block). The analytical sample was recrystallized from the same solvent and showed m.p. 172.5–173°, $[\alpha]D$ −43.1° (*c* 1.06); ν_{max}^{KBr} 1726, 1704, and 1250 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{36}O_3$: C, 76.62; H, 10.07. Found: C, 76.70; H, 10.12.

4,4-Dimethyl-5α-androstan-3β-ol-7-one (XV).—To a solution of potassium hydroxide (400 mg.) in methanol (35 ml.) was added 4,4-dimethyl-5α-androstan-3β-ol-7-one acetate (IX, 1.2 g.) and the mixture was heated under reflux for 2 hr. After cooling to room temperature, the solution was poured into cold water; the flocculent precipitate was collected by filtration and washed with water. The product was dried in a vacuum desiccator over Drierite and then recrystallized from hexane–ethyl acetate giving XV as hair-like needles (1.0 g., 94%), m.p. 195–196°. Further recrystallization from the same solvent system yielded an analytical sample, m.p. 196.5–197°, $[\alpha]D$ −70° (*c* 1.045), $\nu_{max}^{CHCl_3}$ 1700 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{34}O_2$: C, 79.19; H, 10.76; mol. wt., 318.48. Found: C, 79.42; H, 10.54; mol. wt., 318 (mass spec.).

5α-d_1-4,4-Dimethylandrostan-3β-ol-7-one (XVI).—Reduction of the Δ^5-7-ketone XIII using deuterium in place of hydrogen gave 5α,6α-d_2-XIV, which, on treatment with base under the usual conditions, furnished XVI, m.p. 195–196°, whose mass spectrum is reproduced in Fig. 2.

Deuterium Bromide Catalyzed Enolization of 5α-Androstan-7-one (IIIa).—5α-Androstan-7-one (IIIa, 60 mg.) was added to a solution of hydrogen bromide (98 mg.) in deuterioacetic acid (5.5 ml.). The homogeneous solution was kept at room temperature and samples were removed for mass spectrometric and n.m.r. assay as indicated in Table I. These aliquots were worked up by pouring into ether and washing twice with 5% sodium bicarbonate solution and water, drying, and evaporating; the residual, colorless crystals were used directly.

3α,4α,5α,6α-d_4-Androstan-7-one (XX).—Palladium on charcoal (5%, 17 mg.) was added to a solution of 70 mg. of $\Delta^{3,5}$-androstadien-7-one (V) in cyclohexane (5 ml.). The mixture was stirred under an atmosphere of deuterium for 2 hr. and the product isolated by filtration and evaporation of the filtrate. A thin layer chromatogram indicated complete reduction but also the presence of traces of a more polar contaminant. Chromatography through alumina (Merck, Grade III, neutral, 8 g.) in hexane gave crystalline XX (50 mg.), m.p. 69–70.5°. The content of 6α-deuterium was 60% (60% shift of *m/e* 178 to 179).

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

Mass Spectrometry in Structural and Stereochemical Problems. LIII.[1] Fragmentation and Hydrogen Transfer Reactions of a Typical 3-Keto Steroid, 5α-Androstan-3-one[2]

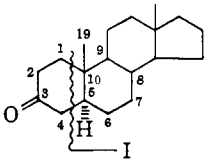
BY ROBERT H. SHAPIRO,[3] D. H. WILLIAMS, H. BUDZIKIEWICZ, AND CARL DJERASSI

RECEIVED MARCH 6, 1964

The multiple origin of the hydrogen migrations, which accompany the two important electron impact fragmentation reactions of 5α-androstan-3-one, has been determined through the measurement of the mass spectra of several deuterated derivatives. The results of this investigation show that a somewhat promiscuous transfer of hydrogen occurs during the formation of the *m/e* 202 and 203 ions. However, some of the itinerant hydrogen atoms, notably those bonded to C-7, C-8, C-9, and C-19, exhibit a migrating preponderance with respect to some others which are involved in the reaction leading to the formation of the *m/e* 202 fragment. The genesis of the adjacent *m/e* 203 ion is less complicated, being accompanied by hydrogen transfers from only three sites (C-5, C-9, and C-19). In addition to the lengthy synthetic sequences leading to the labeled analogs of 5α-androstan-3-one, some suggested fragmentation mechanisms are presented.

Introduction

Since it is now firmly established that the fragmentation processes of saturated steroidal ketones are extremely complex and subject to variations upon rather subtle modifications in the substitution pattern,[4–6] the principal interest in the mass spectral behavior of this class of compounds lies in the reaction mechanisms which involve hydrogen transfers. The spectrum (Fig. 1) of 5α-androstan-3-one (I)[7] contains two major peaks in the high mass range appearing at *m/e* 202 and 203. The formation of both these ions is the consequence of cleavages of the 1–10 and 4–5 bonds. The *m/e* 202 ion results from two hydrogen atoms migrating from the charged hydrocarbon portion, while the *m/e* 203 ion is formed with associated transfer of only one hydrogen atom. It was the object of the present study to identify the itinerant hydrogen atoms through the use of deuteriated analogs of the parent ketone I, in order to explain the fragmentation reactions from a mechanistic point of view. Unfortunately, substituent labels (except for the 1-methyl derivatives) cannot be used in this series as supplementary evidence, as they were for steroidal ethylene ketals,[8] amines,[9] and α,β-unsaturated ketones,[10] because of the sensitivity of the fragmentation pattern to subtle structural changes. For example, 17-substituted compounds such as cholestan-3-one,[7] 5α-androstan-17β-ol-3-one, and 5α-pregnan-3-one undergo principal cleavage in ring D and their spectra show virtually no ions corresponding to the cleavage in ring A. Therefore, the investigation was limited to extensive deuterium



I

(A) 202 (−2H)
(B) 203 (−1H)

(1) Paper LII: C. Djerassi, M. Cereghetti, H. Budzikiewicz, M.-M. Janot, M. Plat, and J. LeMen, *Helv. Chim. Acta*, in press.

(2) Supported in part by Grants No. CA-07195 and AM-04257 from the National Institutes of Health, U. S. Public Health Service.

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

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

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

(6) H. Powell, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *ibid.*, **86**, 2623 (1964).

(7) H. Budzikiewicz and C. Djerassi, *ibid.*, **84**, 1430 (1962).

(8) (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).

(9) (a) W. Vetter, P. Longevialle, F. Khuong-Huu-Lainé, Q. Khuong-Huu, and R. Goutarel, *ibid.*, 1324 (1963); (b) L. Dojejš, V. Hanuš, V. Cerny, and F. Šorm, *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).

(10) R. H. Shapiro and C. Djerassi, *ibid.*, **86**, 2825 (1964).

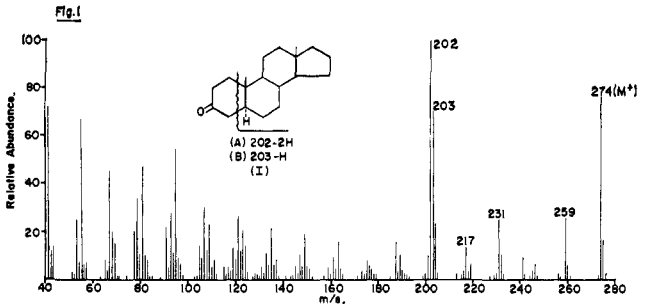
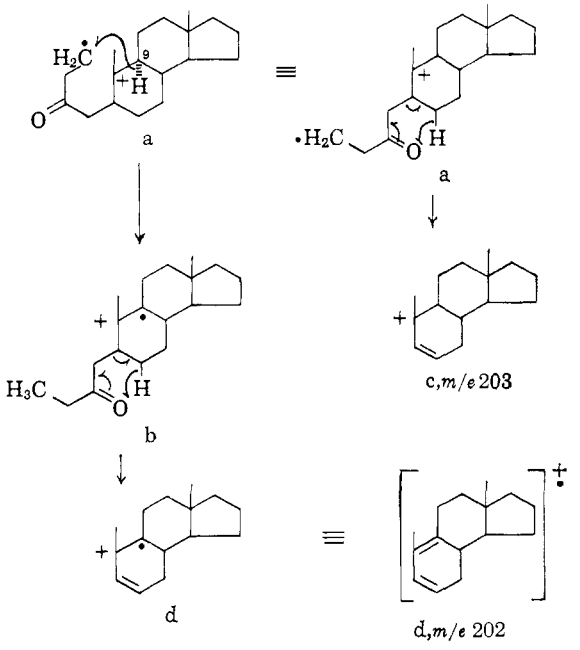


Fig. 1 —Mass spectrum of 5α-androstan-3-one (1).

labeling in a single compound, namely 5α-androstan-3-one (I), in order to gain as much insight as possible into the bond cleavages occurring in the vicinity of ring A in a 3-keto steroid.

A priori, we expected one of the C-6 hydrogen atoms to play a leading role in the formation of both the *m/e* 202 and 203 fragments. An attractive mechanism for the production of these ions involves initial cleavage[11] of the 1–10 bond as in a. Migration of a tertiary hydrogen atom from, say, C-9 to the radical site at C-1 could furnish b containing an ionized olefinic linkage.[11,12] Further decomposition of either a or b can then be pictured as involving the transfer of a C-6 hydrogen atom to the carbonyl oxygen by way of a six-membered cyclic transition state with concomitant cleavage of the 4–5 bond, leading to the relatively stable ions *m/e* 203 (c) and 202 (d), respectively.

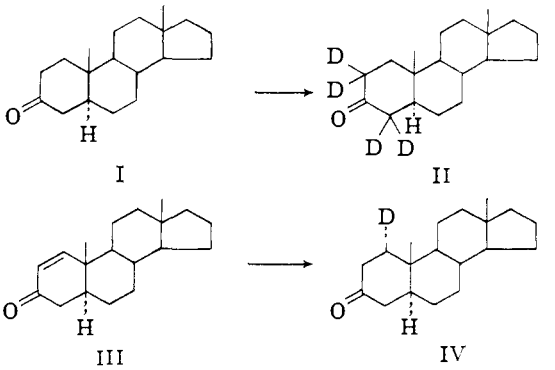


Both the conversions of a to c and b to d involve a β-cleavage with γ-hydrogen transfer, a reaction very common in the fragmentation of straight chain ketones.[13]

However, it was found in the spectrum of 6,6-d_2-5α-androstan-3-one (XII) that only about 10% of the migrating atom originated from C-6 during the formation of the *m/e* 202 and none in the production of the *m/e* 203 ion. Therefore, as observed in other saturated ketone studies,[4–6] the problem of hydrogen migration in the fragmentation processes of 5α-androstan-3-one (I) seemed to be more complex than superficially apparent and, in order to elucidate the mechanisms of any or all the competing decomposition paths, a number of deuterated derivatives of I were prepared and subjected to electron impact.

Results and Discussion

Synthesis of Deuterated Derivatives.—A few ring A labeled ketones were prepared in order to ascertain that both the *m/e* 202 and 203 ions resulted from the loss of carbon atoms 1–4 and also to determine whether any of the ring A hydrogen atoms were involved in a reciprocal transfer process of the type observed in 1-ketones,[6] 11-ketones,[4] and Δ^1-3-ketones.[10] 2,2,4,4-d_4-5α-Androstan-3-one (II) was prepared from the parent ketone I by base-catalyzed equilibration with basic deuteriomethanol–deuterium oxide. Catalytic deuteration of Δ^1-5α-androsten-3-one (III)[10,14] in cyclohexane containing palladized charcoal led to a mixture of epimeric 3-alcohols which was then oxidized[15] to the 3-ketone. Back exchange of the C-2 deuterium atom gave 1α-d_1-5α-androstan-3-one (IV).[16] Since both 1α- and 1β-methylandrostan-3-one-17β-ol were available to us,[17] and the 1-methyl group acted as substituent label, all of the ring A positions had been effectively labeled. The mass spectra of all of the above labeled ketones confirm that carbon atoms 1–4 with their attached hydrogen atoms are lost,[7] in addition to proving the absence of reciprocal transfer processes.



The 5α-d_1-derivative IX of I was prepared from Δ^5-androsten-3β-ol-7-one acetate[18] (V) in four steps in the following way. Catalytic deuteration of the Δ^5-enone V yielded VI, which upon saponification led to 5α-d_1-androstan-3β-ol-7-one (VII). The carbonyl group was reduced by a modified Huang-Minlon reaction[19] giving the alcohol VIII which was converted into 5α-d_1-androstan-3-one (IX) by the procedure of Jones, *et al.*[15]

(11) A fishhook (↷) is used to designate the shift of a single electron, while the movement of an electron pair is signified by an arrow (⇝). See H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra or Organic Compounds," Holden-Day, Inc., San Francisco, Calif., 1964, pp. xi–xiii.

(12) J. S. Shannon, *Australian J. Chem.*, **16**, 683 (1963).

(13) (a) F. W. McLafferty, *Anal. Chem.*, **31**, 82 (1959); (b) K. Biemann, "Mass Spectrometry," McGraw–Hill Book Co., Inc., New York, N. Y., 1962, p. 119; (c) ref. 11, Chapter 1.

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

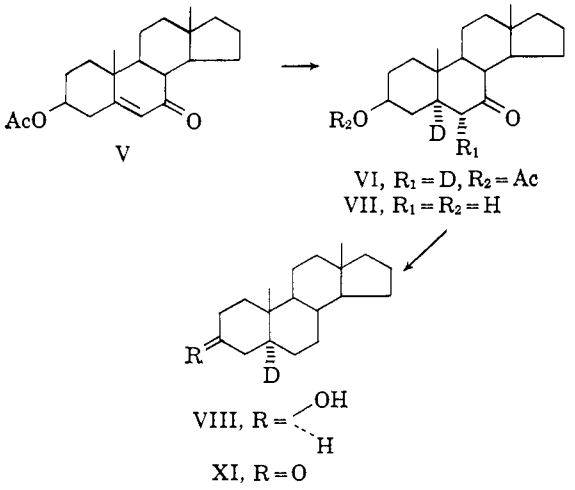
(15) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

(16) For the stereochemistry of catalytic deuteration of other Δ^1-3-keto steroids, see H. J. Ringold, M. Hayano, and V. Stefanovic, *J. Biol. Chem.*, **238**, 1960 (1963); H. J. Ringold, M. Gut, M. Hayano, and A. Turner, *Tetrahedron Letters*, 835 (1962); F. J. Schmitz and W. S. Johnson, *ibid.*, 647 (1962).

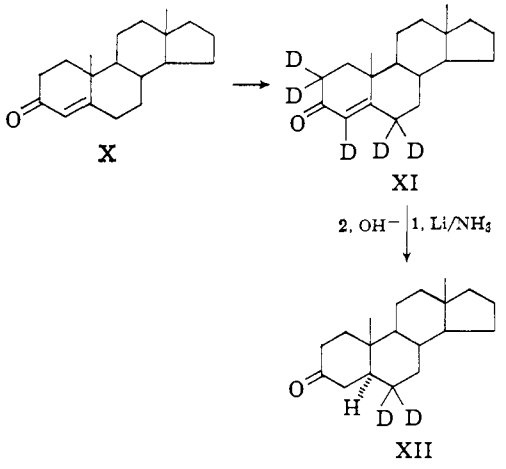
(17) We wish to thank Dr. G. Raspé, Schering A.G., Berlin, for the gift of these materials.

(18) R. D. H. Heard and A. F. MacKay, *J. Biol. Chem.*, **165**, 677 (1946).

(19) Huang-Minlon, *J. Am. Chem. Soc.*, **68**, 2487 (1946).



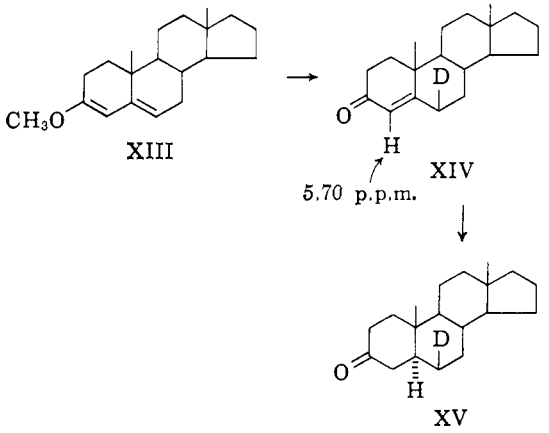
In order to introduce two deuterium atoms at C-6, advantage was taken of the fact that five hydrogens (2,2,4,6,6) can be exchanged in Δ^4-androsten-3-one (X).[14] Equilibration of the unsaturated ketone X in basic deuteriomethanol gave the d_5- derivative XI,[14] in which the double bond was reduced with lithium in liquid ammonia; the desired 6,6-d_2- derivative XII was obtained after back exchanging the deuterium atoms attached to C-2 and C-4.



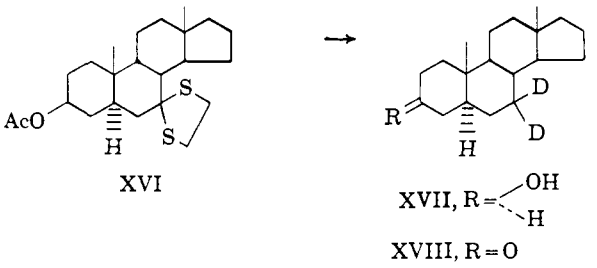
Although the contribution of a C-6 hydrogen atom to the formation of the *m/e* 202 ion was small (see Tables I and II), the 6β-d_1- derivative XV was prepared in order to see if there existed any stereospecificity in such a hydrogen transfer. Treatment of Δ^4-androsten-3-one (X) with methyl orthoformate in acid medium gave the dienol ether XIII, which was hydrolyzed to the 6β-deuterated enone XIV with deuterioacetic acid–deuterium oxide. The deuterium atom incorporated at C-6 was proved to be axial by examination of the n.m.r. spectrum of XIV which showed a sharp singlet at 5.70 p.p.m.[20] Reduction of the double bond with lithium in liquid ammonia resulted in the transformation to 6β-d_1-5α-androstan-3-one (XV).

A simple method which seemed applicable for the preparation of the 7,7-d_2-ketone is the desulfurization of a 7-thioketal with deuterium-containing W-7 Raney nickel, even though this method has occasionally

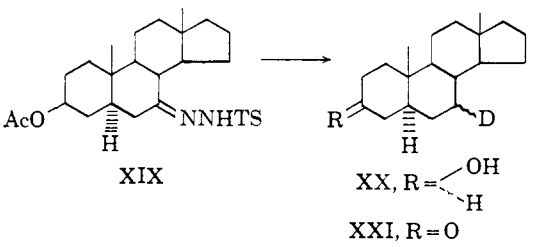
(20) The 6β-hydrogen couples with the olefinic hydrogen bonded to C-4 in Δ^4-3-ketones giving rise to a doublet or at least extensive broadening. See T. A. Wittstruck, S. K. Malhotra, and H. J. Ringold, *J. Am. Chem. Soc.*; **85**, 1699 (1963).



led to compounds of poor isotopic purity.[4] Consequently, the thioketal XVI, prepared from 5α-androstan-3β-ol-7-one acetate[18] with ethanedithiol–boron trifluoride etherate, was exposed to freshly prepared Raney nickel[21] (made with deuterium oxide), followed by base-catalyzed hydrolysis to give the alcohol XVII; subsequent oxidation yielded the 7,7-d_2-3-ketone XVIII.



Since the isotopic purity of the doubly labeled derivative XVIII was rather poor (*ca.* 65% d_2), the extent of hydrogen transfer from C-7 was difficult to determine, and a C-7 monolabeled compound was therefore prepared. The modified[22] method of Caglioti and Magi[23] for introducing a single deuterium atom was used to prepare 7ξ-d_1-5α-androstan-3β-ol (XX), which was transformed to the corresponding ketone XXI.



The key intermediate for the preparation of 8β-d_1-androstan-3-one (XXXII) was thought to be Δ^7-androsten-3β-ol-6-one (XXVII) in which the double bond could be reduced with lithium in liquid d_3-ammonia[4] as a means of introducing the label at C-8.

For this purpose, there was employed the selective hydroboration[24] of the 5–6 double bond in a $\Delta^{5,7}$-diene system. Allylic bromination[25] of Δ^5-androsten-

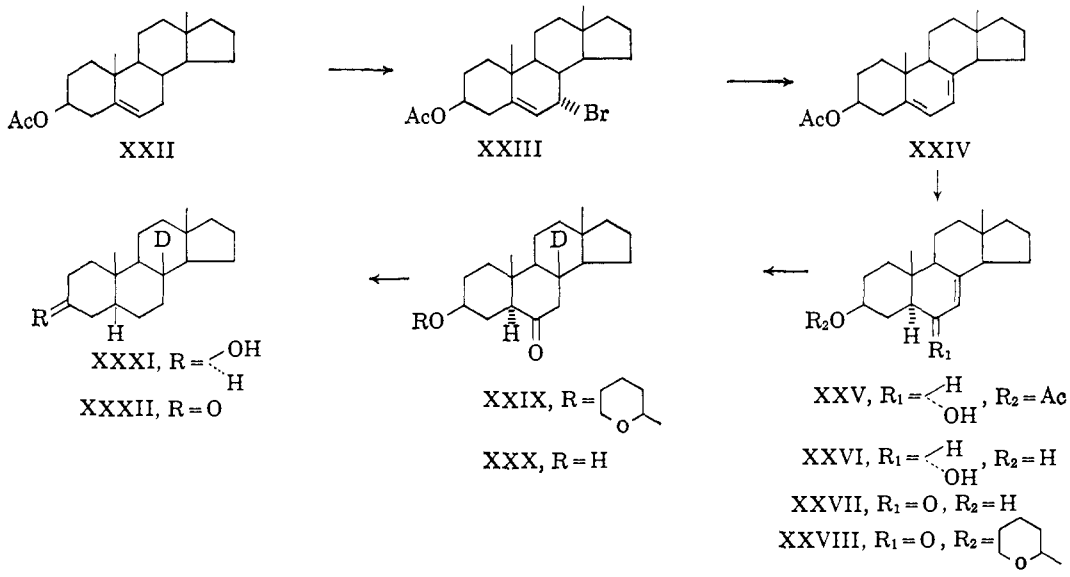
(21) H. R. Billica and H. Adkins, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 176.

(22) Z. Pelah, unpublished observations from this laboratory.

(23) L. Caglioti and M. Magi, *Tetrahedron*, **19**, 1127 (1963); see also L. Caglioti and P. Grasselli, *Chem. Ind.* (London), 153 (1964).

(24) L. Caglioti, G. Cainelli, and G. Maina, *Tetrahedron*, **19**, 1957 (1963).

(25) C. Djerassi, J. Romo, and G. Rosenkranz, *J. Org. Chem.*, **16**, 754 (1951).



3β-ol acetate (XXII)[26] gave the bromide XXIII which was not isolated but directly dehydrobrominated to the diene XXIV. Hydroboration with a very large excess of gaseous diborane followed by brief treatment with basic hydrogen peroxide gave a mixture of the monoacetate XXV and the diol XXVI. After complete saponification, the 6α-hydroxyl group was selectively oxidized with either dichlorodicyanoquinone[27] or manganese dioxide[28] to afford the α,β-unsaturated 6-ketone XXVII. Since the Δ^7-6-keto moiety has been shown to be completely resistant to the catalytic hydrogenation conditions[29] normally used to reduce only the double bond, we resorted to a lithium in liquid d_3-ammonia[4] reduction. However, over-reduction of the carbonyl group is frequently a serious factor in reduction of this type.[30] Therefore, the 3β-hydroxyl group was protected through the tetrahydropyranyl grouping[31] so that in the event of over-reduction of the 6-keto function the resulting 6-hydroxyl function could be back-oxidized without affecting the hydroxyl group attached to C-3. After the label had been incorporated at C-8, the acetal moiety was removed by hydrolysis and the resulting 6-keto-3-alcohol XXX was converted to the 8β-d_1-3-ketone XXXII by procedures already described for the 7-keto analog.

The incorporation of a deuterium atom at C-9 involved a lengthy series of reactions, commencing with 5α-androstan-3β-ol-11-one (XXXIII).[4] Reduction of this material with lithium aluminum hydride gave the 3β,11β-diol XXXIV which was selectively acetylated to the 3-acetate XXXV. Dehydration with phosphorus oxychloride in pyridine[32] led to the $\Delta^{9(11)}$-3β-acetate XXXVI which was subjected to allylic oxidation by means of chromium trioxide in acetic acid[33] furnishing the α,β-unsaturated ketone XXXVII.

(26) A. Butenandt and A. Suranyi, *Ber.*, **75**, 591 (1942).

(27) D. Burn, V. Petrow, and G. O. Weston, *Tetrahedron Letters*, **9**, 14 (1960); D. N. Kirk, V. Petrow, and M. H. Williamson, *J. Chem. Soc.*, 3872 (1960); A. Bowers, P. G. Holton, E. Necoechea, and F. A. Kincl, *ibid.*, 4057 (1961).

(28) F. Sondheimer, C. Amendolla, and G. Rosenkranz, *J. Am. Chem. Soc.*, **75**, 5930 (1953).

(29) D. H. R. Barton and C. H. Robinson, *J. Chem. Soc.*, 3045 (1954).

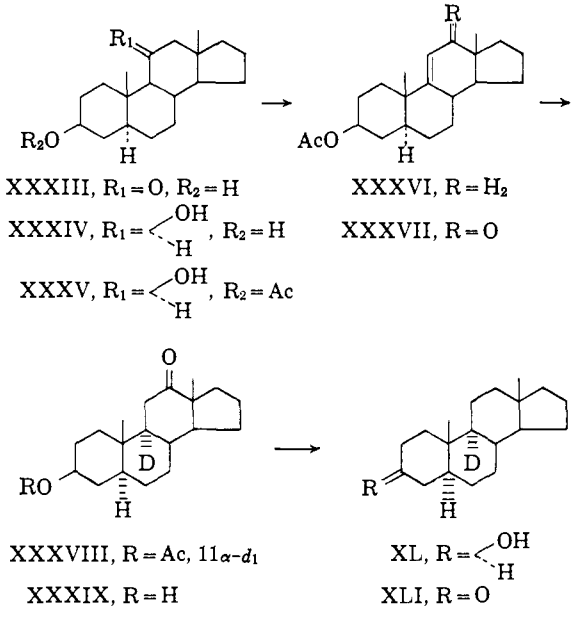
(30) See J. E. Starr in "Steroid Reactions," C. Djerassi, Ed., Holden-Day, Inc., San Francisco, Calif., 1963, Chapter 7.

(31) J. F. W. Keana, ref. 30, Chapter 1.

(32) G. Rosenkranz, O. Mancera, and F. Sondheimer, *J. Am. Chem. Soc.*, **76**, 2227 (1954).

(33) Y. Mazur, N. Danieli, and F. Sondheimer, *ibid.*, **82**, 5889 (1960).

Saturation of the 9–11 double bond with deuterium gas using palladium on charcoal catalyst followed by saponification of the 3β-acetate XXXVIII gave 9α-d_1-5α-androstan-3β-ol-12-one (XXXIX) which was converted to the desired 9α-d_1-3-ketone XLI by the usual Huang-Minlon[19] reduction and subsequent Jones[15] oxidation.



Other labeled 5α-androstan-3-ones which were employed during this study were the 19-d_1- (XLII) and 19,19,19-d_3- (XLIII) derivatives prepared previously by Djerassi and Kielczewski.[34]

Interpretation of the Mass Spectra.—The mass spectrum of 5α-androstan-3-one (I) is reproduced in Fig. 1. After correcting the spectrum for the natural abundance of ^{13}C, the normalized (total ionization of investigated region of spectrum set equal to 100) abundance ratios of the m/e 202 (process A), 203 (process B), and 204 peaks are 61:33:6. These normalized values, along with those obtained for the various isotopic analogs, are recorded in Table I. Isotopic contaminants (*e.g.*, 8% d_2- species in the 5α-d_1- analog IX), in all cases existing in small amounts, have been deleted

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

by simple subtraction, assuming their lack of participation in rearrangement processes. Any errors involved in this assumption have been shown by more sophisticated calculations to be far less than the general accuracy of the method (±5%).

TABLE I

NORMALIZED ABUNDANCES OF PRINCIPAL PEAKS[a]

5α-Androstan-3-one	*m/e* 202	*m/e* 203	*m/e* 204	*m/e* 205	*m/e* 206	*m/e* 207
d_0 (I)	61	33	6			
1α-d_1 (IV)	61	33	6			
2,2,4,4-d_4 (II)	61	33	6			
5α-d_1 (IX)	6	57	30	8		
6β-d_1 (XV)	5	50	37	8		
7ξ-d_1 (XXI)	14	46	35	5		
8β-d_1 (XXXII)	17	38	38	7		
9α-d_1 (XLI)	21	52	17	10		
19-d_1 (XLII)	6	58	29	7		
6,6-d_2 (XII)		8	50	35	7	
7,7-d_2 (XVIII)		28	33	33	6	
19,19,19-d_3 (XLIII)			18	54	21	7

[a] The values given for the deuterated analogs have been corrected for the possible shift of the *m/e* 201 ion (see Fig. 1) on the assumption that this fragment retains the deuterium label.

The method for calculating the extent of transfer from the various labeled positions will be outlined using the values (see Table I) obtained from the spectrum of 5α-d_1-androstan-3-one (IX) as a concrete example. The values for the extent of partial transfer are rounded off to the nearest 0.1 atom per ionized molecule (see Table II). This limit in accuracy is imposed by the observed differences in total ionization in the region *m/e* 202–205 of the spectra of the various deuterated derivatives of I.

TABLE II

EXTENT OF HYDROGEN MIGRATION FROM EACH POSITION[a]

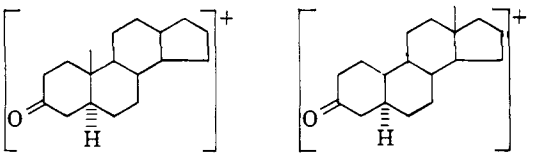
Fragment ion	C-5	C-6	C-7	C-8	C-9	C-19	Total
m/e 202	0.1	0.1[b]	0.4[c]	0.3	0.4	0.3	1.6 (out of 2)
m/e 203	0.1	0.0	0.0	0.0	0.5	0.3	0.9 (out of 1)

[a] Expressed as the fraction of a deuterium atom transferred per ionized molecule. Each value contains a possible error of ±0.05 atom. [b] 6β-d_1 showed about 0.1. [c] 7ξ-d_1 showed about 0.2.

The "normalized" abundance of the *m/e* 202 (A) ion in the spectrum (Table I) of 5α-d_1-androstan-3-one (IX) is 6% as compared with 61% in the parent ketone I. Since an *m/e* 202 ion, formed during the decomposition of IX, can only be $C_{15}H_{22}^+$, which results from the transfer of the deuterium label, the 6% abundance is a direct measure of the extent of transfer of hydrogen from C-5 to the nonionized portion of the molecule. For this example, the extent of transfer amounts to $^6/_{61}$ = 0.098 or *ca.* 0.1 atom. If we assume that the presence of a deuterium atom attached to C-5 does not inhibit this fragmentation process leading to species A (*m/e* 202 from I), then 61 − 6 = 55% will retain the deuterium label and thus be recorded at *m/e* 203. The observed abundance ratio of the *m/e* 203 ion in the spectrum of IX is 57% and, therefore, 57 − 55 = 2% must result from species B (*m/e* 203 from I) which has lost the deuterium label during its formation. Thus the transfer from C-5 during the formation of the *m/e* 203 ion in the decomposition of the parent ketone I is $^2/_{33}$ = 0.06 or *ca.* 0.1 atom.

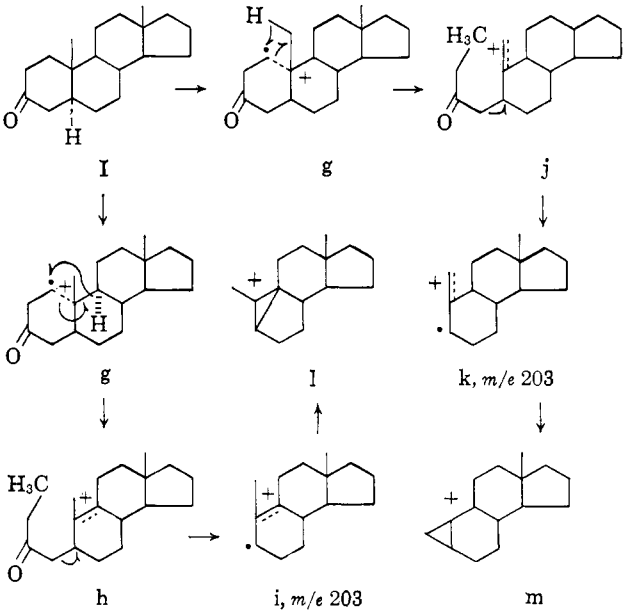
The correctness of these calculations can be checked by measuring the abundance of the *m/e* 204 fragment in the spectrum of the 5α-d_1- derivative IX. This fragment represents ion B ($C_{15}H_{22}D^+$) retaining its deuterium label. The calculated value for the species retaining its label is $^{30}/_{33}$ = 0.91 which is in good agreement with the value obtained by subtracting the transferring portion (*ca.* 0.1 atom) from the total amount (1.0 atom).

M − 15 (*m/e* 259) Ion.—The M − 15 ion is formed from the loss of a methyl radical from the molecular ion. The spectrum of the 19,19,19-d_3- derivative[34] contains equally abundant peaks at M − 18 and M − 15 demonstrating that the *m/e* 259 ion is a composite peak composed of ions e and f.



M − 43 (*m/e* 231) Ion.—The ion resulting from the fissions of the 13–17 and 14–15 bonds accompanied by a hydrogen transfer to the neutral fragment is the most common species found in the spectra of steroids,[7,35] which in the case of 5α-androstan-3-one (I) amounts to the loss of 43 mass units. The spectra of the 5α-androstan-3-ones labeled at carbon atoms 1–9 and C-19 all showed the M − 43 ion, thereby indicating that hydrogens from none of the labeled positions are involved in this process. However, in direct contrast to the corresponding ions found in the spectra of C-17 substituted steroids, the formation of the *m/e* 231 ion does not represent a major process in the decomposition of 5α-androstan-3-one (I).

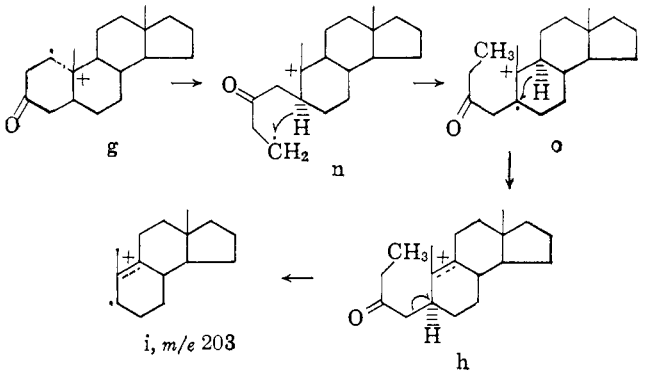
M − 71 (*m/e* 203) Ion.—The values in Table II show that during the formation of the *m/e* 203 ion, the 9α-hydrogen atom is extensively transferred to the neutral fragment while those bonded to C-5 and C-19 play a smaller role. The processes[11] by which the C-9 and C-19 hydrogens may be transferred are probably



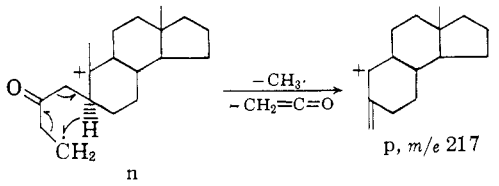
(35) C. Djerassi, *Pure Appl. Chem.*, **6**, 575 (1963).

very similar—both proceeding by way of a four-membered cyclic transition state leading to h and j, respectively. Subsequent cleavage of the 4,5-bond leads to the ions i and k; the driving force for these latter processes is presumably the delocalization of the ionized double bond.[12] Ions i and k can also be visualized as their valence bond tautomers l and m.

The small transfer from C-5 can be rationalized by invoking a six-membered cyclic transition state in n, but it does not seem likely that cleavage of the 4–5 bond would occur in ion o. Therefore, hydrogen rearrangement (as for instance from C-9) is invoked to give ion h in which the 4–5 bond cleavage is now much more favorable.

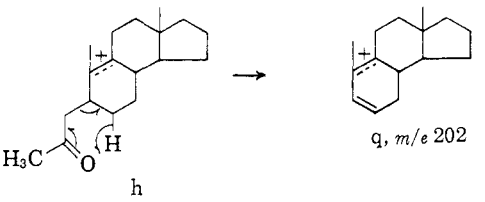


Reasonably strong evidence that ion n is formed is the presence of the *m/e* 217 ion in Fig. 1. This ion is shifted accordingly in the spectra of ketones labeled at C-4, C-5, C-6, C-7, C-8, and C-9. The expected decomposition of n involves the cleavage of the 3–4 bond resulting in the formation of p.

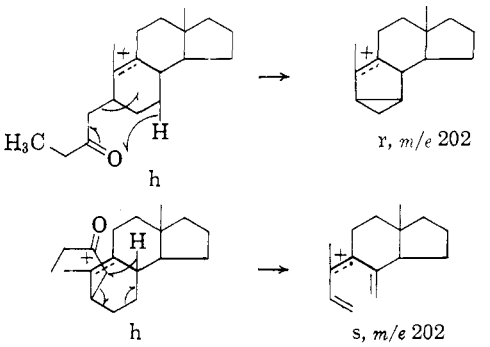


M − 72 (*m/e* 202) Ion.—The most abundant peak (*m/e* 202) in the spectrum (Fig. 1) of 5α-androstan-3-one (I) results from an almost random process, in which hydrogen atoms from at least six positions are transferred to the neutral fragment (see Table II). Since the formation of the *m/e* 202 ion ($C_{15}H_{22}^+$) is accompanied by the migration of two hydrogen atoms from the charge-retaining species and we have already presented a rationalization of the transfer from C-5, C-9, and C-19 for the formation of the *m/e* 203 ion, only formulations for the transfers from C-6, C-7, and C-8 will be represented now. That is, we shall assume that a common intermediate, say ion h, decomposes by a route which involves hydrogen transfer to the expelled neutral fragment during the formation of the *m/e* 202 species. The (minor) migration from C-6 is best represented as proceeding through a six-membered cyclic transition state leading to fragment ion q.

Although the contributions of hydrogen atom migrations from both C-7 and C-8 are pronounced, reasonable representations which correspond to ordinary ground state reaction mechanisms are difficult to formulate



for either process. The following representations have been arbitrarily chosen merely as a means to rationalize the experimental results. The transfer from C-7 invokes a seven-membered cyclic transition state to give ion r, while the migration from C-8 may proceed through the more conventional six-membered complex leading to ion s.



In summary, it may be said that although the rearrangement processes which have been discussed are rather complex, the isotopic labeling has accounted for virtually all the required migrating hydrogen atoms. In fact, if it is assumed that the over-all isotope effect is approximately 0.9 (discrimination against deuterium),[36] the agreement between expected (0.9 and 1.8) and observed (0.9 and 1.6, see Table II) transfers, in the formation of *m/e* 203 and 202, respectively, is very close. A useful generalization would appear to be that rearrangement processes will be complex when charge localization is not specific (contrast the behavior of 5α-androstan-3-one (I) with that of its derived ethylene ketal[38a]).

Experimental[37]

5α-Androstan-3-one (I).—A mixture of 5α-androstan-3β-ol-17-one[38] (8 g.), triethylene glycol (130 ml.), *n*-butyl alcohol (40 ml.), and 95% anhydrous hydrazine (25 ml.) was heated under reflux for 2 hr. The external heating was discontinued while potassium hydroxide (10 g.) was added slowly in portions. The reflux condenser was replaced by a distillation head and the solvent was distilled until the reaction temperature reached 200°. Heating at 200–210° was continued for 4 hr., after which time the mixture was cooled and poured into ice–water (1 l.). The precipitated solid was collected by suction filtration, washed excessively with

(36) D. H. Williams, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **86**, 284 (1964).

(37) Routine melting points were taken in capillary tubes and are uncorrected, while those for analytical samples were determined on a Kofler hot stage and are corrected. Rotations were determined in chloroform solutions. All mass spectra were measured with a Consolidated Electrodynamics Corp. No. 21-103C mass spectrometer 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. Analytical thin layer chromatoplates had a thickness of 0.25 mm. of silica gel G (E. Merck A.G., Darmstadt) and the spots were detected by spraying with 2% ceric sulfate solution in 2 *N* sulfuric acid. Preparative chromatoplates had a thickness of 1.0 mm. of silica gel HF_{254} (E. Merck A.G., Darmstadt) containing a trace of disodium 3,5-dihydroxypyrenedisulfonate (Bayer A.G., Leverkusen) and the compounds were detected by the use of an appropriate ultraviolet lamp.

(38) We are greatly indebted to Syntex S.A., Mexico City, for a generous gift of this material.

water, and dried in a vacuum desiccator over Drierite. The product, 5α-androstan-3β-ol (7 g., 92%), m.p. 150–151° (reported[39] m.p. 151–152°), exhibited a band at 3480 cm.$^{-1}$, but showed no carbonyl absorption in the infrared. This material was oxidized without further purification.

A solution of the above alcohol (7 g.) in reagent grade acetone (600 ml.) was treated with 8 *N* chromic acid solution until a yellow tinge persisted for 3 min. Excess anhydrous magnesium sulfate was added and the suspension was filtered. The filtrate was concentrated under reduced pressure until incipient precipitation and then poured into cold water (125 ml.). The fluffy solid was collected by filtration, washed, and dried over Drierite at 0.1 mm. The white powder (6.9 g.), m.p. 100–102.5°, was crystallized from petroleum ether at −15°, giving 5α-androstan-3-one (I), m.p. 104–105°, $[\alpha]^{25}D$ +25° (*c* 0.5), $\nu_{max}^{CHCl_3}$ 1706 cm.$^{-1}$ (reported[40] m.p. 104.5–105.5°, $[\alpha]^{20}D$ +25.4°).

2,2,4,4-d_4-5α-Androstan-3-one (II).—A clean piece of sodium (40 mg.) was dissolved in deuteriomethanol (2 ml.), and immediately after the evolution of deuterium had subsided, 5α-androstan-3-one (I, 20 mg.) was added. The solution was heated to boiling, and deuterium oxide (*ca.* 0.3 ml.) was added until incipient turbidity. Heating was then continued at reflux for 1.5 hr., after which time crystals of the exchanged ketone II deposited upon cooling to room temperature. The fine needles were collected by filtration, washed with a few drops of heavy water, and dried in air. Mass spectral analysis of this material, m.p. 101–102°, showed the presence of two isotopic species: d_4, 80%, and d_3, 20%.

1α-d_1-5α-Androstan-3-one (IV).[41]—A solution of Δ^1-5α-androsten-3-one[10] (III, 1.0 g.) in methylcyclohexane (50 ml.) containing suspended palladium (10%) on charcoal (0.2 g.) was stirred in an atmosphere of deuterium gas for 48 hr. The catalyst was removed by filtration and the filtrate was evaporated to dryness at reduced pressure leaving a white solid, m.p. 65–95°. A thin layer chromatogram developed with benzene–ethyl acetate (9:1) contained two spots, both of which were more polar than authentic 5α-androstan-3-one (I); the infrared spectrum of the mixture showed a broad band at about 3500 cm.$^{-1}$ and no carbonyl absorption. The mixture was dissolved in acetone (75 ml.) and treated with 8 *N* chromic acid solution until the supernatant liquid remained yellow for 5 min. The product was isolated with ether in the usual manner and heated with basic methanol for 2 hr. After pouring into water, the product was collected by filtration and was crystallized from aqueous methanol as fine needles, m.p. 99–100°, undepressed upon admixture with authentic parent ketone I. Mass spectral analysis gave the following isotopic distribution: d_0, 12%; d_1, 81%; d_2, 7%.

5α-d_1-Androstan-3β-ol-7-one (VII).—In a microhydrogenation apparatus, a solution of Δ^5-androsten-3β-ol-7-one acetate[15] (V, 25 mg.) in cyclohexane (5 ml.) containing 10% palladized charcoal (15 mg.) was treated with deuterium gas at 20° while stirring rapidly. After 4 min., 1.89 ml. of deuterium had been absorbed (theoretical uptake 1.85 ml.) and no additional absorption was noted during a further 8 min. The catalyst was removed by filtration and the filtrate was evaporated to dryness at reduced pressure leaving a crystalline residue (24.2 mg., 98%), m.p. 114–117°; ν_{max}^{KBr} 1727, 1700, and 1290 cm.$^{-1}$. To the crude acetate IV was added a 0.5% solution (5 ml.) of potassium hydroxide in methanol and the resulting solution was heated at reflux temperature for 2 hr. The cooled reaction mixture was poured into cold water and the suspended solid was extracted with three small portions of ether. The organic layer was washed with 1% sulfuric acid, then water, dried over anhydrous magnesium sulfate, and evaporated to dryness at reduced pressure yielding a crystalline residue (20 mg., 97%), m.p. 136–138°, $\nu_{max}^{CHCl_3}$ 1694 cm.$^{-1}$. Mass spectral analysis showed the product VII to contain 4% d_0, 80% d_1, and 16% d_2.

5α-d_1-Androstan-3-one (IX).—The above 7-ketone VII was subjected to Huang-Minlon reduction as described above and the resulting alcohol VIII converted to the ketone IX with 8 *N* chromic acid in acetone solution as described in previous experiments. The isotopic distribution of IX was 7% d_0, 85% d_1, and 8% d_2.

6,6-d_2-5α-Androstan-3-one (XII).—To a stirred solution of lithium (225 mg.) in liquid ammonia (50 ml.) at −78° was added 2,2,4,6,6-d_5-Δ^4-androsten-3-one (XI, 300 mg.)[14] in dry ether (20 ml.) during 20 min. After the addition was complete, the Dry Ice–acetone bath was removed and the deep blue solution was allowed to reflux for 1.5 hr. Ammonium chloride (3 g.) was added carefully to discharge the color, ether (50 ml.) was added, and most of the ammonia was allowed to evaporate. Water was added to dissolve the inorganic material, the layers were separated, and the aqueous phase was extracted with two additional portions of ether. The combined ethereal extracts were washed successively with dilute hydrochloric acid, saturated sodium bicarbonate solution, and several times with water, then dried over anhydrous magnesium sulfate and evaporated to dryness. A thin layer chromatogram of the residue developed with benzene–ethyl acetate (9:1), exhibited three spots, one of which had the same R_f as authentic 5α-androstan-3-one (I), while the other two were of lower R_f. The crude mixture was dissolved in acetone (35 ml.) and titrated with 8 *N* chromic acid solution. As a precautionary measure, the product from the Jones[15] reaction was heated in basic methanol for 1.5 hr. ensuring that the deuterium atoms attached to C-2 and C-4 were exchanged for hydrogen. After pouring into cold water, the crude ketone XII was isolated with ether by the usual procedure. Crystallization of the crude material from petroleum ether at −15° gave 6,6-d_2-5α-androstan-3-one (XII, 210 mg., 70%) as flat blades, m.p. 100–102°. The isotopic purity of XII was 93%—the only contaminant being the d_1- species.

3-Methoxy-Δ^{3,5}-androstadiene (XIII).[41]—To a solution of Δ^4-androsten-3-one (X, 2 g.) in trimethyl orthoformate (5 ml.), methanol (0.5 ml.), and anhydrous dioxane (20 ml.) was added concentrated sulfuric acid (2 drops). During 12 hr. at room temperature, the solution changed color from orange through shades of brown to dark green, and then back to light orange with the addition of pyridine (1 ml.). The solvent was removed with a rotary evaporator, the resulting crystalline residue was suspended in methanol (10 ml.) containing a trace of pyridine, and collected by filtration. Drying at 0.1 mm. gave the enol ether XIII (1.4 g., 67%), m.p. 98–103°. The analytical sample was recrystallized from methanol containing a trace of pyridine and showed m.p. 107–107.5° $[\alpha]^{26}D$ −162° (*c* 1.02, $CHCl_3$ + pyridine), ν_{max}^{KBr} 1640 and 1620 cm.$^{-1}$, λ_{max}^{EtOH} 239 mμ (log ε 4.30).

Anal. Calcd. for $C_{20}H_{30}O$: C, 83.86; H, 10.56. Found: C, 83.96; H, 10.57.

6β-d_1-5α-Androstan-3-one (XV).—A solution of enol ether XIII (300 mg.) in deuterioacetic acid (5 ml.) and deuterium oxide (5 ml.) was prepared by gentle heating on a steam bath. Norit (100 mg.) was added and the suspension was filtered while still hot. The filtrate was allowed to stand at room temperature for 1 hr., excess water was added, and the crystalline precipitate was collected by filtration and washed with water. The 6β-d_1-Δ^4-3-ketone XIV was recrystallized from hexane as flat plates, m.p. 103.5–104°. The n.m.r. spectrum exhibited a sharp (half-width 2.3 c.p.s.) peak at 5.70 p.p.m.

Conversion of the Δ^4-3-ketone XIV to 6β-d_1-5α-androstan-3-one (XV) was effected with lithium in liquid ammonia as previously described. The isotopic composition of ketone XV was 6% d_0, 82% d_1, and 12% d_2.

5α-Androstan-3β-ol-7-one Acetate Ethylene Thioketal (XVI).—To a solution of 5α-androstan-3β-ol-7-one acetate[18] (200 mg.) in ethanedithiol (1.5 ml.) was added freshly distilled boron trifluoride etherate (0.5 ml.) and the mixture was allowed to stand at room temperature for 15 min. The product was precipitated by the addition of methanol (10 ml.), collected by filtration, and was washed thoroughly with ice-cold methanol. Drying at reduced pressure gave fluffy white crystals (176 mg.), m.p. 236–243° (with decomposition). Recrystallization from methanol–chloroform gave the thioketal XVI as short fine needles, m.p. 252–253° (no decomposition), $[\alpha]^{25}D$ −38.1° (*c* 0.97), $\nu_{max}^{CHCl_3}$ 1715 and 1250 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{36}O_2S_2$: C, 67.62; H, 8.88; S, 15.66. Found: C, 67.43; H, 8.90; S, 15.52.

7,7-d_2-5α-Androstan-3-one (XVIII).—To a suspension of the thioketal XVI (40 mg.) in deuteriomethanol (7 ml.) was added freshly prepared deuterium containing Raney nickel[4] (from 1.25 g. of alloy) and the mixture was heated under reflux for 5 hr. The catalyst was removed by filtration through Celite and washed with methanol, while allowing the washings to dilute the filtrate. The filtrate was concentrated to about 8 ml. at reduced pressure on a steam bath; to the concentrate was added a 10%

(39) L. Ruzicka, V. Prelog, and P. Wieland, *Helv. Chim. Acta*, **28**, 1651 (1945).

(40) V. Prelog, L. Ruzicka, P. Meister, and P. Wieland, *ibid.*, **28**, 618 (1945).

(41) We thank Dr. Z. Pelah, Stanford University, for the preparation of this material.

solution of potassium hydroxide in methanol (2 ml.) and the resulting solution was heated at reflux for 2 hr. The cooled solution was poured into water (40 ml.) and the precipitate was partitioned into ether. After washing, drying, and evaporating the organic layer, the labeled alcohol XVII (22 mg., 82%), m.p. 149–151°, was obtained.

As in the cases of other labeled 5α-androstan-3β-ols, this alcohol XVII was converted to the corresponding ketone XVIII by Jones[15] oxidation. The mass spectrum of XVIII showed 16% d_1, 65% d_2, and 19% d_3 as the isotopic distribution.

5α-Androstan-3β-ol-7-one Tosylhydrazone (XIX).—A solution of 5α-androstan-3β-ol-7-one acetate[15] (2.80 g.) and *p*-toluenesulfonylhydrazine (1.66 g.) in ethanol (100 ml.) containing concentrated sulfuric acid (2 ml.) was heated under reflux for 50 min.[42] The solvent was concentrated to about 30 ml. at reduced pressure and the precipitated solid was collected by filtration and washed with cold ethanol. The white crystalline solid (2.0 g., 52%), m.p. 226–229°, was chromatographically homogeneous when developed with ether and was used without further purification.

Anal. Calcd. for $C_{26}H_{38}N_2O_3S$: N, 6.11; S, 6.98. Found: N, 6.16; S, 6.98.

7ξ-d_1-5α-Androstan-3-one (XXI).—To a solution of the tosylhydrazone XIX (250 mg.) in anhydrous tetrahydrofuran (25 ml., freshly distilled from lithium aluminum hydride) was added lithium aluminum deuteride (125 mg.) and the resulting suspension was heated under reflux for 27 hr. in an apparatus protected from atmospheric moisture. After cooling the mixture to room temperature, the excess deuteride was decomposed by the cautious addition of saturated sodium sulfate solution and the residual water was removed with the aid of anhydrous magnesium sulfate. The inorganic material was separated by filtration and washed well with ether, allowing the washings to dilute the filtrate which was then evaporated to dryness yielding a thick foam (210 mg.). A thin layer chromatogram, developed with benzene–ethyl acetate (9:1) indicated that the residue was a mixture of unreacted tosylhydrazone XIX (R_f 0) and alcohol XX, which had the same R_f as authentic 5α-androstan-3β-ol. The foam was dissolved in chloroform (0.5 ml.) and applied to a preparative chromatoplate which was developed with benzene–ethyl acetate (9:1) until the solvent front had traversed through 14 cm. The silica bearing the desired material was scraped from the plate and eluted with three 25-ml. volumes of ether. Removal of the solvent at reduced pressure gave the alcohol XX as a white crystalline solid (20 mg., 15%), m.p. 148–150°.

The corresponding ketone XXI, m.p. 99–101°, was obtained from the alcohol XX by the Jones[15] oxidation and was shown to contain the isotopic distribution 4% d_0, 87% d_1, and 9% d_2.

Δ^{5,7}-Androstadien-3β-ol Acetate (XXIV).—A heterogeneous mixture of Δ^5-androsten-3β-ol acetate (XXII, 10.0 g.), N-bromosuccinimide (5.9 g., freshly recrystallized from water), and benzoyl peroxide (60 mg.) in dry carbon tetrachloride (200 ml.) was heated under reflux for 4 min. The succinimide was removed by filtration and the filtrate was concentrated to about 20 ml. at 0.1 mm. Without attempting to isolate the intermediate allylic bromide XXIII, γ-collidine (5 ml.) was added and most of the remaining solvent was removed. To the yellow residue was added γ-collidine (30 ml.) and dry xylene (250 ml.) and the resulting solution was heated under reflux for 1.5 hr. After cooling to room temperature, the mixture was filtered to remove the collidine hydrobromide and washed with dilute hydrochloric acid until the washings were acidic to blue litmus. The organic layer was washed with water and then steam distilled to remove the xylene. The distillation residue was extracted with ether and the ether layer was washed with water, dried over anhydrous magnesium sulfate, and evaporated to dryness at reduced pressure, leaving a dark brown, yet crystalline, residue. Treatment of the dark material with Norit in hot methanol resulted in the deposition of the diene XXIV as glistening colorless plates (2.6 g., 26%), m.p. 113–116°. The analytical sample which was recrystallized from methanol exhibited m.p. 118.5–119.5°, $[\alpha]^{28}D$ −149° (*c* 1.05), $\nu_{max}^{CHCl_3}$ 1715 and 1250 cm.$^{-1}$; λ_{max}^{EtOH} 270 (4.14), 280 (4.15), and 292 mμ (log ε 3.82).

Anal. Calcd. for $C_{21}H_{30}O_2$: C, 80.21; H, 9.69. Found: C, 79.94; H, 9.69.

Δ^7-Androstene-3β,6α-diol (XXVI).—A rapidly stirring suspension of Δ^{5,7}-androstadien-3β-ol acetate (XXIV, 2.7 g.) in diglyme (40 ml., freshly distilled from lithium aluminum hydride) was treated at 0° with a 50-fold excess of diborane under an atmosphere of dry nitrogen. The diborane was generated in a separate flask according to the procedure of Brown and Subba Rao.[43] After the addition was complete (*ca.* 30 min.), the ice bath was removed and the clear solution was allowed to stir at room temperature for 2 hr. To the solution was added 5% sodium hydroxide solution (20 ml.) and then, cautiously, 30% hydrogen peroxide solution (5 ml.). The resulting turbid mixture was allowed to stand at ambient temperature for 15 min., and then the product was precipitated with water. After several extractions with ether the organic phase was washed successively with water, saturated ferrous sulfate solution, and several times with water. Drying the ethereal solution over anhydrous magnesium sulfate and removing the solvent at reduced pressure led to a white solid, m.p. 146–184°. A thin layer chromatogram, developed with hexane–ether (1:9), showed two spots of equal intensity with R_f values of about 0.1 and 0.5. The crude mixture was dissolved in 2% sodium hydroxide in methanol (100 ml.) and the solution was heated under reflux for 1 hr. The cooled solution was poured into water and the precipitate was partitioned into ether. The organic layer was washed with 5% hydrochloric acid and then water, dried over anhydrous magnesium sulfate, and evaporated to dryness at reduced pressure yielding the diol XXVI as a white crystalline solid (1.4 g., 57%), m.p. 182–188°. The analytical sample was recrystallized from aqueous methanol and showed m.p. 189–190°, $[\alpha]^{28}D$ +8.3° (*c* 0.48).

Anal. Calcd. for $C_{19}H_{30}O$: C, 78.57; H, 10.41. Found: C, 78.46; H, 10.49.

Δ^7-5α-Androsten-3β-ol-6-one (XXVII). (A).—A solution of the diol XXVI (716 mg.) and 2,3-dichloro-5,6-dicyanobenzoquinone (780 mg.) in dry dioxane (17 ml.) was allowed to stand at room temperature for 20 hr. The precipitated hydroquinone was removed by filtration and the filtrate was evaporated to dryness leaving a dark brown oil which was redissolved in chloroform. The chloroform solution was washed very carefully (to prevent emulsification) with 5% sodium hydroxide solution until no additional color was transferred into the aqueous phase and then with water until the washings were neutral. The solution was dried over anhydrous sodium sulfate and evaporated to dryness leaving a dark oil which was absorbed on neutral alumina (30 g., Activity II) and eluted with chloroform. Evaporation of the chloroform gave the enone XXVII (360 mg., 50%), m.p. 162.5–164.5°. Recrystallization from methanol gave needles, m.p. 163.5–164.5°, $[\alpha]^{29}D$ −45.6° (*c* 1.03), λ_{max}^{EtOH} 244 mμ (log ε 4.08); $\nu_{max}^{CHCl_3}$ 3580, 1653, and 1610 cm.$^{-1}$.

(B).—To a solution of the diol XXVI (103 mg.) in chloroform (10 ml.) was added activated manganese dioxide (1.0 g., Beacon Chemical Co.) and the resulting suspension was allowed to stir vigorously at room temperature for 5 hr. The inorganic material was removed by filtration through Celite and the filtrate was evaporated to dryness at reduced pressure yielding a white crystalline solid, m.p. 148–158°. A single recrystallization from methanol gave the enone XXVII as needles (90 mg., 87%), m.p. 163–165°. The analytical sample which was recrystallized from methanol showed m.p. 164–165°, $[\alpha]^{33}D$ −47.5° (*c* 1.03), spectral characteristics identical with the compound obtained in part A.

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.12; H, 9.79. Found: C, 79.14; H, 9.89.

Δ^7-5α-Androsten-3β-ol-6-one Tetrahydropyranyl Ether (XXVIII).—To a solution of Δ^7-5α-androsten-3β-ol-6-one (XXVII, 194 mg.) in 2,3-dihydropyran (2 ml., freshly distilled from sodium hydroxide) was added 1 drop of a 2% solution of phosphorus oxychloride in 2,3-dihydropyran. After standing at room temperature for 30 min., the reaction mixture was made basic with a few drops of 5% methanolic sodium hydroxide solution and then poured into water (20 ml.). The precipitate was separated with ether and the organic layer was washed with water, dried, and evaporated to dryness leaving an amorphous mass. The residue was crystallized from petroleum ether giving the desired tetrahydropyranyl ether XXVIII as plates (185 mg., 75%), m.p. 161–165°, $[\alpha]^{33}D$ −22.5° (*c* 0.89), λ_{max}^{EtOH} 244 mμ (log ε 4.07), $\nu_{max}^{CHCl_3}$ 1651 and 1612 cm.$^{-1}$.

8β-d_1-5α-Androstan-3-one (XXXII).—To a vigorously stirring suspension of finely divided magnesium nitride (20 g.) in mineral oil (100 ml.) was added deuterium oxide (24 g.) during 3.5 hr. The liberated d_3-ammonia was passed through a water-cooled reflux condenser, then through a cold trap maintained at −10° with an ice–ammonium chloride bath, and finally collected in a 50

(42) E. J. Corey and R. A. Sneen, *J. Am. Chem. Soc.*, **78**, 6269 (1956).

(43) H. C. Brown and B. C. Subba Rao, *J. Org. Chem.*, **22**, 1135 (1957).

ml., three-necked flask immersed in a Dry Ice–acetone bath and equipped with a reflux condenser containing the same freezing mixture. To the collected d_3-ammonia (*ca.* 8 ml.) was added clean lithium metal (30 mg.) and to the resulting blue solution was added the tetrahydropyranyl ether XXVIII (76 mg.) in tetrahydrofuran (3 ml., freshly distilled from lithium aluminum hydride) during 3 min. After the addition was complete, the Dry Ice bath was removed and the reaction mixture was allowed to reflux for 1 hr. After the addition of ammonium chloride (0.5 g.) followed by ether (10 ml.) and water (10 ml.), the layers were separated and the aqueous phase was extracted with two additional small volumes of ether. The combined extracts were washed with 5% hydrochloric acid, saturated sodium bicarbonate solution, and several times with water. After drying the ethereal solution and evaporation of the solvent, the saturated ketone XXIX was obtained as a semicrystalline solid which showed $\nu_{max}^{CHCl_3}$ 1709 cm.$^{-1}$ and was chromatographically homogeneous when developed with benzene–ethyl acetate (19:1).

The crude material XXIX was dissolved in 95% ethanol (4 ml.) containing concentrated hydrochloric acid (0.5 ml.) and the resulting solution was heated under reflux for 1 hr. After cooling, the solution was poured into water (20 ml.) and the suspended keto alcohol XXX was isolated with ether in the usual manner. The mass spectrum of XXX showed a molecular ion at *m/e* 291 (d_1, 92%) with a small (8%) peak at *m/e* 290.

A solution of the crude keto alcohol XXX (54 mg.) in diethylene glycol (10 ml.), *n*-butyl alcohol (3 ml.), and 95% anhydrous hydrazine was heated at 137° for 30 min. Potassium hydroxide (150 mg.) was added, the solvents were codistilled until the reaction temperature reached 206°, and the mixture was heated at 206–210° for 4 hr. The 8β-d_1-5α-androstan-3β-ol (XXXI) was isolated with ether and converted to the corresponding ketone XXXII as previously described. The product showed m.p. 101–102° and an isotopic purity of 92%. The remainder (8%) was unlabeled 5α-androstan-3-one (I).

5α-Androstane-3β,11β-diol (XXXIV).—To a solution of 5α-androstan-3β-ol-11-one (XXXIII,[4] 1.64 g.) in anhydrous ether (50 ml.) was added lithium aluminum hydride (2 g.) and the resulting suspension was heated under reflux for 1 hr. After cooling the mixture to 10°, the excess hydride was decomposed by the cautious addition of saturated sodium sulfate solution and the residual water was absorbed with anhydrous sodium sulfate. The inorganic salts were removed by filtration and washed exhaustively with dry ether. Evaporation of the filtrate to dryness at reduced pressure gave a crystalline solid, m.p. 157–163°. Recrystallization from hexane–chloroform gave the pure diol XXXIV as small plates (1.46 g., 89%), m.p. 165.5–167°, $[\alpha]^{26}D$ +24° (*c* 1.00).

Anal. Calcd. for $C_{19}H_{32}O_2$: C, 78.03; H, 11.03. Found: C, 77.85; H, 11.01.

5α-Androstane-3β,11β-diol 3-Acetate (XXXV).—To a solution of diol XXXIV (1.24 g.) in anhydrous pyridine was added acetic anhydride (5 ml.) and the mixture was allowed to stand at room temperature for 15 hr. After processing in the usual way and recrystallizing from petroleum ether, there were obtained short needles (1.27 g., 90%), m.p. 149–150°. The analytical sample was recrystallized from petroleum ether and showed m.p. 149–150°, $[\alpha]^{27}D$ +12.3° (*c* 1.05); $\nu_{max}^{CHCl_3}$ 3600, 1710, and 1250 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{34}O_3$: C, 75.40; H, 10.25. Found: C, 75.48; H, 10.26.

Δ$^{9(11)}$-5α-Androsten-3β-ol Acetate (XXXVI).—To a cold (0°) solution of the diol monoacetate XXXV (325 mg.) in anhydrous pyridine (3 ml.) was added phosphorus oxychloride[32] (0.9 ml., freshly distilled) and the mixture was allowed to stand at room temperature for 21 hr. After cooling to 0°, the mixture was cautiously added to crushed ice and the resulting suspension was extracted with ether. The product (315 mg.) obtained after evaporating the washed and dried ether solution showed two components in a thin layer chromatogram developed with benzene–ethyl acetate (19:1). The preponderant spot was less polar than the other, which had an identical R_f with that of authentic starting material XXXV. The residue was adsorbed on neutral alumina (16 g., Activity II) and eluted with petroleum ether yielding a chromatographically homogeneous colorless oil (256 mg., 84%). The oil was miscible with all the common organic solvents and separated as a liquid from aqueous methanol. However, the neat material crystallized upon standing for 3 days and showed m.p. 72.5–74°, $[\alpha]^{25}D$ +4° (*c* 1.04), $\nu_{max}^{CHCl_3}$ 1720 and 1250 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19. Found: C, 79.72; H, 10.21.

Δ$^{9(11)}$-5α-Androsten-3β-ol-12-one Acetate (XXXVII).—To a solution of Δ$^{9(11)}$-5α-androsten-3β-ol acetate (XXXVI, 250 mg.) in acetic acid (15 ml.) was added a solution of chromium trioxide (363 mg.) in 85% acetic acid (13 ml.).[33] The orange solution was allowed to stand at 40 ± 2° (oil bath) for 8 hr. and then poured into cold water (125 ml.). The gummy material which separated was dissolved in ether and the ethereal solution was washed with water, saturated sodium bicarbonate solution, and again with water. After drying over anhydrous magnesium sulfate, the solution was evaporated to dryness giving a colorless oil (209 mg.) which, in a thin layer chromatogram developed with benzene–ethyl acetate (19:1), showed four spots, all of which had a smaller R_f than starting material. The oil was adsorbed on neutral alumina (10 g., Activity II). Elution with hexane–ether (9:1) gave a mixture (29 mg.) of the two least polar components which showed a broad band at about 1720 cm.$^{-1}$ and was discarded. Further elution with the same solvent gave a mixture (73 mg.) of the two polar components, which showed a strong absorption at 1669 as well as 1720 cm.$^{-1}$. The mixture was applied to a preparative chromatoplate with the aid of ether and the chromatogram was developed with benzene–ethyl acetate (19:1), dried, and redeveloped with the same solvent. This treatment did not completely separate the components which appeared as a broad band under ultraviolet light. The silica gel containing the upper portion of the band was removed and eluted with three 15-ml. portions of ether. Removal of the solvent at reduced pressure gave an oil which was applied to a fresh preparative chromatoplate; this chromatogram was developed with benzene and then four times with benzene–ethyl acetate (19:1), drying after each elution. However, complete separation was still not effected and only the faster moving portion of the band was removed and extracted with ether. Evaporation of the solvent gave a crystalline residue (36 mg., 15%), m.p. 89–94°. Recrystallization from petroleum ether did not alter the melting range and, therefore, the crude Δ$^{9(11)}$-12 ketone XXXVII ($\nu_{max}^{CHCl_3}$ 1720, 1667, and 1250 cm.$^{-1}$; λ_{max} 238 mμ (log ε 4.18)) was used without further purification.

9α-d_1-5α-Androstan-3-one (XLI).—In a microhydrogenation apparatus, a suspension of palladium (10%) on charcoal in cyclohexane (5 ml.) was allowed to stir in an atmosphere of deuterium for 30 min. at 17° and then the crude enone XXXVII (34 mg.) was added. After 6 min., 2.46 ml. of deuterium had been absorbed (theoretical 2.44 ml.) and no further uptake was noted during an additional 40 min. The catalyst was removed by filtration and the filtrate was evaporated to dryness at reduced pressure leaving a semicrystalline mass, $\nu_{max}^{CHCl_3}$ 1720, 1706, and 1250 cm.$^{-1}$.

The residue XXXVIII was dissolved in methanol (10 ml.) containing previously dissolved potassium hydroxide (125 mg.) and the resulting solution was heated under reflux for 1.5 hr. After pouring into water, the product was isolated with ether in the usual manner as a crystalline solid, m.p. 148–153°. A thin layer chromatogram developed with hexane–ether (1:9) contained two spots, but the mixture was not separated for the next step.

The crude intermediate XXXIX (27 mg.) was dissolved in a mixture of diethylene glycol (10 ml.), *n*-butyl alcohol (3 ml.), and 95% anhydrous hydrazine (2 ml.) and the resulting solution was heated at 125° for 40 min. Potassium hydroxide (600 mg.) was added and, after codistillation of the solvents, the reaction mixture was heated at 195–198° for 9.5 hr. Isolation with ether gave a two-component crystalline residue (26 mg.), m.p. 135–139°, which was recrystallized from acetone at −15° giving chromatographically homogeneous 9α-d_1-5α-androstan-3β-ol (XL, 20 mg.), m.p. 147–149°.

Conversion of the alcohol XL to the corresponding ketone XLI utilized the Jones[15] oxidation as in previous experiments. The labeled ketone XLI, m.p. 99–101°, had an isotopic purity of 87%, the remainder being the d_0 contaminant.