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

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

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

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

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

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

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

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

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

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

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

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

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

RECEIVED FEBRUARY 7, 1964

A mass spectrometric study of four 7-keto-5α-androstanes and a number of deuterated analogs has demonstrated that these compounds show two characteristic fragmentation modes. Mass spectrometry and nuclear magnetic resonance have been employed to illustrate that, in contrast to the enolization of the analogous steroidal 11-ketones, two deuterium atoms can be introduced at C-6 before introduction of a third deuterium atom at C-8. Moreover, under certain conditions, it has been shown that equatorial ketonization of the Δ8-enol prevails over the alternative axial ketonization.

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

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

m/e 178
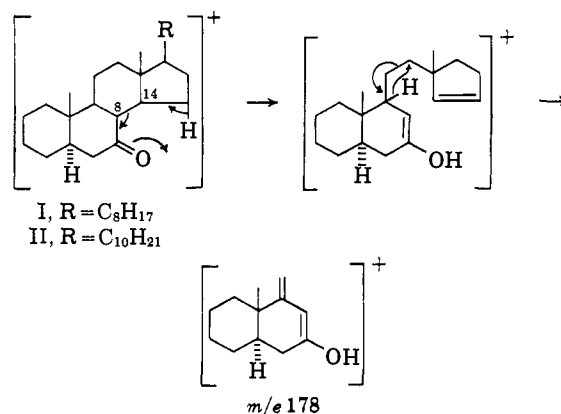
(1) Paper L: R. H. Shapiro and C. Djerassi, *J. Am. Chem. Soc.*, **86**, 2825 (1964).

(2) Financial support by the National Institutes of Health (Grants No. AM-04257 and CA-07195), National Science Foundation Cooperative Fellowship (to R. H. S.), and N.A.T.O., Paris (travel grant to R. B.) is gratefully acknowledged.
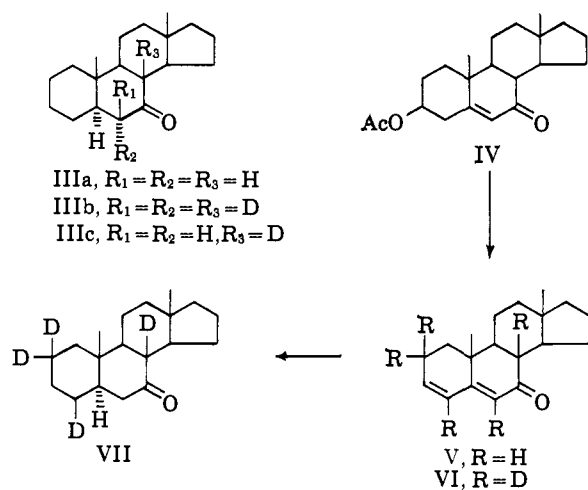
(3) H. Budzikiewicz and C. Djerassi, *J. Am. Chem. Soc.*, **84**, 1430 (1962).

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

allylic hydrogen radical could then furnish the m/e 178 ion. Although such a mechanism appears very

attractive, it is very unlikely that it is in fact operative[4b] in view of the failure of analogous six-membered cyclic transfers to operate in 11-ketones[5] and 15-ketones.[6]

It is, nevertheless, rather surprising that 5α-androstan-7-ones fragment in a somewhat different manner. The mass spectrum of 5α-androstan-7-one (IIIa) is reproduced in Fig. 1 and it can be seen that the most prominent features of the higher mass range are ions at m/e 135 and 178. In the spectrum of the 6,6,8-d_3-derivative IIIb, obtained by prolonged exchange (4 days) in basic deuterium oxide–deuteriomethanol, these ions are shifted to m/e 136 (predominantly, 55%) and 181, respectively. Additional isotope labels in ring A are *not* retained in these fragments. The introduction of deuterium into ring A of 5α-androstan-7-one (IIIa) could be effected *via* the dienone V, which was available through acid-catalyzed elimination[7] of the oxygen function at C-3 from Δ[5]-androsten-3β-ol-7-one acetate (IV). Base-catalyzed enolization of V in deuterated solvents gave the 2,2,4,6,8β-d_5- derivative VI, which on catalytic hydrogenation and subsequent limited treatment with aqueous methanolic base furnished 2,2,4-d_3-5α-androstan-7-one (VII), containing one additional deuterium atom, later shown to be located at C-8 (VII).

Fig. 1.—Mass spectrum of 5α-androstan-7-one (IIIa).
Fig. 2.—Mass spectrum of 4,4-dimethyl-5α-d_1-androstan-3β-ol-7-one (XVI).
Fig. 3.—Mass spectrum of 8β-d_1-5α-androstan-7-one (IIIc).

IIIa, $R_1 = R_2 = R_3 = H$
IIIb, $R_1 = R_2 = R_3 = D$
IIIc, $R_1 = R_2 = H, R_3 = D$

IV

VII

V, R = H
VI, R = D

Evidently, m/e 135 and 178 arise through the cleavages indicated in Fig. 1, the former occurring with loss of one additional hydrogen atom from the hydrocarbon fragment. Since the m/e 135 fragment partly (45%) loses deuterium from C-8 in its genesis, at least two mechanisms must be operative in its formation and possibilities are indicated below, along with a plausible path for the production of m/e 178.[8]

Probably a will be an abundant form of the molecular ion[9] and can undergo the favored α-cleavage to b.[9] Hydrogen migration from C-14 can then furnish the aldehyde containing the ionized olefinic linkage (c), which can undergo homolysis of the 9–10 bond to yield the allylic carbonium ion d (m/e 135). Those

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

(6) G. von Mutzenbecher, J. Fajkos, N. S. Bhacca, D. H. Williams, H. Budzikiewicz, and C. Djerassi, in preparation.

(7) A. Nickon and J. F. Bagli, *J. Am. Chem Soc.*, **83**, 1498 (1961).

(8) In this and all subsequent publications from this laboratory, a fishhook (⌒) is employed to indicate a one-electron shift while an arrow (⌒) implies a two-electron movement (see ref. 9, pp. xi–xiii).

(9) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra in Organic Compounds," Holden–Day, San Francisco, Calif., 1964, Chapter 1.
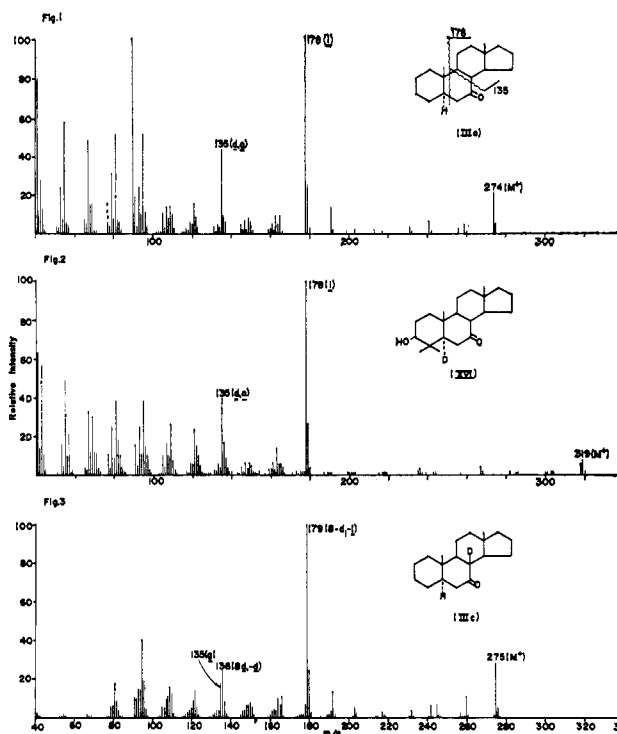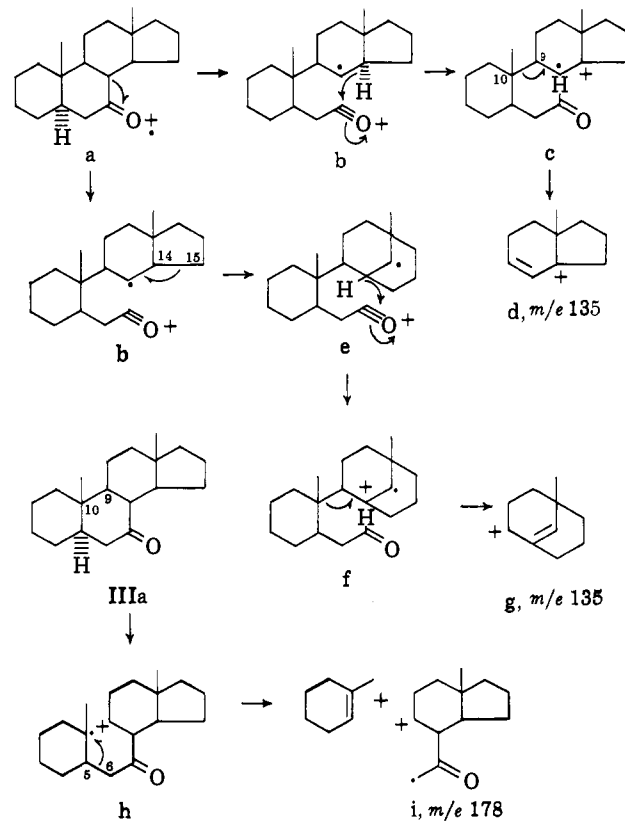
species in which hydrogen is lost from C-8 must be formed in a more complex manner if the unfavorable process of simultaneously breaking two bonds to C-8 is

a

b

c

b
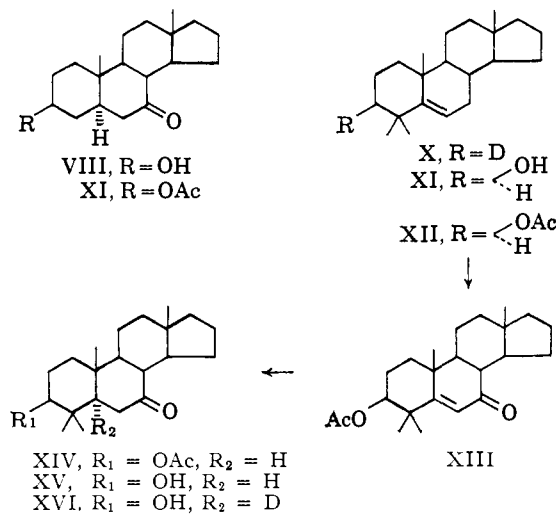
e

d, m/e 135

IIIa

f

g, m/e 135

h

i, m/e 178

to be avoided. One possibility is that b may rearrange by migration of the 14–15 bond, thus relieving the strain inherent in the C/D-*trans* ring juncture. Hydrogen migration from C-8 in the product so obtained (e) is

then a very reasonable process, furnishing f. Homolysis of the 9–10 bond gives m/e 135 in which orbital overlap (g) constitutes a formal violation of Bredt's rule. However, as has been recently pointed out,[10] a clear definition of the qualitative validity of the rule is not yet available and species such as g can by no means be regarded as "impossible" at high energies.

In the formation of m/e 178, it is very plausible that fission of the highly substituted 9–10 bond can occur as a primary process yielding h. Homolysis of the 5–6 bond in h leads to i (m/e 178), the neutral fragment being stabilized by double bond formation. It will be noted that resonance delocalization of the radical in i is possible on the carbonyl oxygen.

Considering the sensitivity of the mass spectra of steroidal ketones to subtle structural changes,[11] the general appearance of the spectra of a number of other 7-keto androstanes are remarkably similar and, in particular, the peaks m/e 135 and 178 remain dominant in an approximately constant ratio. Thus, the spectra of 5α-androstan-3β-ol-7-one (VIII)[12] and 5α-androstan-3β-ol-7-one acetate (IX)[12] both show the features alluded to above, as does also that of 4,4-dimethyl-5α-androstan-3β-ol-7-one (XV), prepared by the standard reaction sequence X → XV (for details see Experimental section). Furthermore, the spectra of several deuterated analogs of these compounds, variously labeled at C-5 [see, for example, the mass spectrum of 4,4-dimethyl-5α-d_1-androstan-3β-ol-7-one (XVI), Fig. 2], C-6, and C-8, are all consistent with the formation of m/e 135 and 178 ions in the manner outlined for 5α-androstan-7-one (IIIa). These ions may therefore be of utility in recognizing this type of skeleton.

VIII, R = OH
XI, R = OAc

X, R = D
XI, R = <$\stackrel{OH}{H}$
XII, R = <$\stackrel{OAc}{H}$

XIV, R₁ = OAc, R₂ = H
XV, R₁ = OH, R₂ = H
XVI, R₁ = OH, R₂ = D

XIII

It has previously been reported[12] that brief treatment (30 min. reflux) of 7-ketones with basic deuteriomethanol and deuterium oxide yields a d_2- derivative. It was noted[12] that if the preference for axial over equatorial ketonization of the Δ^6-enol is very great and the enolization to C-8 only slightly less facile than to C-6, then the 6β,8β-d_2- derivative would be obtained. This case would parallel the behavior of 11-ketones,[5] in which axial deuterium atoms at C-9 and C-12 are the first two heavy isotopes to be introduced. However,

if the preference for axial over equatorial ketonization of the Δ^6-enol is not very great and enolization to C-8 considerably more difficult than to C-6, then the 6,6-d_2- derivative would result. The mass spectrum of d_2-5α-androstan-3β-ol-7-one,[12] obtained by the brief base treatment outlined above, retained the m/e 135 ion, indicating that the compound is in fact a 6,6-d_2- derivative. This conclusion is supported by the fact that when 6,6,8β-d_3-5α-androstan-7-one (IIIb) is treated for a limited period with aqueous methanolic base, the mass spectrum (Fig. 3) of the resulting d_1-ketone shows 55% of the original m/e 135 ion at m/e 136; i.e., it contains deuterium at C-8 (IIIc). Thus the ease of replacement of enolizable hydrogens adjacent to a 7-ketone under our conditions of base catalysis is 6α and 6β >> 8β. This accounts for the retention of one enolizable deuterium at C-8 in compound VII.

It is pertinent to note that the acid-catalyzed enolization and ketonization of 7-ketones has previously been studied.[13,14] The results will be briefly summarized below, but it should be remembered that, a priori, no extrapolation of acid-catalyzed enolization and ketonization to the corresponding base-induced changes, which we have investigated, is possible. For example, the predominant stereochemical course of the ketonization of a Δ^6-enol may vary even with the nature of the acid.[13] Moreover, the stereochemistry of homoketonization is completely reversed on passing from base to acid catalysis.[15] The results of Corey and Sneen[13] show that axial and equatorial ketonization of Δ^6-cholestene-3β,7-diol 3β-acetate (XVII) occur to approximately the same extent in chloroform containing hydrogen bromide, but that the axial process is preferred (9:1) in acetic acid. It was also pointed out[13] that a 7-ketone, under acid conditions, enolizes predominantly to C-6 rather than to C-8. However, since acid-catalyzed enol-acetylation of cholestan-3β-ol-7-one acetate, under essentially kinetic control, gives mixtures containing the Δ^6-enol acetate XVIII and Δ^7-enol acetate XIX in ratios within the range 1.2:2.1,[14] the two possible enols may be almost equally favorable under certain conditions.

XVII, R = H
XVIII, R = Ac

XIX
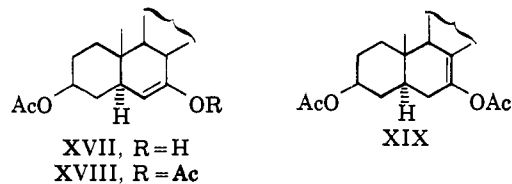
By means of n.m.r. and mass spectrometry, we have now investigated these enolization processes under another set of conditions, namely under the influence of 2% deuterium bromide in deuterioacetic acid. The rate of deuterium incorporation into 5α-androstan-7-one (IIIa) was followed by mass spectral analysis of samples isolated at various time intervals and the results are summarized in Table I.

It can be seen from the table that incorporation of one deuterium atom proceeds quite rapidly (see 0.25-hr. sample) while a second heavy isotope is introduced slightly more slowly, giving after 4.5–5.0 hr. a reason-

(10) Reference 4, pp. 246–247.
(11) Reference 9, chapter 8.
(12) D. H. Williams, N. S. Bhacca, and C. Djerassi, J. Am. Chem. Soc., 85, 2810 (1963).

(13) E. J. Corey and R. A. Sneen, ibid., 78, 6269 (1956).
(14) E. R. H. Jones and D. J. Wluka, J. Chem. Soc., 911 (1959).
(15) A. Nickon, J. H. Hammons, J. L. Lambert, and R. O. Williams, J. Am. Chem. Soc., 85, 3714 (1963).

TABLE I

INCORPORATION OF DEUTERIUM INTO 5α-ANDROSTAN-7-ONE
(IIIa) UNDER THE INFLUENCE OF 2% DBr–AcOD

Sample	Time, hr.	d_0	d_1	d_2	d_3	Atoms
		Deuterium content, %[a]				
1	0.25	48	45	6	1	0.60
2	.50	38	48	13	1	0.77
3	.75	20	50	27	3	1.13
4	2.0	3	30	61	6	1.70
5	4.5	3	26	63	8	1.74
6	5.0	3	19	66	12	1.87
7	20.0	2	12	51	35	2.19

[a] The percentages are corrected for the ^{13}C isotope.

ably clean dideuterated species. A third deuterium atom has only partially entered the molecule even after 20 hr. The results are consistent with three distinct modes of deuterium incorporation at very approximate relative rates of 100:50:1. A study of the m/e 135 region in the mass spectra of the eight samples shows that the shift m/e 135 → 136 is only perceptible in sample 7. Thus, as with base catalysis, two deuteriums are first introduced at C-6 and isotope incorporation at C-8 proceeds only with difficulty.

The positions of the deuterium atoms in the various labeled 5α-androstan-7-ones (IIIa) have been independently confirmed by 100 Mc. n.m.r. spectroscopy (Fig. 4). In the n.m.r. spectrum of 5α-androstan-7-one (IIIa), the region δ = 1.9–2.6 p.p.m. contains signals due to four protons.[16] The 6,6,8β-d_3-analog IIIb exhibits in this region a one-proton multiplet, assumed to be due to the equatorial proton at C-15 (see IIIa), which suffers long range deshielding by the carbonyl group in analogy to 11-ketones.[12] As this signal persists throughout the series, the chemical shifts reported for overlapping resonances in the sequel are approximate.

The n.m.r. spectrum of 6,6-d_2-5α-androstan-7-one (sample 5, Table I) shows an approximate triplet (J = 10.7 c.p.s.) at δ = 2.26–2.27 p.p.m., superimposed on the 15α-H signal. The splitting is consistent with that expected for a proton at C-8 with two axial–axial interactions with hydrogens at C-9 and C-14 and dihedral angles approaching 180° (Fig. 4).

The equatorial 6α-proton is responsible for the δ = 2.02 p.p.m. signal on the basis of its coupling pattern (A of an ABX system). A large, geminal coupling ($J \sim$ 12.5 c.p.s.) with the 6β-proton is observed and some broadening, approximately 2 c.p.s., due to the equatorial–axial coupling to the C-5 axial proton is also evident. The 6β-axial proton appears as a pair of doublets (B of ABX), the larger coupling ($J \sim$ 12.5 c.p.s.) being the geminal interaction and the slightly smaller splitting (10–11 c.p.s.) being due to the axial–axial interaction with the 5α-proton. The signals arising from the C-6 protons are most easily seen in the spectrum of the 8β-d_1- analog (IIIc, Fig. 4). The assignments are strongly supported by the spectrum (Fig. 4) of 3α,4α,5α,6α-d_4-5α-androstan-7-one (XX), prepared by catalytic deuteration of the Δ^{3,5}-dien-7-one V. Although deuteration was incomplete (see Experimental) owing to the incorporation of hydrogen adsorbed on the catalyst, the signal centered at δ = 2.02 p.p.m. almost completely disappears. More-

(16) The n.m.r. spectrum of 5α-androstan-3β-ol-7-one (VIII) also contains four protons in this region and not, as erroneously reported earlier (ref. 12), three protons.
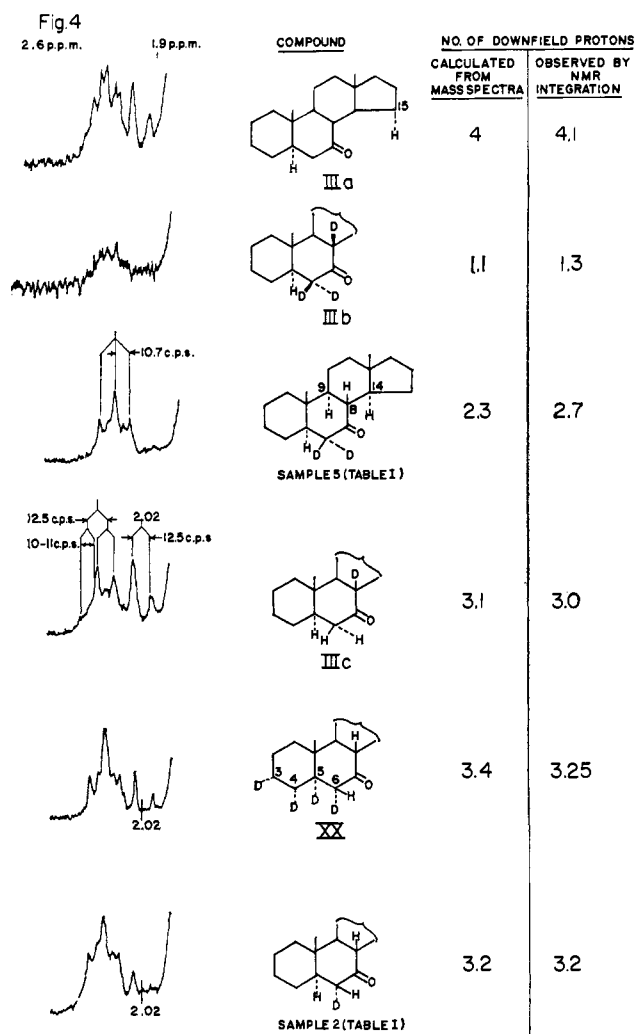

Fig. 4.—100 Mc. n.m.r. spectra of 5α-androstan-7-one and deuterated analogs in δ = 1.9–2.6 p.p.m. region.

over, the portion of the signal remaining is considerably sharpened because axial–equatorial coupling to the 5α-proton is lessened on partial introduction of deuterium at C-5. Close examination of the spectrum of this compound also indicates that the 6β-axial resonance is, as expected, simplified to a singlet, but the effect is somewhat difficult to see overlying the signals due to 8β- and 15α-protons. It is pertinent to note that the appearance of the resonance due to the 6α-equatorial proton at higher field (2.02 p.p.m.) than its axial counterpart ($\sim$ 2.3 p.p.m.) is consistent with the relative positions of these protons in the corresponding epimeric 6-bromocholestan-7-ones.[17]

Finally, the n.m.r. spectrum of the product of deuterium bromide–deuterioacetic acid monodeuteration (sample 2, Table I) indicates that the 6α-equatorial proton is that which has been predominantly replaced by deuterium, because the doublet centered at δ = 2.02 p.p.m. has practically disappeared. This result provides an interesting confirmation of Corey's proposal[13] that the extent of stereoelectronic control in ketonization will decrease with increasing acid strength of the proton donor. Thus, acetic acid causes axial ketonization,[13] the stronger acid hydrogen bromide in chloroform causes approximately equal ratios of axial

(17) A. Nickon, M. A. Castle, R. Harada, C. E. Berkoff, and R. O. Williams, J. Am. Chem. Soc., **85**, 2185 (1963).

and equatorial ketonization,[13] and, as shown in the present work, the strongest acid of the three, hydrogen bromide in acetic acid, causes predominantly equatorial ketonization.

Experimental[18]

$\Delta^{3,5}$-**Androstadien-7-one** (V).—A solution of Δ^{5}-androsten-3β-ol-7-one acetate[12] (IV, 509 mg.) in absolute ethanol (11 ml.) containing concentrated hydrochloric acid (0.1 ml.) was heated under reflux for 1 hr. Heating was discontinued, and water was added until the solution became turbid and then it was homogenized by heating to the boiling point. Upon cooling to room temperature, long, fine needles separated and were collected by filtration. A single recrystallization from aqueous ethanol gave 376 mg. (90%) of the dienone V, m.p. 140–140.5°, unchanged on recrystallization; $[\alpha]^{27}$D −416 ° (c 1.03), λ_{max}^{EtOH} 279 mμ (log ϵ 4.47), ν_{max}^{KBr} 1655, 1621, and 1594 cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{26}O$: C, 84.39; H, 9.69; mol. wt., 270.40. Found: C, 84.27; H, 9.79; mol. wt., 270 (mass spec.).

5α-**Androstan-7-one** (IIIa).—A solution of $\Delta^{3,5}$-androstadien-7-one (V, 100 mg.) in absolute ethanol (20 ml.) containing suspended 10% palladized charcoal (50 mg.) was treated with hydrogen at atmospheric pressure. During 20 min., 16.5 ml. of hydrogen was absorbed (theoretical uptake, 16 ml.). The catalyst was removed by filtration and the filtrate evaporated to dryness at reduced pressure leaving a crystalline solid, m.p. 56–66°. A thin layer chromatogram, developed with benzene–ethyl acetate (19:1), indicated the presence of predominantly one material and a small amount of a more polar contaminant. Adsorption on neutral alumina (5.0 g., Activity I) and elution with hexane–ether (9:1) gave chromatographically homogeneous 5α-androstan-7-one (91 mg., 91%), m.p. 70–71.5°. The analytical sample was recrystallized three times from methanol at −15° and showed m.p. 74–74.5°, $[\alpha]^{25}$D −110° (c 1.00), $\nu_{max}^{CHCl_3}$ 1691 cm.$^{-1}$. The optical rotatory dispersion curves exhibited the expected[19] negative Cotton effect: $[\alpha]$D −112°, $[\alpha]_{308}$ −866° (trough), $[\alpha]_{265}$ +208° (peak) (c 0.0813, methanol).

Anal. Calcd. for $C_{19}H_{30}O$: C, 83.15; H, 11.12; mol. wt., 274.43. Found: C, 83.16; H, 11.09; mol. wt., 274 (mass spec.).

2,2,4,8-d_4-5α-**Androstan-7-one** (VII).—A piece of clean sodium (25 mg.) was dissolved in deuteriomethanol (1.5 ml.) and $\Delta^{3,5}$-androstadien-7-one (10 mg.) was added. The solution was heated to boiling, the heating was then discontinued, and deuterium oxide added dropwise until incipient turbidity. Homogeneity was attained upon boiling and the solution was heated at reflux for 2 hr. Fine needles crystallized upon cooling to room temperature and were collected by suction filtration, washed with a few drops of heavy water, and dried in a vacuum desiccator over Drierite.

The exchanged dienone was dissolved in cyclohexane (5 ml.), 10% palladized charcoal added, and the suspension stirred vigorously in an atmosphere of hydrogen for 20 min. Removal of the catalyst by filtration and evaporation of the solvent yielded a crystalline solid which was dissolved in methanol (2 ml.). A few drops of 2.5 N sodium hydroxide solution was added and the solution was heated at reflux temperature for 1 hr. After pouring into water, the product was isolated with ether and recrystallized from aqueous methanol. The material so obtained (5 mg.) had m.p. 71–72° and an isotopic distribution of d_0, 2%; d_1, 3%; d_2, 4%; d_3, 36%; d_4, 45%.

6,6,8β-d_3-5α-**Androstan-7-one** (IIIb).—A solution of 5α-androstan-7-one (IIIa, 50 mg.) in deuteriomethanol (4 ml.) and deuterium oxide (2.0 ml.) containing dissolved sodium (8 mg.) was

heated under reflux for 4 days and then poured into ether (10 ml.). The ether phase was washed with water, dried, and evaporated, and the residue recrystallized from deuterium oxide–deuteriomethanol giving IIIb (42 mg.), m.p. 70–71° (d_2, 6%; d_3, 94%).

8β-d_1-5α-**Androstan-7-one** (IIIc).—A solution of the above deuterated ketone IIIb (40 mg.) in 70% aqueous methanol (2 cc.) containing sodium hydroxide (100 mg.) was heated under reflux for 1 hr. The product was isolated in the usual manner and recrystallized from aqueous methanol giving IIIc (30 mg.), m.p. 70–71° (d_0, 7%; d_1, 93%).

4,4-**Dimethyl**-Δ^{5}-**androsten**-3β-**ol** (XI).—4,4-Dimethyl-Δ^{5}-androsten-3-one[20] (1.4 g.) was placed in a porous thimble in a Soxhlet extractor and a suspension of lithium aluminum hydride (1.0 g.) in anhydrous ether (100 ml.) was allowed to reflux in the apparatus for 4 hr. After cooling the mixture to room temperature, the excess hydride was decomposed by the cautious addition of a saturated solution of sodium sulfate and the residual water was absorbed with solid anhydrous sodium sulfate. The inorganic salts were removed by filtration and washed well with dry ether, allowing the washings to dilute the filtrate. A thin layer chromatogram developed with benzene–ethyl acetate (9:1) showed the reaction product to be predominantly a single compound, but demonstrated the presence of a trace contaminant of higher R_f value. Evaporation of the solvent at reduced pressure gave a white crystalline solid, m.p. 155–160°, which showed no carbonyl absorption in the infrared. Two recrystallizations from methanol yielded an analytical sample of XI (1.3 g., 93%) as large, flat blades, m.p. 165–166°, $[\alpha]^{26}$D −4.4° (c 1.00).

Anal. Calcd. for $C_{21}H_{34}O$: C, 83.38; H, 11.33; mol. wt., 302.48. Calcd. for $C_{21}H_{34}O \cdot 0.5CH_3OH$: C, 81.07; H, 11.39. Found: C, 81.31; H, 11.36; mol. wt., 302 (mass spec.).

4,4-**Dimethyl**-Δ^{5}-**androsten**-3β-**ol Acetate** (XII).—A solution of the above alcohol XI (1 g.) in anhydrous pyridine (10 ml.) and acetic anhydride (10 ml.) was allowed to stand at room temperature for 16 hr. and then poured onto crushed ice. After all of the ice had melted the suspended solid was extracted with three 50-ml. portions of ether. The combined ethereal extracts were washed consecutively with dilute hydrochloric acid, saturated sodium bicarbonate solution, several times with water, and dried over anhydrous magnesium sulfate. Evaporation of the solvent gave a crystalline residue, m.p. 90–101°, which was, however, homogeneous in a thin layer chromatogram developed with hexane–benzene (1:3). Two recrystallizations from petroleum ether gave the desired acetate XII (0.908 g., 80%) as large needles, m.p. 114–115°, $[\alpha]^{26}$D − 80 3° (c 0.585), $\nu_{max}^{CHCl_3}$ 1715 and 1250 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{36}O_2$: C, 80.18; H, 10.53; mol. wt., 344.52. Found: C, 80.06; H, 10.41; mol. wt., 344 (mass spec.).

4,4-**Dimethyl**-Δ^{5}-**androsten**-3β-**ol**-7-**one Acetate** (XIII).—To a solution of 4,4-dimethyl-Δ^{5}-androsten-3β-ol acetate (XI, 2.57 g.) in acetic acid (30 ml.) and acetic anhydride (25 ml.), previously heated to 40°, sodium chromate tetrahydrate[21] (2.57 g.) was added at such a rate so as to maintain the temperature at 40 ± 2°. After the addition was complete, the yellow-orange solution was heated at 40 ± 2° by means of a water bath for 15 min., then cooled to room temperature and allowed to stand for an additional 72 hr. The resulting greenish brown solution was poured into cold water (300 ml.) and the suspension so produced was allowed to stand at room temperature for 3 hr. The precipitated solid was collected by filtration, washed with water, and dried in a vacuum desiccator over Drierite. Crystallization from aqueous ethanol gave XIII as flat plates (2.20 g., 84%), m.p. 134–135°. Two recrystallizations furnished an analytical sample, m.p. 134–135°, $[\alpha]^{27}$D −136° (c 1.04), λ_{max}^{EtOH} 238 mμ (log ϵ 4.11); $\nu_{max}^{CHCl_3}$ 1719, 1650, 1599, and 1250 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56; mol. wt., 358.50. Found: C, 76.87; H, 9.56; mol. wt., 358 (mass spec.).

4,4-**Dimethyl**-5α-**androstan**-3β-**ol**-7-**one Acetate** (XIV).—A solution of the above Δ^{5}-7-ketone XIII (1.80 g.) in absolute ethanol (50 ml.) containing suspended palladium (10%) on charcoal (400 mg.) was stirred vigorously in an atmosphere of hydrogen at ambient temperature and pressure. During 16 min., exactly 1 equivalent of hydrogen (119 ml.) was absorbed and no further uptake was observed during an additional 15 min. The catalyst was removed by filtration and the filtrate was evaporated to dryness giving a crystalline residue. Recrystallization from hexane gave the saturated ketone XIV (1.72 g., 96%) as irregular plates, m.p. 172.5–174.5° (uncorrected on Kofler

(18) Melting points are corrected and were determined in capillaries unless otherwise stated. Rotations were measured in chloroform and ultraviolet absorption spectra in 95% ethanol solution. The optical rotatory dispersion curves were obtained by Mrs. Ruth Records using a Nippon Bunko (Japan Spectroscopic Manufacturing Co., Ltd.) automatically recording spectropolarimeter (Model ORD-5). Thin layer chromatography (t.l.c.) was performed on silica gel G (E. Merck A.G., Darmstadt), the spots being developed by spraying with a 2% solution of ceric sulfate in 2 N sulfuric acid and subsequent heating. All mass spectra were determined with a Consolidated Electrodynamics Corp. 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. All microanalyses were carried out by Messrs. E. Meier and J. Consul.

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block). The analytical sample was recrystallized from the same solvent and showed m.p. 172.5–173°, [α]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°, [α]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-d₁-4,4-Dimethylandrostan-3β-ol-7-one (XVI).—Reduction of the Δ⁵-7-ketone XIII using deuterium in place of hydrogen

gave 5α,6α-d₂-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-d₄-Androstan-7-one (XX).—Palladium on charcoal (5%, 17 mg.) was added to a solution of 70 mg. of Δ³,⁵-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).

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]

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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
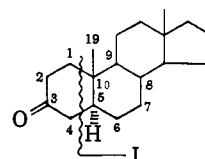
(A) 202 (−2H)
(B) 203 (−1H)

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