however, indicated the presence of some unsaturated contaminant which was about the same amount as was found in the unlabeled synthetic sample provided by Professor W. S. Johnson.[10] When the preparation of 18-d_3-*dl*-epiandrosterone from methyl ester VII (R = CH_3, free of olefin contaminants according to its mass spectrum) was carried out without degassing the reaction mixtures and by using water-pumped nitrogen the resulting purified product (76% crude yield) showed about three times the amount of unsaturated contaminant as otherwise, judging by the relative intensity of the M − 2 ion in its mass spectrum.

5α-Androstan-17-one (X) and 18,18,18-d_3-*dl*-5α-Androstan-17-one (XI).—A solution of 5α-androstan-3β-ol-17-one (VIII)[19,29] in 2 ml of dry pyridine was treated with 100 mg of *p*-toluenesulfonyl chloride at ice-bath temperature. The colorless solution was left to stand at room temperature, in the dark, for 20 hr, then it was poured into a mixture of ice and hydrochloric acid. The resulting suspension was extracted with ether. The ether phase was washed with dilute hydrochloric acid solution, then with water and dried over magnesium sulfate. Evaporation of the solvent gave 153 mg (100%) of colorless crude 5α-androstan-3β-ol-17-one tosylate, mp 159–160° (lit.[30] 163–164°). This product was sufficiently pure for the next reaction step.

The crude 18-d_3-*dl*-epiandrosterone (IX, 140 mg) was converted into the tosylate derivative as described above for the unlabeled analog. The resulting semicrystalline product (215 mg, 100%) exhibited the same R_f value on tlc as the unlabeled analog.

A solution of the crude, unlabeled tosylate (153 mg) in 6 ml of dry tetrahydrofuran was added dropwise to a boiling suspen-

(28) Melting points (uncorrected) were determined on the Kofler block. Optical rotations and infrared spectra were measured in chloroform solution and ultraviolet absorption spectra were measured in ethanol and methanol. Thin layer chromatography (tlc) was performed on silica gels, H, G, and GF_{254} (B. Merck, A. G. Darmstadt). Spots or bands were developed with ultraviolet light, iodine vapor, or spraying with a 2% ceric sulfate solution, 2 *N* in sulfuric acid, and heating for optimum development of colored spots. Mass spectra were determined, except where indicated, with a CEC Model 21-103C mass spectrometer using an all-glass inlet system heated to 200° with the isotron temperature maintained at 270° while the ionizing energy was kept at 70 ev and the ionizing current at 50 μa. Mass spectra were determined by Messrs. John Smith and Nelson Garcia except for the high-resolution mass measurements (A.E.I. MS-9 double-focussing mass spectrometer) which are due to Mr. R. Ross.

(29) L. Ruzicka, M. W. Goldberg, and H. Brungger, *Helv. Chim. Acta*, **17**, 1389 (1934); I. Salamon, *ibid.*, **32**, 1306 (1949).

(30) J. Iriarte, G. Rosenkranz, and F. Sondheimer, *J. Org. Chem.*, **20**, 542 (1955).

sion of 160 mg of lithium aluminum hydride in 10 ml of tetrahydrofuran. The reaction mixture was heated under reflux for 6 hr and then the excess hydride was decomposed with water. After cooling, the white precipitate was removed by filtration and was washed well with ether. The solvents from the collected tetrahydrofuran and ether solutions were removed under reduced pressure, and the residue was dissolved in ether which was washed with dilute hydrochloric acid, and with water and dried over anhydrous magnesium sulfate. Evaporation of solvent produced a crystalline residue which was oxidized under Jones conditions.[12] The 91 mg of crude product was purified by tlc on silica gel H, which contained about 10% silver nitrate[31] in a mixture of hexane–ether (9:1). The resulting pure 5α-androstan-17-one (X) amounted to 89 mg (94% over-all yield), which exhibited mp 121.5–122° (lit.[32] 119.5–120.5°). The conversion of the crude labeled tosylate (215 mg) into XI was carried out in the same way as described above for the unlabeled analog. The resulting crude product (130 mg) was purified by tlc on silica gel H, containing about 10% silver nitrate[31] in a mixture of hexane–ether (9:1). Obtained was 101 mg (77% over-all yield from IX) of pure, olefin-free 18-d_3-*dl*-5α-androstan-17-one (XI), mp 91–92°. Recrystallization from methanol gave mp 91.5–92°; ν_{max} 1730 (C=O), 2064, 2162, 2210, and 2230 cm^{-1} (CD). This product exhibited the correct molecular weight (*m/e* 277) and fragmentation pattern in its mass spectrum.

12,12-d_2-5α-Androstan-17-one (XIV).—By analogy to the procedure of Marshall, *et al.*,[13] for a similar degradation, a mixture of 12,12-d_2-5α-pregnan-20-one (XII, 17 mg),[7] *p*-toluenesulfonic acid monohydrate (12 mg), and acetic anhydride (10 ml) was distilled slowly during a period of 5 hr. The residual solution (about 1 ml) was poured onto ice and was left to stand for 2 hr. Ether extraction, followed by washing with dilute sodium bicarbonate and water and drying gave a glassy residue (25 mg) which was purified by tlc in benzene. The resulting mixture of enol acetates amounted to 15 mg [ν_{max} 1750, 1225 (acetate), 1690 cm^{-1} (C=C)] and was treated with an excess of ozone in 30 ml of methanol–ethyl acetate mixture (1:1) at −40°. After the ozonolysis the solution was allowed to warm slowly to room temperature under a slow stream of nitrogen, then 10 mg of palladized calcium carbonate was added and the resulting suspension was hydrogenated for 30 min at room temperature and atmospheric pressure. The catalyst was removed by filtration, the solvent was evaporated, and the residue was purified by tlc in benzene–ether mixture (9:1), yielding 7 mg (45% over-all yield) of **12,12-d_2-5α-androstan-17-one** (XIV), mp 119.5–121° (from methanol). This product exhibited the correct fragmentation pattern and 2% d_0, 21% d_1, 68% d_2, and 9% d_3 isotope composition in its mass spectrum.

8β-d_1-5α-Androstan-17-one (XVII).—To a solution of 25 mg of 8β-d_1-5α-pregnan-20-one (XV)[7] in 3 ml of anhydrous ether was added 25 mg of *m*-chloroperbenzoic acid. The resulting solution was allowed to stand in the dark at room temperature for 7 days with additional quantities (5–10 mg) of *m*-chloroperbenzoic acid added every second day. Additional ether was added and the solution was worked up with dilute sodium bicarbonate solution and water and then dried. Evaporation of the ether followed by tlc separation of the product mixture afforded 12 mg of unconverted pregnanone (XV) and 8 mg of 8β-d_1-5α-androstan-17β-ol acetate (XVI), characterized by tlc and infrared comparison with an authentic, unlabeled specimen. The labeled acetate (XVI) was treated with lithium aluminum hydride in dry ether and the resulting product was oxidized with Jones reagent.[12] After tlc purification 4 mg of 8β-d_1-5α-androstan-17-one (XXVII) was obtained, mp 116–117°, whose isotopic purity is given in Table I.

7β-d_1-5α-Androstan-17-one (XX).—7β-d_1-5α-Androstan-17-one 17-ethylene ketal (XIX,[6c] 3 mg) was dissolved in 10 ml of methanol and 0.1 ml of 2 *N* hydrochloric acid solution was added to it. After heating under reflux for 30 min most of the solvent was evaporated under reduced pressure and the residue was dissolved in ether. The ether solution was washed with dilute sodium bicarbonate and water and dried over magnesium sulfate. Evaporation of the ether afforded 2.5 mg of crude ketone, mp 112–118°. Purification by tlc in benzene–ether mixture (9:1) gave 2.1 mg of 7β-d_1-5α-androstan-17-one (XX), mp 120.5–122° (from methanol). This product exhibited the correct mass spectrometric fragmentation pattern and 95% d_1 isotopic purity.

3α-d_1-5α-Androstan-17-one (XXI).—A solution of the tosylate of 5α-androstan-3β-ol-17-one (VIII, 60 mg, mp 160–162°) in 10 ml of tetrahydrofuran (freshly distilled from lithium aluminum hydride) was added dropwise to a boiling suspension of 60 mg of lithium aluminum deuteride in 10 ml of tetrahydrofuran. The reaction mixture was heated under reflux for 30 min, then the excess deuteride was decomposed by the addition of water. The inorganic salts were removed by filtration and the crystalline residue, obtained upon evaporation of the solvent, was oxidized under Jones conditions.[12] The resulting 35 mg (95%) of crude product, mp 116–118°, was recrystallized from methanol, yielding the pure **3α-d_1-5α-androstan-17-one** (XXI), mp 121–122°, which showed no depression when mixed with the unlabeled sample (X). Mass spectrometric analysis indicated 2% d_0 and 98% d_1 isotopic purity.

16,16-d_2-5α-Androstan-17-one (XXII).—A solution of 170 mg of 5α-androstan-17-one and 150 mg of sodium in 5 ml of methanol-*O*-d_1 and 0.5 ml of heavy water was heated under reflux for 4 hr. Upon cooling colorless plates of the product crystallized out and these were filtered and used directly for mass spectrometry (see Table I), mp 121–122°.

15α-d_1-5α-Androstan-17-one (XXV).[33]—A sample (52 mg) of Δ^{15}-5α-androstan-17-one (XXIII)[6c] was catalytically (5% palladium on charcoal) deuterated at room temperature and atmospheric pressure in ethyl acetate solution (10 ml) and after 2 hr the catalyst was filtered, the solvent was evaporated, and the 15α,16α-d_2-5α-androstan-17-one (XXIV) was recrystallized from methanol to yield 44 mg, mp 120–121°.

Half of the product was back exchanged by heating under reflux for 7 hr with 200 mg of sodium hydroxide in 24 ml of 33% aqueous methanol. Dilution with ether, washing with water, drying, evaporation, and two recrystallizations from methanol provided 20 mg of colorless plates of XXV, mp 120–122°.

5β-Androstan-17-one (XXVI).—5β-Androstan-17β-ol-3-one acetate (XXVIa,[17,18] 500 mg, mp 147–149°) was subjected to a modified Wolff-Kishner reduction.[16] The resulting crude alcohol (XXVIb) was oxidized directly under Jones conditions,[12] yielding 388 mg (94% over-all yield) of **5β-androstan-17-one** (XXVI), mp 97–100°. Recrystallization from methanol provided the analytical sample: mp 101–102°, $[\alpha]^{25}D$ +99.3° (*c* 3.5), ν_{max} 1735 cm^{-1} (C=O). *Anal.* Calcd for $C_{19}H_{30}O$: C, 83.15; H, 11.01. Found: C, 83.32; H, 11.03.

Registry No.—IV, 7718-30-1; V, 7695-49-0; VI, 7695-50-3; VII, 7721-34-8; IX, 7718-31-2; X, 963-74-6; XI, 7695-51-4; XIV, 7703-53-9; XVII, 7756-98-1; XX, 7695-52-5; XXI, 7695-53-6; XXII, 7721-35-9; XXV, 7695-54-7; XXVI, 1912-61-4.

(31) E. Dunn and P. Robson, *Chromatography*, **17**, 501 (1965), and references cited therein.

(32) L. Ruzicka and A. C. Mhr, *Helv. Chim. Acta*, **27**, 503 (1944).

(33) This experiment was performed by Dr. G. von Mutzenbecher.