carbons with no directly bonded protons are found. However, in each case one must be alert to variations in χ. Further, an estimate of the contribution of the competing relaxation mechanisms can be obtained using this method provided enhancement data are available for a sufficient number of different carbon atoms in the molecule.

Acknowledgment. This work was supported primarily by Grant No. GM08521-08 from the National Institutes of Health.

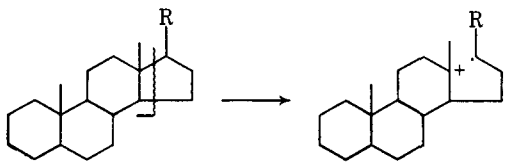
Mass Spectrometry in Structural and Stereochemical Problems. CLXXVI.[1] The Course of the Electron Impact Induced Fragmentation of Androstane[2]

L. Tökés[3] and Carl Djerassi

Contribution from the Department of Chemistry, Stanford University, Stanford, California 94305. *Received March 24, 1969*

Abstract: The fragmentation of the androstane skeleton differs drastically from that of C-17-substituted steroidal hydrocarbons such as cholestane or pregnane. The characteristic ring D fragmentation of the latter leading to peaks at *m/e* 217 and 218 is relatively unimportant in the absence of a C-17 substituent. Instead, androstane suffers appreciable fragmentation in ring A and through extensive deuterium labeling, the nature of this fragmentation—notably the course of a reciprocal hydrogen transfer—has been elucidated. Another striking difference between the mass spectrum of androstane and 17β-ethylandrostane (pregnane) is the origin of the expelled methyl radical associated with the characteristic M − 15 peak. Mechanistic rationalizations for the various fragmentation processes are presented as are synthetic details for the preparation of the various deuterated analogs.

Steroids possessing a saturated alkyl substituent at C-17, such as cholestane (I) or pregnane (II), undergo a very characteristic electron impact induced fragmentation of ring D, which is schematically illustrated by the wavy line in the structural formula. The precise course of this long-known fragmentation process has recently been elucidated[4] through extensive deuterium labeling and could best be rationalized through the intervention of the molecular ion a.



I, R = C_8H_{17}
II, R = C_2H_5
III, R = H

a

The presence of a double bond in various locations in the side chain (R in I) drastically affects[5] this ring D fragmentation, which is not surprising, since preferred charge localization at the double bond may be anticipated at the expense of contributions from species a. Work on the elucidation of the mass spectral fragmentation of these hydrocarbons was required to provide the basic information upon which the interpretation of the mass spectra of more highly substituted steroids rests. Consequently, we felt that a similar detailed study of the simplest steroidal hydrocarbon, androstane (III), was definitely warranted, especially since even a cursory examination[6] of its mass spectrum without any isotopic labeling indicated major differences between its fragmentation and that of cholestane (I). The presently described investigation involving extensive deuterium labeling proved well worth the effort, because in addition to defining in some detail the course of the major mass spectral fragmentation paths of the androstane skeleton, it uncovered two processes which are undetectable except through isotopic labeling. The first refers to the origin of the loss of a methyl radical from the molecular ion, which in the case of pregnane (II) had been found[4] to involve the C-19 angular methyl group[7] to the extent of 80%, but which in androstane (III) has now been found (see Table I) to proceed to a more nearly equal extent by elimination of either angular substituent. The second and even more interesting observation concerns the discovery of a reciprocal hydrogen transfer associated with fission of ring A, which is discussed in detail in the sequel.

Synthesis of Deuterium-Labeled Analogs of 5α-Androstane

During the course of this study all hydrogen-bearing carbon atoms in 5α-androstane (III) except for positions 1, 11, and 15 were labeled with deuterium atoms

(1) For paper CLXXV see C. A. Brown and C. Djerassi, *J. Chem. Soc.*, in press.
(2) Financial assistance from the National Institutes of Health (Grant No. AM-12758) is gratefully acknowledged.
(3) Taken in part from the Ph.D. thesis of L. T.
(4) L. Tökés, G. Jones, and C. Djerassi, *J. Am. Chem. Soc.*, **90**, 5465 (1968).
(5) S. G. Wyllie and C. Djerassi, *J. Org. Chem.*, **33**, 305 (1968).
(6) H. Budzikiewicz and C. Djerassi, *J. Am. Chem. Soc.*, **84**, 1430 (1962).
(7) In this connection, attention should be called to a misprint in Table I of ref 4 in that the M − CH_3 peak in 19,19-d_2-5α-pregnane is distributed to the extent of 80/20% between *m/e* 273 and 275, rather than *m/e* 273 and 276. Another misprint in Table I of ref 4 refers to the *m/e* 219 peak in the 14α-d_1 analog, which by mistake is listed as *m/e* 218.

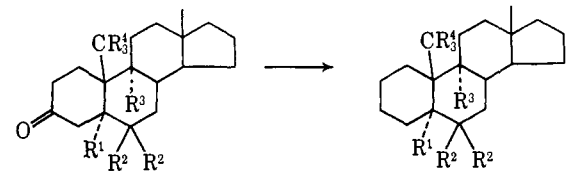
Table I. Shifts[a] of Mass Spectral Peaks of Deuterated Analogs of 5α-Androstanes (III)

5α-Androstanes	Isotopic purity, %	M+	M − CH_3 (%)	M − C_2H_4 (%)	M − C_3H_6 (%)	M − C_3H_7 (%)	M − C_4H_8 (%)	M − C_4H_9 (%)	M − C_5H_{10} (%)
d_0 (III)		260	245	232	218	217	204	203	190
2,2,4,4-d_4 (XXIX)	1 d_2 8 d_3 91 d_4	264	249	236	222(82)[b]	221(93)[b]	204[c]	203(~90) 207(7)	194[d]
3α-d_1 (XVI)	2 d_0 98 d_1	261	246	233	219(81) 218(19)	218(95) 217(5)	204(55) 205(45)	203(~95)	191[c]
5α-d_1 (VIII)	39 d_0 53 d_1 6 d_2 2 d_3	261	246	233[c]	219[c]	218[c]	205	203(~70) 204(~30)	191[d]
6,6-d_2 (IX)	2 d_0 7 d_1 91 d_2	262	247	234[c]	220	219	206	205	192[c]
7β-d_1 (XXX)	9 d_0 90 d_1 1 d_2	261	246	233	219(91) 218(9)	218(98) 217(2)	205[c]	204	191[d]
8β-d_1 (XIII)	3 d_0 96 d_1 1 d_2	261	246	233	219(90) 218(10)	218(90) 217(10)	205	204(95) 203(5)	191[c]
9α-d_1 (X)	5 d_0 91 d_1 4 d_2	261	246	233	219(96) 218(4)	218(80) 217(20)	205	204(95) 203(5)	191[d]
12,12-d_2 (XXII)	1 d_0 18 d_1 72 d_2 8 d_3 1 d_4	262	247	234[c]	220[c]	219(98) 218(2)	206	205	192[c]
14α-d_1 (XIX)	18 d_0 82 d_1	261	246	233[c]	219	218(75) 217(25)	205	204	190(60) 191 (40)
16,16-d_2 (XXV)	3 d_1 97 d_2	262	247	232(90) 234(10)	219(70) 220(26) 218(4)	217(94) 219(6)	206	205(95) 203(~5)	190[d]
17,17-d_2 (XXVIII)	1 d_0 4 d_1 93 d_2 2 d_3	262	247	234(60) 232(40)	218(76) 220(24)	217(88) 219(12)	206	205(95) 203(5)	190[d]
18,18,18-d_3 (XVII)	2 d_2 98 d_3	263	245(60) 248(40)	235	220(65) 221(35)	220(95) 219(1) 217(4)	207	206(94) 203(6)	190[c]
19,19,19-d_3 (XI)	2 d_0 4 d_1 24 d_2 67 d_3 3 d_4	263	248(63) 245(37)	235[c]	221(98)[b]	220(86) 217(14)	206(~75)[e] 207(~25)[e]	206[c] 203(~5)	193[d]

[a] The shift values are corrected for isotopic impurity as well as for ^{13}C contributions and are certain to ±5% for all peaks except the M+ − C_5H_{10} ions where the uncertainty is ±10%. [b] No definite shift assignment could be made for the rest of these ions. [c] At least 90% is at the indicated shift value but exact calculation was impossible because of isotopic contaminants or low intensity of peak. [d] At least 80% is at the indicated shift value, but the low intensity of this peak and the uncertain shifts of the adjacent *m/e* 189 peak prevented exact calculations. [e] The uncertainty of these values is high due to the low isotopic purity of the sample; hence the implicated ~75% loss of deuterium may in fact be much higher to counterbalance the 90% retention of label from C-3.

(see Table I for isotopic purity of these analogs). Many of these samples were prepared by a modified microscale Huang-Minlon reduction of previously reported labeled androstanone derivatives. For example, the synthesis of the 5α-d_1 (IV), 6,6-d_2 (V), 9α-d_1 (VI), and 19,19,19-d_3 (VII) labeled 5α-androstan-3-ones has been reported from this laboratory in conjunction with the study of the mass spectral behavior of androstan-3-one.[8] Reduction of these samples gave the corresponding labeled 5α-androstane analogs (VIII–XI).

Similarly, reduction of 8β-d_1-5α-androstan-12-one[9] (XII) and of the 3α-d_1 (XIV) and 18,18,18-d_3-*dl* (XV) 5α-androstan-17-ones[10] provided the 8β-d_1 (XIII), 3α-d_1 (XVI), and 18,18,18-d_3-*dl* (XVII) labeled 5α-androstanes.

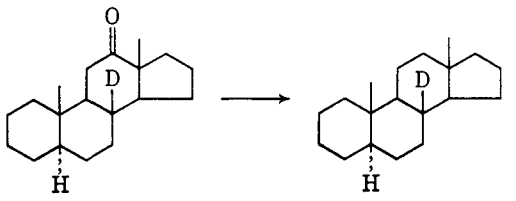


IV, R^1 = D; R^2 = R^3 = R^4 = H
V, R^1 = R^3 = R^4 = H; R^2 = D
VI, R^1 = R^2 = R^4 = H; R^3 = D
VII, R^1 = R^2 = R^3 = H; R^4 = D

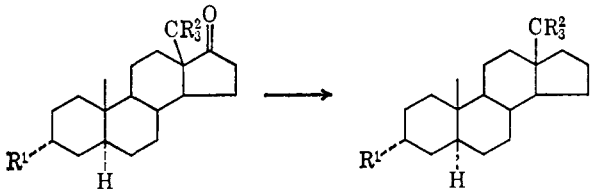
VIII, R^1 = D; R^2 = R^3 = R^4 = H
IX, R^1 = R^3 = R^4 = H; R^2 = D
X, R^1 = R^2 = R^4 = H; R^3 = D
XI, R^1 = R^2 = R^3 = H; R^4 = D

The 14α-d_1-5α-androstane (XIX) was obtained by the reduction of 14α-d_1-5α-androstane-3,17-dione[11] (XVIII) but this reaction required more forcing conditions

(8) R. H. Shapiro, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **86**, 2837 (1964).
(9) C. Djerassi and L. Tökés, *ibid.*, **88**, 536 (1966).
(10) L. Tökés, R. T. LaLonde, and C. Djerassi, *J. Org. Chem.*, **32**, 1012 (1967).
(11) G. Jones and C. Djerassi, *Steroids*, **10**, 653 (1967).



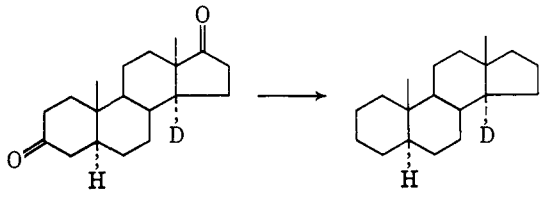
XII XIII



XIV, $R^1 = D; R^2 = H$
XV, $R^1 = H; R^2 = D$

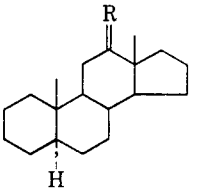
XVI, $R^1 = D; R^2 = H$
XVII, $R^1 = H; R^2 = D$

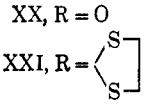
(higher temperature and longer time) than the previous monoketo derivatives.



XVIII XIX

We reported previously[4,12] that deuterated Raney nickel desulfurization (usually accompanied by very extensive isotopic scrambling)[13] of the ethylene thioketal derivative of 12-keto steroids provides a reasonable means for labeling the C-12 position. By analogy, 5α-androstan-12-one[9] (XX) was converted into its ethylene thioketal derivative (XXI) which upon desulfurization gave 12,12-d_2-5α-androstane (XXII) in 72% d_2 isotopic purity.



XX, R = O
XXI, R = 
XXII, R = 2D

Incorporation of deuterium atoms onto the steroid skeleton *via* lithium aluminum deuteride treatment of the tosylhydrazone derivative of steroidal ketones[14] is seriously limited by the formation in certain cases of olefinic side products which are hard to separate by conventional methods. This reaction, however, can provide labeled derivatives with excellent isotopic purity when the unsaturated products are separated by chromatography on silver nitrate impregnated silica gel

(12) L. Tökés, R. T. LaLonde, and C. Djerassi, *J. Org. Chem.*, **32**, 1020 (1967).
(13) See C. Djerassi, "Proceedings of the Second International Congress of Hormonal Steroids," Excerpta Medica Foundation, Amsterdam, 1967, pp 261–268.
(14) (a) L. Caglioti and M. Magi, *Tetrahedron*, **19**, 1127 (1963); (b) M. Fischer, Z. Pelah, D. H. Williams, and C. Djerassi, *Ber.*, **98**, 3236 (1965).

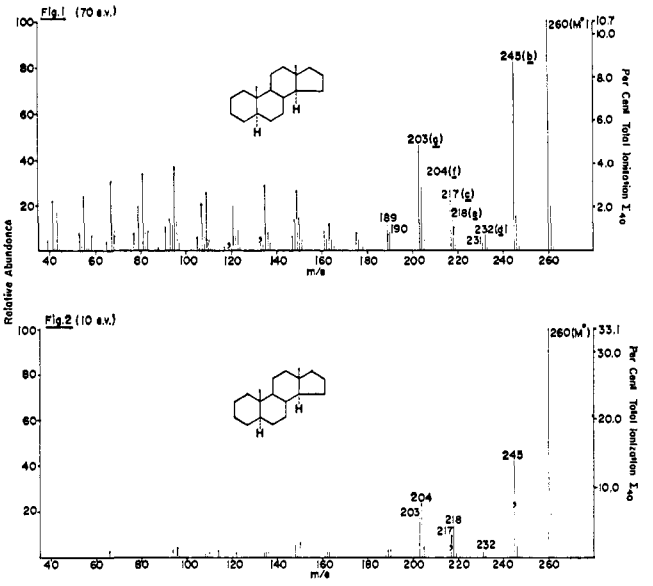
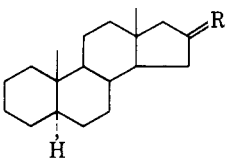


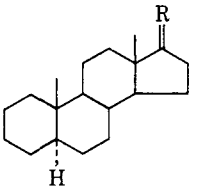
Figure 1. Mass spectrum (70 eV) of 5α-androstane (III).

Figure 2. Mass spectrum (10 eV) of 5α-androstane (III).

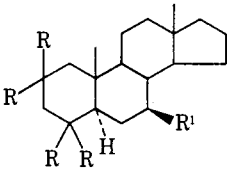
plates. The application of this reduction to the tosylhydrazone derivatives (XXIV and XXVII) of 5α-androstan-16-one[15] (XXIII) and -17-one[10] (XXVI) followed by separation of the olefinic by-products gave the 16,16-d_2 (XXV) and 17,17-d_2 (XXVIII) 5α-androstanes in 97 and 93% isotopic purity, respectively.



XXIII, R = O
XXIV, R = NNHTs
XXV, R = 2D



XXVI, R = O
XXVII, R = NNHTs
XXVIII, R = 2D



XXIX, R = D; R^1 = H
XXX, R = H; R^1 = D

The synthesis of 2,2,4,4-d_4-5α-androstane (XXIX) has been reported previously.[14b] The 7β-d_1-5α-androstane (XXX) was obtained inadvertently as a minor side product from the Huang-Minlon reduction of 7β-d_1-5α-androstane-3,17-dione 17-ethylene ketal[16] and hence no attempt was made to synthesize it by an alternative route.

Discussion of Mass Spectral Fragmentation Processes

Preliminary examination of the mass spectrum of 5α-androstane (Figure 1) has been reported previously both

(15) C. Djerassi and D. Herbst, *J. Org. Chem.*, **26**, 4675 (1961).
(16) Dr. G. von Mutzenbecher, unpublished results.

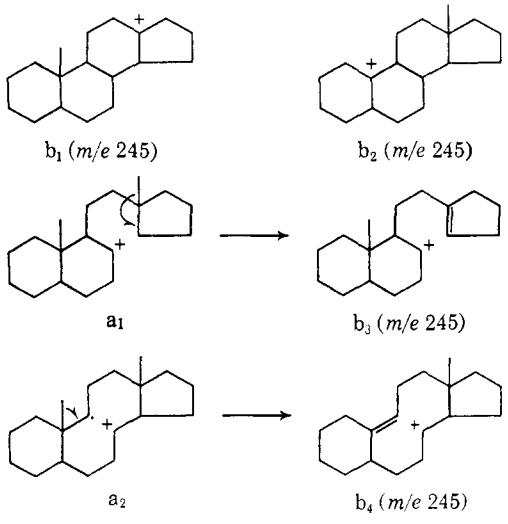
from this[6] and from other[17] laboratories. These studies led to the proposal of certain cleavage patterns which may be responsible for some of the fragment ions in the mass spectrum, but these conclusions were reached mainly by comparing the spectral features of androstane with those of other substituted derivatives, without any isotope-labeling evidence.

It was noted[17] that the mass spectrum of 5α-androstane (III) is quite void of any prominent fragmentation patterns such as the characteristic ring D cleavage[4] of compounds such as pregnane (II) and cholestane (I). This observation can be attributed to the lack of any exceptionally strained, highly substituted bond in the androstane skeleton whose preferential rupture could trigger a main fragmentation sequence. In pregnane, cholestane, and other C-17 substituent bearing steroids the presence of this substituent introduces enough strain in the 13–17 bond to cause its rupture, forming molecular ion a in which the radical site is further stabilized by the substituent at C-17. The intervention of molecular ion a then leads to the intense loss of ring D in these compounds.

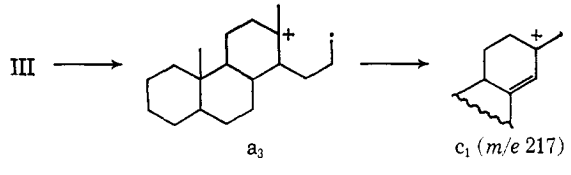
In general, in the unsubstituted tertracyclic steroid skeleton typified by androstane (III), formation of a fragment ion requires the fission of two bonds. The only exception is the ejection of one of the two angular methyl groups. It is, therefore, not surprising to find that in the mass spectrum (Figure 1) of androstane the only two intense peaks are the molecular ion and the M − CH_3 peak. Smaller peaks of fairly similar intensity can be found at 14 mass unit intervals, indicating that fission of virtually all other bonds occurs to an approximately equal extent at 70 eV.

The M − CH_3 peak is present in all spectra of angular methyl-bearing steroids. It was clearly established[4] that in 5α-pregnane (II) the expelled methyl radical originates exclusively from the C-18 and C-19 methyl functions in a ratio of about 1:4. The strong preference for the loss of C-19 is most likely due to the abundant cleavage of the 13–17 bond in this compound, forming molecular ion a from which the loss of C-18 would be an unfavorable process. Our current deuterium-labeling results (see Table I) confirmed that in 5α-androstane (III) the two angular methyl groups are responsible entirely for the expelled methyl radical, but the ratio is now reversed (3:2) in favor of C-18.

In attempting to explain these results, it should be kept in mind that there is no evidence to differentiate between a fragmentation mechanism involving a single bond scission, yielding ions b_1 and b_2 directly, and another alternative involving multiple bond cleavage in which the loss of the methyl radical is due to a secondary process from molecular ions such as a_1 and a_2. However, in view of the fact that tetrasubstituted carbon centers in aliphatic compounds are known[18] to undergo facile rupture of one of the adjoining C–C bonds, it is tempting to consider ions b_1 and b_2 as more plausible candidates for this process. The slight preference for the loss of C-18 may be due to the release of higher strain inherent in the *trans*-hydrindan system in forming ion b_1. The same reasoning, namely release of ring strain, also can be applied to the preferential formation of molecular ion a_1 (as compared to a_2) which would account for the higher abundance of ion b_3 than b_4 in the alternative mechanism.



As already mentioned previously, the intense peaks at *m/e* 217 and 218, associated with the characteristic ring D cleavage in the mass spectrum of pregnane[4] (II) and cholestane[4] (I), are of only minor importance in the spectrum (Figure 1) of 5α-androstane (III). Analogous ring D cleavages also have been proposed previously[6,17] for the genesis of these ions in androstane and the Spitellers[17] suggested that the mechanism of this fragmentation may be the same as that reported[4,19] for the C-17 side chain bearing hydrocarbons (III → a_3 → c_1).



With the aid of the current deuterium-labeling results (Table I), it is now revealed that there are several different cleavage patterns which contribute to the genesis of both the *m/e* 217 and 218 peaks. At least three different types of processes are involved in the formation of the ion of mass 217, the most important (75–77%) being the loss of ring D with an additional hydrogen atom. About 28% of the *m/e* 217 peak is generated by partial ring D cleavage followed by the loss of either the C-18 (4%) or the C-19 (14%) angular methyl groups, and there is evidence for about 5–7% of ring A fragmentation of hitherto unknown nature.

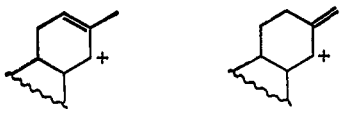
The abstraction of the extra hydrogen in conjunction with the loss of ring D is a somewhat random process since loss of deuterium label was observed from the 7β (2%), 8β (10%), 9α (20%), 12α and β (2%), 14α (25%), and 18 (1%) positions. These results are similar to our previous observations in 5α-pregnane[4] that only minor amounts are transferred from the 8β, 12β, and 18 positions which could be considered *a priori* as prime candi-

(17) M. Spiteller-Friedman and G. Spiteller, *Org. Mass Spectry.*, 1, 231 (1968).

(18) R. Ryhage and E. Stenhagen, *Arkiv Kemi*, **15**, 333 (1960); J. Diebler, *J. Res. Natl. Bur. Stand.*, **49**, 235 (1952).

(19) Prior to the publication of our results (see ref 4) the mechanism of these fragmentations has been reported by C. Djerassi at the International Mass Spectrometry Conference in Berlin, 1967 (see C. Djerassi, *Advan. Mass Spectry.*, 4, 199 (1968)).

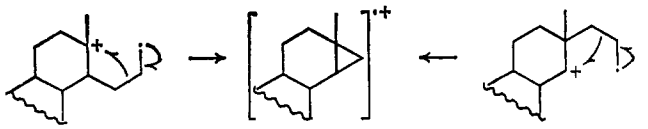
dates since transfer from these sites yields allylically stabilized carbonium ions (c_1, c_2, and c_3).



c_2 (*m/e* 217) c_3 (*m/e* 217)

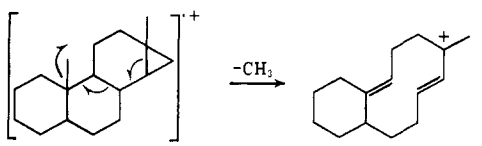
The dominant transfer site is the 14α position where the loss of 25% of label represents about 33% of the total transfer associated with the ring D cleavage. The mechanisms which may be responsible for the transfers from the 7β, 8β, 12, and 14α positions are probably the same as those which have already been explained in ref 4 for this fragmentation in pregnane and cholestane. The significance of the substantial loss of deuterium from the 9α position (20%) is not obvious. It may indicate the participation of other mechanisms; in fact, an undetermined portion of it may be associated with the ring A fragmentations. Major intervention of a possible two-step mechanism involving the subsequent loss of a hydrogen atom from the ion of mass 218 is deemed unlikely by metastable peak evidence. Clearly identifiable metastable peaks were observed for both $M^+ \rightarrow$ *m/e* 218 (at *m/e* 182.8) and $M^+ \rightarrow$ *m/e* 217 (at *m/e* 181.2), while no metastable peak could be seen around *m/e* 216 which would correspond to *m/e* 218 $\rightarrow$ *m/e* 217.

The second major cleavage pattern, which contributes to the extent of 14% to the genesis of the *m/e* 217 peak, involves the partial loss of ring D followed by the expulsion of the C-19 methyl group. This fragmentation may proceed *via* an intermediate ion d which is apparently responsible for about 90% of the *m/e* 232 peak in Figure 1 as judged by the 90% loss of label in 16,16-d_2-5α-androstane (Table I). Considering the differences in the loss of the C-16 and C-17 labels it seems that ion d may be formed by two different paths: $a_3 \rightarrow$ d (~40%) and $a_4 \rightarrow$ d (~50%).



a_3 d (*m/e* 232) a_4

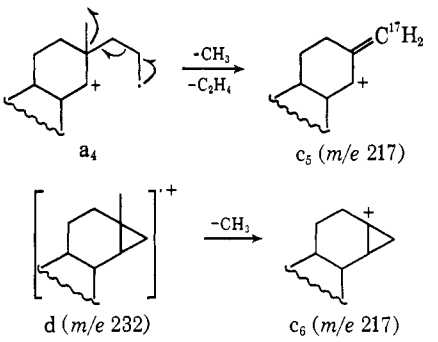
The expulsion of the C-19 methyl function triggered by the concerted cleavage of the 13–14 and 8–9 bonds, as indicated in d_1, can then yield ion c_4 (*m/e* 217).



d_1 (*m/e* 232) c_4 (*m/e* 217)

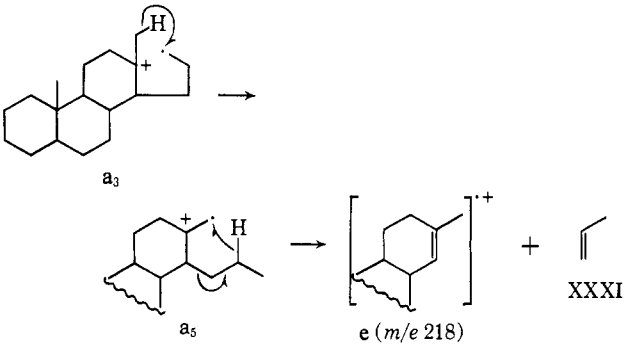
The minor (4%) contributor to the *m/e* 217 ion, which is derived by expelling the 18-angular methyl group in conjunction with the partial ring D cleavage, may originate either from molecular ion a_4 by the concerted rupture of the 16–17 and 13–18 bonds (see $a_4 \rightarrow c_5$), or from ion d by the simple loss of C-18 (see d $\rightarrow c_6$).

Judging from the ring A and ring D labeling results (Table I), it is apparent that about 18–26% of the *m/e* 218 species originates from a ring A fragmentation, the nature of which could not be deduced from the currently available results. The remaining 74–82% of this ion is formed by the loss of ring D as indicated in formula III. Such a cleavage can be easily envisaged by the simple rupture of the activated 14–15 bond in molecular ion a_3; however, the deuterium-labeling results revealed that this cleavage is associated with a reciprocal double hydrogen transfer. These results are in excellent agreement with previous observations in pregnane,[4] showing high degree of site specificity in both hydrogen transfers. Thus, 65% of deuterium loss was observed from C-18 which was counterbalanced by 70% retention of the C-16 label from the expelled neutral fragment. These values represent 80–88% and 85–95% of the hydrogen transfers associated with the ring D cleavage. Small amounts of deuterium losses were observed from the 7β, 8β, and 9α positions also, but these are of little significance compared to that of C-18 and some of it may be associated with the unidentified minor ring A cleavage processes.



a_4 c_5 (*m/e* 217) d (*m/e* 232) c_6 (*m/e* 217)

A mechanism which is consistent with these results can be depicted as follows. Transfer of a sterically easily accessible hydrogen from C-18 to relieve the C-17 primary radical site in molecular ion a_3 yields an ionized olefin a_5. A second hydrogen transfer from C-16 in a six-membered transition state to the terminal end of the double bond in ion a_5 is in accord with the general fragmentation behavior of olefins[20] and in this case it triggers cleavage of the 14–15 bond to form an ionized (e) and a neutral (XXXI) olefin.

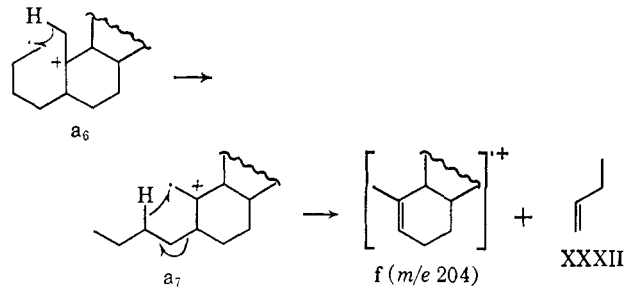


a_3 a_5 e (*m/e* 218)

A close analogy for such a site-specific reciprocal hydrogen transfer in association with ring cleavage is found in the genesis of the ion of mass 204. According to the labeling results (Table I) this ion is almost entirely due to the loss of ring A. Furthermore, it was revealed that 45% of the deuterium label from the 3α position is retained in the charge-bearing fragment while about 75% of a deuterium is lost from C-19. This

(20) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day, Inc., San Francisco, Calif., 1967, pp 55–60.

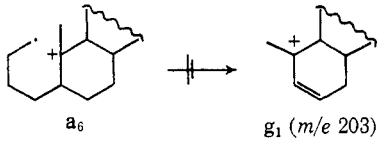
can be explained only by a reciprocal double hydrogen transfer which is completely analogous to the ring D process $a_3 \rightarrow e$ and can be formulated as follows.



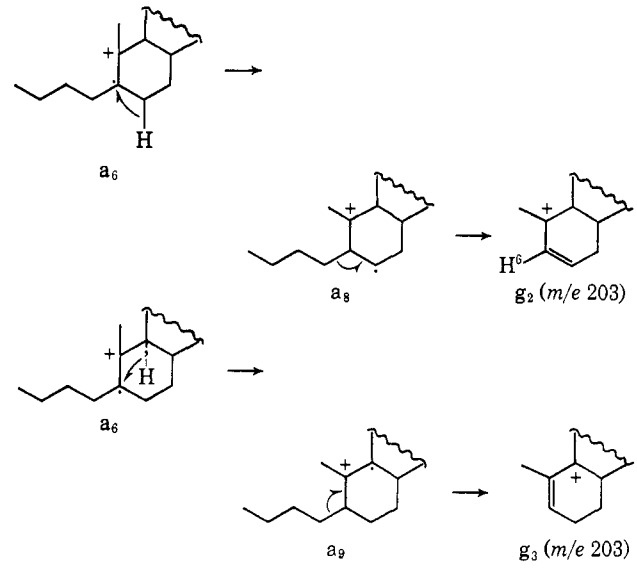
Transfer of a hydrogen atom from C-19 in molecular ion a_6 yields the ionized olefin a_7 in which the 3α-deuterium lost its stereochemical significance. Hence the observed 45% deuterium retention from the monolabeled C-3 position is indicative of at least 90% transfer from this site. The transfer of the C-3 hydrogen atoms together with fission of the 4–5 bond then yields an ionized (f, *m/e* 204) and a neutral (XXXII) olefin. It should be pointed out that such reciprocal hydrogen transfers in conjunction with cleavage patterns which involve no net migration of hydrogen can be revealed only by isotope-labeling techniques and that their occurrence in organic mass spectrometry may be much more prevalent than has been suspected.

The most intense ion in Figure 1, next to the M^+ and $M^+ - CH_3$ ions, is the ion of mass 203. The loss of ring A together with a hydrogen atom has been proposed earlier[6,17] to explain the origin of this ion. The current labeling results confirmed that such a cleavage is indeed responsible for a major portion (~90%) of this ion, but in addition bond fissions involving the loss of carbon atoms 16, 17, 18, and 19 must also be operative to the extent of 5–10%. At low electron voltage (Figure 2) the *m/e* 204 peak predominates over the *m/e* 203 one, exactly as has been observed previously[4] for the *m/e* 217 and 218 peak group in I and II (as well as in III).

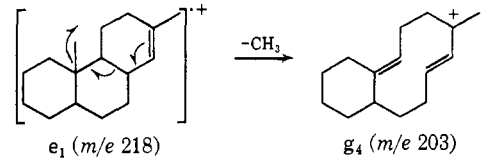
The Spitellers[17] proposed a fragmentation mechanism for this cleavage which implies the transfer of a C-6 hydrogen atom ($a_6 \rightarrow g_1$), but they provided no isotopic labeling evidence for its occurrence.



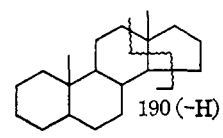
We labeled, therefore, the C-6 position with two deuterium atoms and found that in fact no detectable hydrogen transfer occurred from this position. Rather, the major transfer site is the tertiary position (C-5) adjacent to the carbonium center in molecular ion a_6, an observation which is analogous to the transfer of the 14α-hydrogen in association with the loss of ring D (*m/e* 217) in both androstane (III) and pregnane (II).[4] In general, processes which involve formally the cleavage of two bonds connected to the same carbon atom (C-5 in the present instance) are suggestive of an intramolecular rearrangement. In the ring A fission of androstane, such a rearrangement can be best understood in terms of a hydrogen migration from either C-6 or C-9 to C-5, yielding intermediates a_8 and a_9 which can then undergo facile cleavage of the 4–5 bond to form ions g_2 and g_3, respectively.



A minor portion (~5%) of the ion of mass 203 in which the 16, 17, and 19 carbon atoms are expelled may originate from the ejection of the C-19 methyl radical from ion e (*m/e* 218), in a fragmentation similar to the one which was responsible in part for the *m/e* 203 peak in the mass spectrum of pregnane[4] (see $e_1 \rightarrow g_4$).



Examination of the shifts of other peaks in the mass spectra of the labeled samples shows that all peaks are due to several different fragmentations of comparable intensity, involving different parts of the molecule. These deuterium labeling results alone do not provide sufficient evidence for unequivocal interpretation of the fractional shifts of these peaks and analyses of these ions are, therefore, not included in Table I. There was, however, one additional peak at *m/e* 190 whose shift values could be assigned and analyzed with a reasonable degree of certainty. According to the labeling results this ion is formed by expulsion of C_5H_{10} moiety involving mainly the fragmentation of the C/D ring junction. The loss of the C-16, -17, and -18 labels and retention of the C-12 label suggest that the expelled five-carbon fragment consists most probably of carbon atoms 13, 15, 16, 17, and 18 as indicated in formula XXXIII. This fragmentation is further associated with the loss of an extra hydrogen which may in part (~60%) originate from the 14α position. A cleavage pattern which is consistent with these labeling results is obviously a very complicated one and proposal of a fragmentation mechanism, based on the currently available results, is not warranted.



XXXIII

In conclusion, the present work affords an impressive illustration of how a minor molecular change (absence or presence of C-17 alkyl substituent: I and II *vs.* III) can have a major effect on the mass spectral fragmentation and how indispensable deuterium labeling is for a proper mechanistic interpretation of the results.

Experimental Section[21]

Reduction of Labeled Androstanones. The reduction of the various labeled androstanones (1.9–10-mg samples) was carried out by using a previously reported general procedure.[4] The products were purified by thin layer chromatography (tlc) on silica gel H, followed by recrystallization from methanol, yielding the following labeled 5α-androstane samples (the position of the keto function in the starting materials is indicated in parentheses): 3α-d_1 (XVI, from 17-one[10] XIV), mp 51–52°; 5α-d_1 (VIII, from 3-one[8] IV), mp 52°; 6,6-d_2 (IX, from 3-one[8] V), mp 51–52°; 8β-d_1 (XIII, from 12-one[9] XII), mp 50–50.5°; 9α-d_1 (X, from 3-one[8] VI), mp 52–53°; 18,18,18-d_3-*dl* (XVII, from *dl*-17-one[10] XV), mp 77–79°; 19,19,19-d_3 (XI, from 3-one[8] VII), mp 51–52°.

14α-d_1-5α-Androstane (XIX). A mixture of 14α-d_1-5α-androstane-3,17-dione[11] (XVIII, 5 mg), 2 ml of diethylene glycol, and 1 ml of 85% hydrazine hydrate was heated under reflux for 1.5 hr, then cooled to 100°. One pellet (~150 mg) of potassium hydroxide was added and the temperature was raised gradually, boiling off the hydrazine hydrate. The heating was continued for 11 hr at 205–210°, then the resulting brown solution was cooled and diluted with water. Ether extraction, washing the ether phase with water, and drying over anhydrous sodium sulfate gave a glassy product (5 mg). Purification by tlc on silica gel H in hexane yielded the pure, partially crystalline 14α-d_1-5α-androstane (XIX, 2 mg) which exhibited a mass spectrum identical with that of an authentic unlabeled sample with the exception of mass shifts of the deuterium containing peaks.

Reduction of an unlabeled sample (15 mg) under identical conditions, followed by recrystallization from methanol, gave 5α-androstane (III, 8 mg), mp 51–52°.

5α-Androstan-12-one Ethylene Thioketal (XXI). Boron trifluoride etherate (0.1 ml) was added to a solution of 5α-androstan-12-one[9] (XX, 20 mg) in ethanedithiol (0.1 ml). After storing at room temperature for 10 min, the reaction mixture was diluted with ether, washed with plenty of dilute sodium hydroxide solution, and dried over anhydrous magnesium sulfate. Evaporation of the ether gave the crude mercaptal which was purified by tlc on silica gel H in benzene–ether (9:1), yielding the pure 5α-androstan-12-one ethylene thioketal (XXI, 20 mg, 79%), mp 110–111° (methanol).

Anal. Calcd for $C_{21}H_{34}S_2$: C, 71.93; H, 9.77. Found: C, 71.91; H, 9.79.

12,12-d_2-5α-Androstane (XXII). Freshly prepared[22] deuterium-containing Raney nickel (from 0.4 g of alloy) was added to a solution of 5α-androstan-12-one ethylene thioketal (XXI, 7 mg) in d_1-methanol (3 ml). The resulting suspension was stirred and heated under reflux for 4 hr, then the nickel was removed by filtration and the solvent was evaporated. The residue was purified by tlc on 10% silver nitrate containing silica gel H in hexane, yielding pure 12,12-d_2-5α-androstane (XXII, 5 mg, 95%), mp 53–54° (methanol); the melting point showed no depression when mixed with authentic unlabeled 5α-androstane. For isotopic purity see Table I.

5α-Androstan-16-one Tosylhydrazone (XXIV). A solution of 5α-androstan-16-one[15] (XXIII, 20 mg) and *p*-toluenesulfonylhydrazide (20 mg) in methanol (1.5 ml), containing a microdrop of concentrated sulfuric acid, was heated under reflux for 2 hr. A few drops of water were added and the solution was cooled in an ice bath. The crystalline precipitate after filtration and drying under reduced pressure (0.1 mm) provided 5α-androstan-16-one tosylhydrazone (XXIV, 27 mg, 84%), mp 188–190°; ν_{max} 3290, 3210 (N—H), 1660 (C=C), and 1160 (S=O) cm^{-1}, but no carbonyl absorption.

Anal. Calcd for $C_{26}H_{38}N_2O_2S$: C, 70.55; H, 8.65. Found: C, 71.00; H, 8.60.

5α-Androstan-17-one Tosylhydrazone (XXVII). Conversion of 5α-androstan-17-one (XXVI, 25 mg) by the same procedure as described above for the preparation of XXIV gave the crystalline tosylhydrazone derivative XXVII, mp 200–202° (lit.[14b] 201–202°), which was used in the next step without further purification.

16,16-d_2-5α-Androstane (XXV). A suspension of 5α-androstan-16-one tosylhydrazone (XXIV, 14 mg) and lithium aluminum deuteride (20 mg) in dry dioxane (2 ml, reagent grade) was heated under reflux for 15 hr, and the excess deuteride decomposed by the addition of a few drops of deuterium oxide. After cooling, the inorganic salts were removed by filtration, the solvent was evaporated under reduced pressure, and the residue was subjected to tlc analysis on 10% silver nitrate containing silica gel H in hexane. The resulting olefin-free 16,16-d_2-5α-androstane (XXV) amounted to 4.5 mg (45%), mp 53–54° (methanol). For isotopic purity see Table I.

17,17-d_2-5α-Androstane (XXVIII). The preparation and purification of 17,17-d_2-5α-androstane (XXVIII) were performed as described above for the 16-labeled analog XXV, starting with 34 mg of the tosylhydrazone XXVII. The yield of the olefin-free product was 6.5 mg (32%), mp 52–54° (methanol).

(21) Melting points (uncorrected) were determined on a Kofler block and the infrared spectra were measured in chloroform solution on a Perkin-Elmer Model 137 Infracord spectrophotometer. The mass spectra were measured by Mr. J. W. Smith and Dr. A. M. Duffield on a CEC 21-103C and Atlas CH-4 (with TO-4 ion source) mass spectrometers at 70 eV unless otherwise indicated. The elemental analyses are due to Messrs. E. Meier and J. Consul.

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

Some "Steric Effects" of Methyl in Mass Spectral Fragmentations

Maurice M. Bursey and Michael K. Hoffman

Contribution from the Venable Chemical Laboratory, The University of North Carolina, Chapel Hill, North Carolina 27514. *Received February 4, 1969*

Abstract: Substituent effects of methyl groups *ortho* to resonance donors were studied for the loss of NO by substituted nitrobenzene ions in the mass spectrometer. The results were found to be consistent with an interpretation as simple steric effects at high voltages. *o*-Methyl groups seem to prevent coplanarity of the dimethylamino group in this system, but not of the methoxy group. Both peak intensity comparisons and metastable ion characteristics support this conclusion. At low voltage an additional complication, possibly associated with ring expansion, appears. Methyl is not completely inert as a hindering group for this reaction, especially at low voltage, and it may be preferable to use halogen substituents for studies of steric effects.

The interpretation of steric effects in mass spectrometry must be made with caution. Interesting parallels between mass spectral substituent effects and Hammett substituent constants suggest that the electronic effects of substituents in mass spectral decompositions resemble those of solution chemistry in some