sibly lose methoxyl for a substituent of low ionization potential in the present work. However, we are not in a position to evaluate this possibility at present. It is noteworthy that in terms of the tenets of the QET, the concept of charge localization[7] has proved a useful expedient (with even predictive power) due to a combination of the following reasons. (a) The larger majority of accessible electronic states are in rapid reversible equilibrium, but lower lying electronic states are more highly populated due to the large number of associated vibrational states. (b) For a given internal energy, the lower lying electronic states have more vibrational energy and on these grounds (as well as in terms of population) decomposition from them is more probable given comparable activation energies for decomposition in comparison with higher electronic states—see (c). (c) The electronic state corresponding to an electron missing from the highest occupied molecular orbital can lead to fragmentation *via* processes of low activation energy in those cases where the charge localization concept has been most successful (*e.g.*, ketals[24] and dimethylamino compounds[25]).

(24) (a) H. Audier, M. Fetizon, J.-C. Gramain, J. Schalbar, and B. Waegel, *Bull. Soc. Chim. France*, 1880 (1964); (b) J. T. B. Marshall and D. H. Williams, *Tetrahedron*, 23, 321 (1967); (c) Z. Pelah, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **86**, 3722 (1964).

However, it is important to remember that the equilibrium hypothesis would appear to be capable of explaining existing data, and therefore the charge is not necessarily localized in the classical sense, except perhaps in a small fraction of ions of relatively low internal energy.[26]

Experimental Section

All spectra were determined using an AEI MS9 mass spectrometer operating at a source temperature of 170°, a heated inlet temperature of ~150°, electron beam energy of 14 eV, and an accelerator potential of 8 kV. All samples were introduced *via* the heated inlet system.

The esters were either commercially available or preparable from the corresponding commercially available acids with diazomethane. Purity was checked by melting point or vapor phase chromatography and, where impure, the compounds were purified by recrystallization and vapor phase chromatography, respectively.

(25) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol II, Holden-Day, Inc., San Francisco, Calif., 1964, Chapters 17 and 18.

(26) NOTE ADDED IN PROOF. Since this paper was submitted, other relevant papers dealing with "charge localization" and substituent effects have been published (or are in press) see A. Mandelbaum and K. Biemann, *J. Am. Chem. Soc.*, **90**, 2975 (1968); R. G. Cooks, R. S. Ward, I. Howe, and D. H. Williams, *Chem. Commun.*, in press; F. W. McLafferty, *ibid.*, in press. We wish to thank Professor McLafferty for sending to us a copy of his manuscript *prior to* publication.

Mass Spectrometry in Structural and Stereochemical Problems. CLXI.[1] Elucidation of the Course of the Characteristic Ring D Fragmentation of Steroids[2]

L. Tökes,[3] G. Jones,[4] and Carl Djerassi

Contribution from the Department of Chemistry, Stanford University, Stanford, California 94305. *Received May 9, 1968*

Abstract: The electron impact induced fragmentation of ring D in steroids, which involves loss of carbon atoms 15, 16, and 17 together with their substituents, is of considerable mechanistic as well as structural significance. It appears to be the most general fragmentation of steroids and lends itself to a convenient determination of the length of the C-17 side chain. These fissions of the 13–17 and 14–15 bonds occur with and without the apparent transfer of a hydrogen atom, and several mechanistic proposals have been made during the past 10 years about the nature of these processes. Extensive deuterium labeling of virtually all carbon atoms in 5α-cholestane or 5α-pregnane has shown that all of the earlier proposals were partly or totally incorrect. It has now been found that the cleavage of the 13–17 and 14–15 bonds is not a simple reaction but rather involves the reciprocal transfer of hydrogens from C-16 and C-18, apparently to make possible the expulsion of an olefin rather than of a cyclopropane. The single hydrogen transfer accompanying the alternative ring D fragmentation has been shown to originate largely from C-14, and a rationalization for such a (generally unfavorable) fission of two bonds connected to one carbon atom is offered. The availability of the various deuterated analogs, whose syntheses are described in this paper, has also made possible mechanistic assignments to many of the other fragment ions.

The potential application of mass spectrometry to the structure elucation of steroids was noted over 10 years ago,[5] and shortly thereafter it was recognized that one of the most general fragmentations of sterols and related C-17-substituted steroids is the loss of 42 mass units together with the C-17 side chain. It is interesting to note that the variety of proposals offered as rationalizations for this characteristic fragmentation encompass in chronology as well as in terms of conceptual approach virtually the entire history of organic mass spectrometry. In the case of steroid hydrocarbons such as 5α-cholestane (X) and 5α-pregnane

(1) For paper CLX, see T. Muraski and C. Djerassi, *J. Org. Chem.*, 33, 2962 (1968).

(2) We are indebted to the National Institutes of Health for financial assistance (Grant No. CA-07195). The purchase of the Atlas CH-4 mass spectrometer was made possible by NASA Grant NsG 81-60.

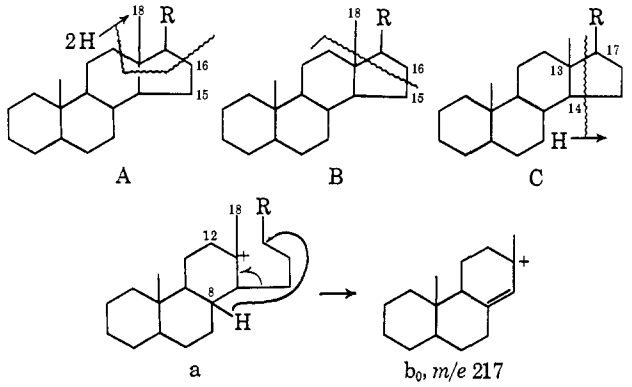
(3) Taken in part from the Ph.D. thesis (1965) of L. T.

(4) Postdoctoral fellow (1966–1967) and recipient of a Fulbright Travel Grant from the U. S. Educational Commission in the United Kingdom.

(5) P. de Mayo and R. I. Reed, *Chem. Ind.* (London), 1481 (1956).

(I) this fragmentation leads to a peak at *m/e* 217 (see Figures 1–5), which Reed[6] depicted intuitively in terms of A without attributing a structure to the fragment ion or concerning himself with the source of the two ejected hydrogen atoms. Independently, Friedland and collaborators[7] recognized the general nature of the *m/e* 217 peak among steroid hydrocarbons and visualized it in terms of B, again without assigning a structure to the fragment species. Their choice, which was also accepted by Fitches,[8a] was presumably based on a desire to invoke bond fissions, which do not require hydrogen migrations.[8b] It should be noted, however, that both A and B suffer from the serious defect of incorporating the cleavage of two bonds connected to one carbon atom—a process which is rare in mass spectrometry and whose occurrence generally can be considered to constitute evidence in favor of some rearrangement reaction.[9]

A third alternative explanation for the genesis of the mass 217 ion was offered by Ryhage and Stenhagen[10] who envisaged the process in terms of C and who were the first to attribute a structure (b) to the resulting fragment ion. This structural assignment implies that the C-8 hydrogen atom is transferred during the fission process—a sequence which in modern terminology[9] can be written as a → b_0.



The virtue of this last proposal[10] is that it formulates the fragment of mass 217 as an allylic carbonium ion (b). However, it was soon noted[11,12] that transfer of hydrogen from C-12 or C-18, rather than from C-8, would also lead to highly favored allylic carbonium ion species and that isotopic labeling with deuterium would be required to settle this question unambiguously.[13] That such deuterium labeling would have mechanistic as well as structural consequences becomes obvious from an inspection of the three main alternatives A, B, and C. It will be noted that none of them encompass the identical carbon atoms of the steroid nucleus.

Thus if A were correct, substituents at both C-15 and C-16 would be retained in the ionized fragment, while according to B, only a C-15 substituent would remain. Fragmentation C, on the other hand, implies the complete loss of ring D but the retention of the C-18 angular methyl group, which in turn is expelled in both A and B.

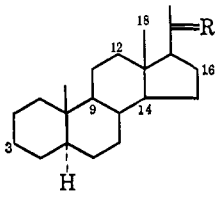
There existed two other justifications for undertaking the extensive and at times also laborious work of labeling the various carbon atoms with deuterium (see following sections on synthesis). The first was that this typical "hydrocarbon" fragmentation prevailed in steroids even in the presence of heteroatomic substituents (*e.g.*, the important 3-keto steroids) and remained even at low electron voltages. Clearly, we are faced here with an energetically very favorable and important fragmentation. Second, since the publication of the original articles[6–8,10] on this particular steroid cleavage, a veritable flood of paper dealing with the mass spectrometry of a wide variety of steroids has appeared[14] and it was therefore of crucial importance that the electron impact induced behavior of the fundamental steroid skeleton be as fully understood as possible. As will be shown in the sequel, the effort expended on the synthesis of the deuterated steroids proved to be well worthwhile in view of the light that their mass spectra shed on many unsolved and unsuspected problems dealing with the mass spectrometry of the steroid hydrocarbon framework.

Synthesis of Labeled and Unlabeled Pregnanes and Cholestanes. To study the inherent fragmentation characteristics of the steroid skeleton without complications due to the presence of other functional groups, we examined the three most important steroidal hydrocarbons—androstane, pregnane, and cholestane—and their deuterium-labeled analogs. In the combination of these three series every possible position on the steroid nucleus was labeled with deuterium atoms, but since the presence of a C-17 side chain as in 5α-pregnane (I) or 5α-cholestane (X) is an essential prerequisite for the typical ring D fragmentation, our discussion in this paper will be limited to these two series only.[15]

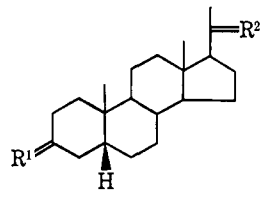
The unlabeled hydrocarbons, 5α-pregnane (I) and 5α-cholestane (X), were easily accessible by a modified Huang-Minlon reduction of the 20- (II) and 3-keto (XI) derivatives, respectively. The preparation of 5β-pregnane (III) was effected by Jones' oxidation of 5β-pregnan-3β-ol-20-one (IV), followed by reduction of the resulting 3,20-dione (V). For precise evaluation of the labeling results both labeled and unlabeled samples had to be pure and especially free of any olefin contaminants. Separation of traces of olefins from hydrocarbons by means of recrystallization or by chromatography on conventional supporting media is generally unsatisfactory. Thin layer chromatography on silver nitrate impregnated silica gel plates,[16] however,

(6) R. I. Reed, *J. Chem. Soc.*, 3432 (1958).
(7) S. S. Friedland, G. H. Lane, R. T. Longman, K. E. Train, and M. J. O'Neal, Jr., *Anal. Chem.*, **31**, 169 (1959).
(8) H. J. M. Fitches in "Advances in Mass Spectrometry," Vol. II, R. M. Elliott, Ed., Pergamon Press, London, 1962: (a) p 434; (b) p 454.
(9) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day, Inc., San Francisco, Calif., 1967, p 18.
(10) R. Ryhage and E. Stenhagen, *J. Lipid Res.*, **1**, 361 (1960).
(11) K. Biemann, "Mass Spectrometry-Organic Chemical Applications," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, pp 338–347.
(12) C. Djerassi, J. M. Wilson, H. Budzikiewicz, and J. W. Chamberlin, *J. Amer. Chem. Soc.*, **84**, 4544 (1962).
(13) C. Djerassi, *Pure Appl. Chem.*, **9**, 159 (1964).
(14) For leading references, see (a) H. Budzikiewicz, C. Djerassi' and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, Inc., San Francisco, Calif., 1964, Chapters 17–22; (b) C. Djerassi in "Proceedings of the Second International Congress of Hormonal Steroids," Excerpta Medical Foundation, Amsterdam, 1967, pp 3–15; (c) J. Diekman and C. Djerassi, *J. Org. Chem.*, **32**, 1005 (1967); (d) S. G. Wyllie and C. Djerassi, *ibid.*, **33**, 305 (1968).
(15) The work on the mass spectrometric fragmentation of androstane will be reported subsequently.
(16) E. Dunn and P. Robson, *Chromatography*, **17**, 501 (1965).

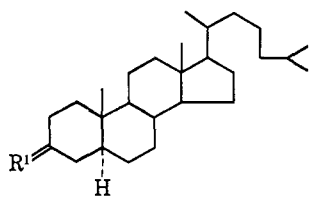
has proven quite effective and was used on most samples with the exception of a few labeled derivatives.



I, R = H_2
II, R = O



III, $R^1 = R^2 = H_2$
IV, R^1 =  ; R^2 = O
V, $R^1 = R^2$ = O
VI, R^1 =  ; R^2 = NNHTs
VII, R^1 =  ; $R^2 = D_2$
VIII, R^1 = O; $R^2 = D_2$
IX, $R^1 = H_2$; $R^2 = D_2$



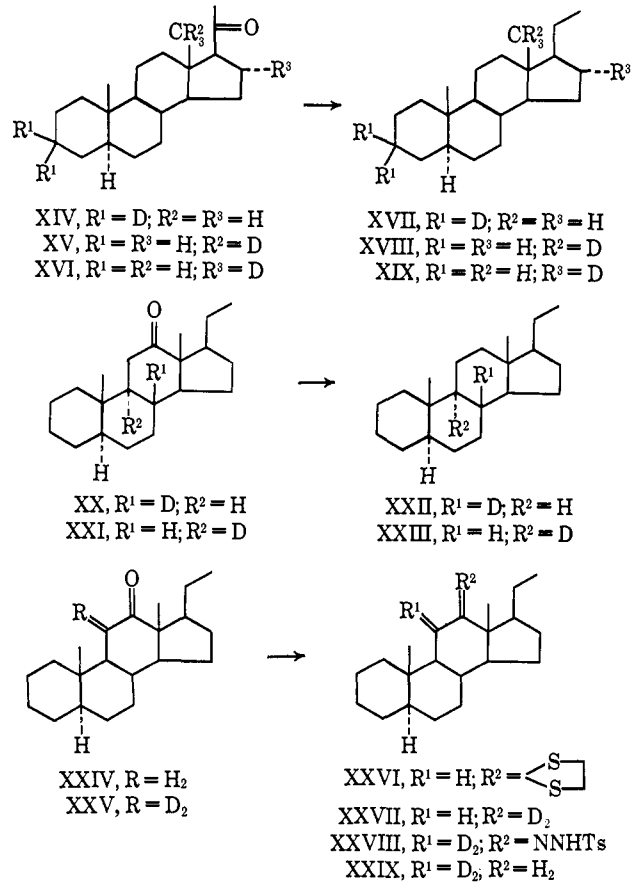
X, R = H_2
XI, R = O
XII, R = NNHTs
XIII, R = 

To differentiate between the previously proposed three ring D cleavage patterns A, B, and C, it was necessary to label the C-16 and C-18 positions. In addition, to find the source of the extra hydrogen atom associated with the genesis of the *m/e* 217 ion, it was essential to label the prime candidate transfer sites, such as carbon atoms 7, 8, 12, and 14. Further labeling in rings A and B enabled us to study other important fragmentation mechanisms as well.

Many of these labeled hydrocarbons were accessible by reduction of the appropriately labeled keto derivatives whose preparation has been already reported in connection with earlier mass spectrometric studies. Thus, modified Huang-Minlon reduction of the pregnan-20-one 3,3-d_2 (XIV), 16α-d_1 (XVI), and *dl*-18,18,18-d_3 (XV) derivatives[17] provided the labeled 5α-pregnane 3,3-d_2 (XVII), 16α-d_1 (XIX), and *dl*-18,18,18-d_3 (XVIII) samples. Similarly, the previously reported[18] 12-keto-5α-pregnane samples, XX and XXI, upon reduction furnished the 5α-pregnanes 8β-d_1 and 9α-d_1 (XXII and XXIII, respectively).

Labeling of carbon atom 12 was achieved by treatment[19] of the ethylene thioketal derivative (XXVI) of 5α-pregnan-12-one[18] (XXIV) with deuterated Raney nickel. The relatively high isotopic purity (75% d_2) of the resulting 5α-pregnane-12,12-d_2 (XXVII), compared to the desulfurization of other thioketals[19,20] can be explained by the lack of easily accessible protons in the vicinity of the C-12 mercaptan.

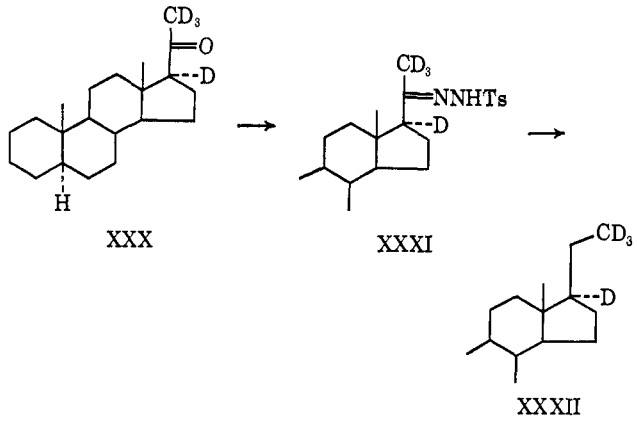
(17) L. Tokes, R. T. LaLonde, and C. Djerassi, *J. Org. Chem.*, **32**, 1020 (1967).
(18) C. Djerassi and L. Tökes, *J. Amer. Chem. Soc.*, **88**, 536 (1966).
(19) D. H. Williams, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *ibid.*, **85**, 2091 (1963).
(20) C. Djerassi, ref 14b, p 265.



XIV, R^1 = D; $R^2 = R^3$ = H
XV, $R^1 = R^3$ = H; R^2 = D
XVI, $R^1 = R^2$ = H; R^3 = D
XVII, R^1 = D; $R^2 = R^3$ = H
XVIII, $R^1 = R^3$ = H; R^2 = D
XIX, $R^1 = R^2$ = H; R^3 = D
XX, R^1 = D; R^2 = H
XXI, R^1 = H; R^2 = D
XXII, R^1 = D; R^2 = H
XXIII, R^1 = H; R^2 = D
XXIV, R = H_2
XXV, R = D_2
XXVI, R^1 = H; R^2 =
XXVII, R^1 = H; $R^2 = D_2$
XXVIII, $R^1 = D_2$; R^2 = NNHTs
XXIX, $R^1 = D_2$; $R^2 = H_2$

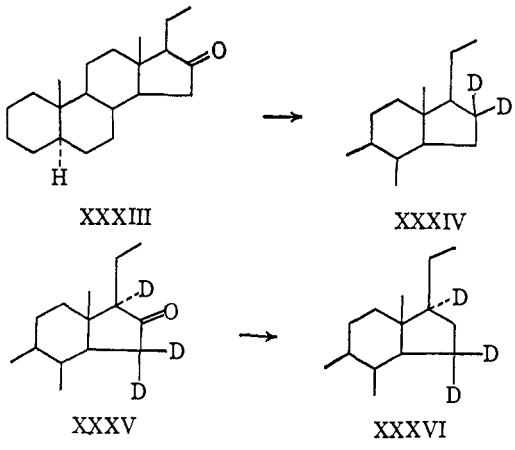
The reduction of tosylhydrazones with hydrides, developed by Caglioti, *et al.*,[21] constitutes a very useful reaction for the introduction of deuterium atoms into steroids when metal deuterides are used in place of hydrides and the products are chromatographed on silver nitrate impregnated plates[16] to separate the olefinic by-products. It is possible to introduce one or two deuterium atoms in place of a carbonyl function, depending on the reaction conditions,[22] or to insert them on adjacent carbon atoms if the enolizable protons are exchanged prior to tosylhydrazone formation. Application of this technique to the preparation of 5α-cholestane-3α-d_1 (XIII) by reduction of 5α-cholestan-3-one tosylhydrazone (XII) with lithium aluminum deuteride has been already reported from this laboratory.[22] Similarly, sodium borodeuteride reduction of 5β-pregnan-3β-ol-20-one tosylhydrazone (VI) gave 5β-pregnan-3β-ol-20,20-d_2 (VII) which on oxidation, followed by reduction to remove the resulting 3-keto function (VIII), provided 5β-pregnane-20,20-d_2 (IX). 5α-Pregnane-11,11-d_2 (XXIX) was obtained in high isotopic purity (91% d_2) by hydride reduction of the tosylhydrazone derivative (XXVIII) of the previously reported[18] 5α-pregnan-12-one-11,11-d_2 (XXV). A similar reaction sequence with 5α-pregnan-20-one-17α,21,-21,21-d_4 (XXX) *via* its tosylhydrazone derivative (XXXI) gave 5α-pregnane (XXXII) labeled at the 17α and 21 positions. However, this reduction is very slow and due to extensive back exchange the isotopic purity of the product turned out to be very poor (see Table I).

(21) L. Caglioti and M. Magi, *Tetrahedron Lett.*, 1261 (1962); *Tetrahedron*, **19**, 1127 (1963); L. Caglioti and P. Grasselli, *Chem. Ind.* (London), 153 (1964).
(22) M. Fischer, Z. Pelah, D. H. Williams, and C. Djerassi, *Ber.*, **98**, 3236 (1965).



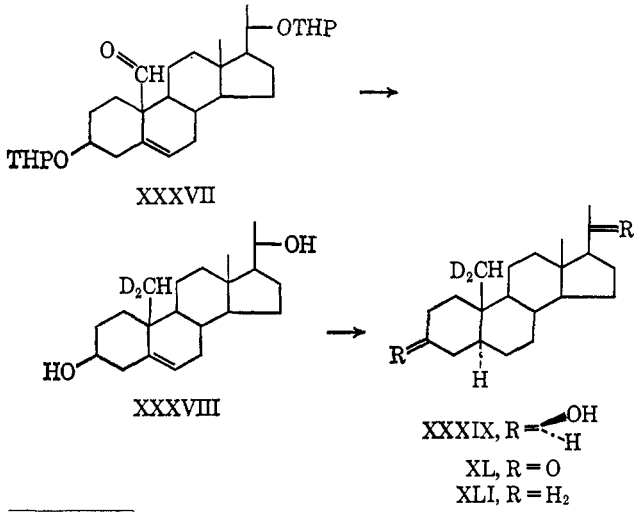
XXX XXXI XXXII

Deuterium labeling of the C-15 and C-16 positions in pregnane was achieved by the electrochemical reduction[23] of the appropriate ketone. Reduction of 5α-pregnan-16-one (XXXIII) in deuterium oxide–deuteriosulfuric acid gave 5α-pregnane-16,16-d_2 (XXXIV) with 87% d_2 isotopic purity. When the previously exchanged 15,15,17α-d_3 ketone XXXV was reduced in a protic medium, the resulting 5α-pregnane-15,15,17α-d_3 (XXXVI) was obtained with 92% d_3 isotopic purity.



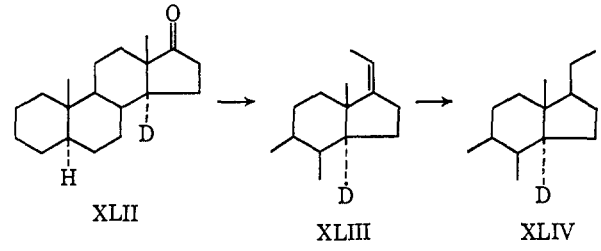
XXXIII XXXIV XXXV XXXVI

Electrochemical reduction[23] also provided a relatively easy means for labeling the C-19 angular methyl group, which otherwise would require a tedious sequence of reactions.[24] Reduction of the bistetrahydropyranyl ether XXXVII of pregn-5-ene-3β,20β-diol-19-al in a deuteric medium provided Δ⁵-pregnene-3β,20β-diol-19,19-d_2 (XXXVIII) in better than 85% yield and with 95% d_2 isotopic purity.[25] A conventional three-step transformation of this product by catalytic hydrogenation of the Δ⁵ double bond, Jones' oxidation and Huang-Minlon reduction of the labeled dione XL gave the desired 5α-pregnane-19,19-d_2 (XLI).



XXXVII XXXVIII XXXIX, R = OH, H XL, R = O XLI, R = H_2

Utilization of 5α-androstan-17-one-14α-d_1 (XLII), whose preparation has been reported recently from this laboratory,[26] was the most direct way for the preparation of 5α-pregnane-14α-d_1 (XLIV). This conversion was achieved in high yield by a Wittig reaction with ethyl triphenylphosphonium iodide followed by catalytic hydrogenation. The *cis* configuration of the olefin intermediate XLIII follows from the observation that when a part of it was hydroborated, 5α-pregnan-20α-ol was the sole product and no 20β epimer could be detected.[27]

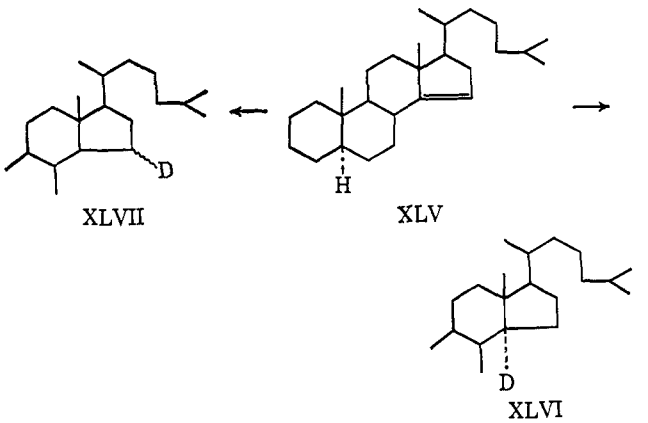


XLII XLIII XLIV

Attempts to label cholestane in the 14α and 15 positions by catalytic deuteration of Δ¹⁴-cholestene[28] (XLV) were unsuccessful. The Δ¹⁴ double bond was resistant to homogeneous catalytic reduction[29] and with heterogeneous catalysts the product exhibited very poor isotopic purity due to extensive isotope scrambling. Quite satisfactory results were obtained, however, by deuterioboration of Δ¹⁴-cholestene (XLV). The 5α-cholestane-14α-d_1 (XLVI) sample was prepared in high isotopic purity (90% d_1) by using deuteriodiborane, followed by hydrolytic cleavage of the alkylborane intermediate with propionic acid. Similarly, when XLV was treated with diborane and the hydrolysis was carried out with O-deuteriopropionic acid the resulting cholestane (XLVII) had a deuterium label on C-15. Identity (other than in terms of isotopic composition) of the labeled samples XLVI and XLVII with 5α-cholestane (X) confirmed the findings of Sondheimer, *et al.*,[30] that under these reaction conditions the newly formed asymmetric center has the 14α configuration.

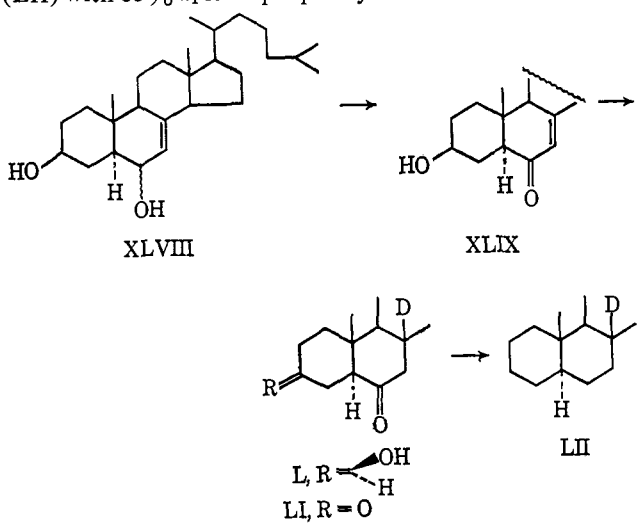
The preparation of cholestane-8-d_1 (LII) was accomplished by the following sequence. Selective oxidation of the allylic alcohol system of Δ⁷-5α-cholestene-3β,6-diol[31] (XLVIII) with manganese dioxide gave the α,β-unsaturated ketone XLIX. Deuteration of the

(23) L. Throop and L. Tökés, *J. Amer. Chem. Soc.*, **89**, 4789 (1967).
(24) C. Djerassi and M. A. Kielczewski, *Steroids*, 2, 125 (1963).
(25) We are grateful to Dr. D. Babbe of Syntex Laboratories, Inc., Palo Alto, Calif., for supplying us with this sample.
(26) G. Jones and C. Djerassi, *Steroids*, **10**, 653 (1967).
(27) B. Zeeh, G. Jones, and C. Djerassi, *Ber.*, **101**, 1018 (1968); hydroboration of the Δ¹⁷⁽²⁰⁾ double bond proceeds almost exclusively from the α face.
(28) J. C. Eck and E. W. Hollingsworth, *J. Amer. Chem. Soc.*, **63**, 2986 (1941).
(29) (a) J. F. Young, J. A. Osborn, F. H. Jardine, and G. Wilkinson, *Chem. Commun.*, 131 (1965); (b) W. Voelter and C. Djerassi, *Ber.*, **101**, 58 (1968), and references cited therein.
(30) M. Nussim, Y. Mazur, and F. Sondheimer, *J. Org. Chem.*, **29**, 1120 (1964).
(31) L. Caglioti, G. Cainelli, and G. Maina, *Tetrahedron*, **19**, 1057 (1963).



XLVII XLV XLVI

Δ^7 double bond with lithium in liquid ammonia-d_3,[19] followed by Jones' oxidation and back-exchange of the C-7 deuterium atom, were carried out in a manner analogous of that reported for the synthesis of the androstane analog.[32] Huang Minlon reduction of the resulting dione LI provided 5α-cholestane-8β-d_1 (LII) with 83% d_1 isotopic purity.

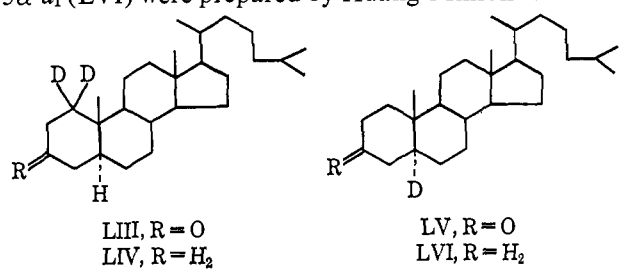


XLVIII XLIX

L, R = OH, H
LI, R = O

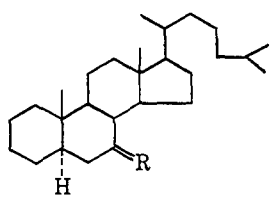
LII

For mechanistic interpretations three additional positions (1, 5, and 7) had to be tagged isotopically in the cholestane series. Cholestane-1,1-d_2 (LIV) and -5α-d_1 (LVI) were prepared by Huang-Minlon reduction



LIII, R = O
LIV, R = H_2

LV, R = O
LVI, R = H_2



LVII, R = O
LVIII, R = NNHTs
LIX, R = D_2

(32) R. H. Shapiro, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *J. Amer. Chem. Soc.*, **86**, 2837 (1964).

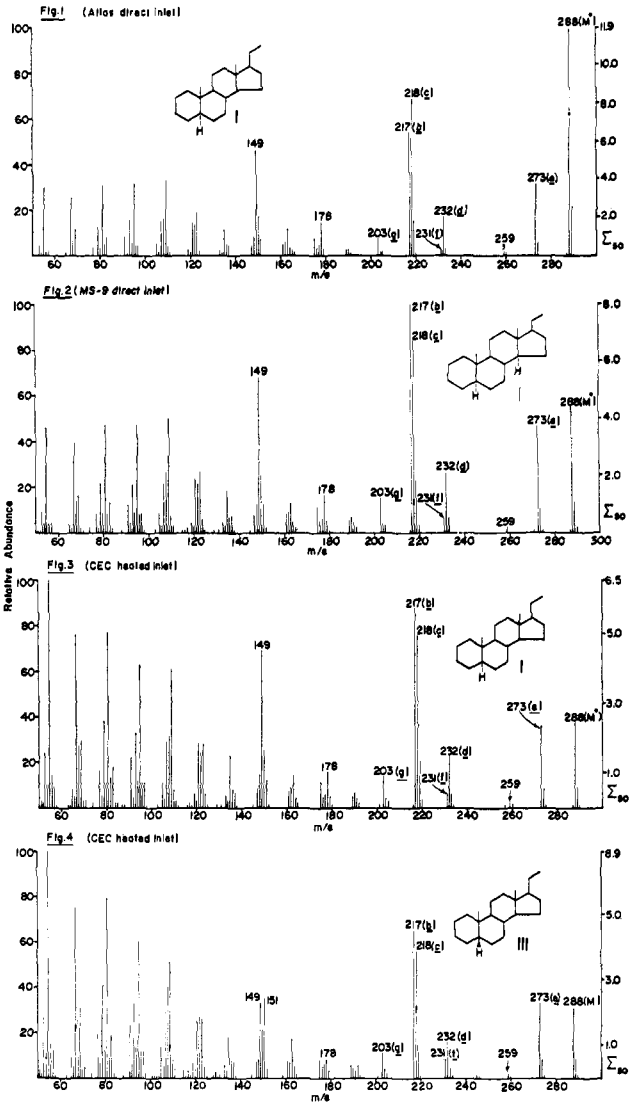


Figure 1. Mass spectrum of 5α-pregnane (I), measured with an Atlas CH-4 spectrometer at 70 eV.
Figure 2. Mass spectrum of 5α-pregnane (I), measured with AEI MS-9 spectrometer at 70 eV.
Figure 3. Mass spectrum of 5α-pregnane (I), measured with a CEC 21-103C spectrometer at 70 eV.
Figure 4. Mass spectrum of 5β-pregnane (III), measured with a CEC 21-103C spectrometer at 70 eV.

of the previously reported labeled 3-keto derivatives LIII[33] and LV.[29b] Introduction of two deuteriums at C-7 was achieved by lithium aluminum deuteride treatment[21,22] of the tosylhydrazone derivative of 5α-cholestan-7-one (LVIII). The olefinic side products were separated by chromatography on silver nitrate impregnated plates[16] yielding the pure 7,7-d_2 sample (LIX) with 85% isotopic purity.

Discussion of Mass Spectral Fragmentation Processes

The mass spectra of 5α- and 5β-pregnanes (I and III) and of 5α-cholestane (X) are shown in Figures 1–5. These spectra are subject to significant variations depending on the nature of the mass spectrometer as illustrated by the three different spectra (Figures 1–3) of 5α-pregnane. The spectra recorded on Models

(33) J. Karliner, H. Budzikiewicz, and C. Djerassi, *J. Org. Chem.*, **31**, 710 (1966).

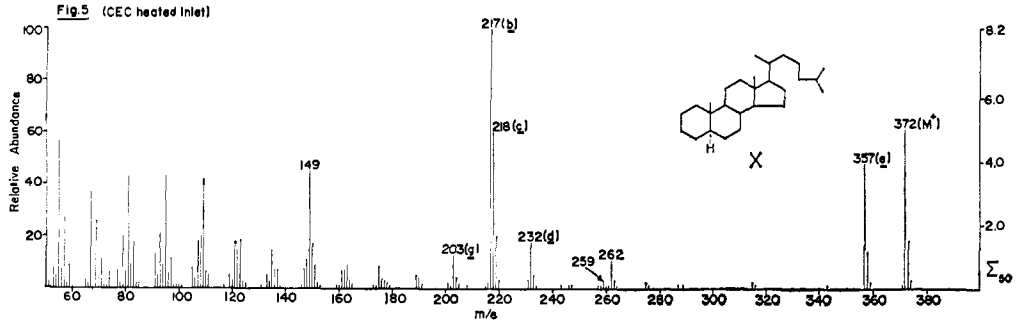


Figure 5. Mass spectrum of 5α-cholestane (X), measured with CEC 21-103C spectrometer at 70 eV.

AEI MS-9 and CEC 21-103C (Figures 2 and 3) are quite similar with the exception of the less abundant thermal "cracking" products (low mass range) in the former. The Atlas CH-4 spectrum (Figure 1), however, shows not only a reduced abundance of the low mass fragments but the relative intensity of the molecular ion

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

5α-Pregnanes	Isotopic purity, %	M+	M − CH_3	M − C_2H_5	M − C_4H_8	M − C_4H_9	M − C_5H_{10}	M − C_5H_{11}	M − C_6H_{13}	M − $C_{10}H_{19}$
d_0 (I)[b-d]	..	288	273	259	232	231	218	217	203	149
3,3-d_2 (XVII)[b]	d_0 2	290	275	261[f]	234 (90%)	231 (80%)	220	219	205 (>90%)	151 (90%)
	d_1 6				233 (10%)	233 (20%)				149 (10%)
	d_2 92									
8β-d_1 (XXII)[b]	d_0 3	289	274	260	233	232	219 (86%)	218 (94%)	204 (70%)	149 (80%)
	d_1 88						218 (14%)	217 (6%)	203 (30%)	150 (20%)
	d_2 9									
9α-d_1 (XXIII)[b,d]	d_0 3	289	274	260	233	232	219	218 (92%)	204 (88%)	150 (90%)
	d_1 96							217 (8%)	203 (12%)	149 (10%)
	d_2 1									
11,11-d_2 (XXIX)[b]	d_0 1	290	275	261[f]	234	233	220	219	205	149 (90%)
	d_1 5									151 (10%)
	d_2 91									
	d_3 3									
12,12-d_2 (XXVII)[b]	d_0 1	290	275	261	234	233	220 (97%)	219 (97%)	205	149 (90%)
	d_1 16						219 (3%)	218 (3%)		151 (10%)
	d_2 75									
	d_3 8									
14α-d_1 (XLIV)[c]	d_0 19	289	274	260[f]	233	232 (85%)	218	218 (51%)	204 (75%)	150 (65%)
	d_1 81					231 (15%)		217 (49%)	203 (25%)	149 (45%)
15,15,17α-d_3	d_1 1	291	276	262	234 (90%)	334 (>70%)	218	217 (76%)	203[f]	149 (90%)
(XXXVI)[c]	d_2 7				235 (10%)			219 (24%)		
	d_3 92									
16α-d_1 (XIX)[b]	d_0 19	289	274	260	232 (90%)	232 (75%)	218 (59%)	217	203 (75%)	149 (90%)
	d_1 77				233 (10%)	231 (25%)	219 (41%)		204 (25%)	150 (10%)
	d_2 2									
	d_3 2									
16,16-d_2 (XXXIV)[c]	d_1 9	290	275	261	232 (90%)	233 (80%)	219 (89%)	217	203 (60%)	149
	d_2 87				234 (10%)	231 (20%)	218 (11%)		204 (40%)	
	d_3 4									
17α,21,21,21-d_4[b]	d_0 12	292	277	260[f]	232[f]		218	217	203[f]	149[f]
(XXXII)	d_1 32									
	d_2 33									
	d_3 18									
	d_4 5									
18,18,18-d_3	d_2 2	291	276 (83%)	262	235	234	220 (82%)	220 (92%)	206 (63%)	149 (90%)
(XVIII)[b,c]	d_3 98		273 (17%)				221 (18%)	219 (6%)	205 (24%)	152 (10%)
								217 (2%)	203 (13%)	
19,19-d_2 (XLI)[c]	d_0 1	291	273 (80%)	261[f]	234 (97%)	233 (68%)	220	219 (81%)	205 (55%)	151 (90%)
	d_1 4		276 (20%)		233 (3%)	231 (24%)		217 (19%)	203 (45%)	149 (10%)
	d_2 95					232 (8%)				
20,20-d_2 (IX)[b,e]	d_0 5	290	275	259[f]	232 (~90%)	233 (85%)	218 (97%)	217 (97%)	203 (90%)	149
	d_1 20					231 (15%)	220 (3%)	219 (3%)	205 (10%)	
	d_2 65									
	d_3 8									
	d_4 2									

[a] The shift values are corrected for isotopic impurity as well as for ^{13}C contributions and are reliable to ±5% for all peaks except *m/e* 149 in which the uncertainty is ±10%. Blank spaces indicate that no unambiguous assignment could be made. The spectra were measured at 70 eV. [b] CEC 21-103C spectrum. [c] AEI MS-9 spectrum. [d] Atlas CH-4 spectrum with TO-4 ion source. [e] 5β isomer. [f] Mainly at indicated *m/e* value, but exact calculation was impossible because of isotopic contaminants or low intensity of peak.

Table II. Shifts[a] of Mass Spectral Peaks of Deuterated Analogs of 5α-Cholestane (X)

5α-Cholestanes	Isotopic purity, %	M+	M − CH_3	M − C_8H_{14}	M − C_8H_{17}	M − $C_{10}H_{20}$	M − $C_{11}H_{22}$	M − $C_{11}H_{23}$	M − $C_{12}H_{25}$	M − $C_{16}H_{31}$
d_0 (X)[b-d]	...	372	357	262	259	232	218	217	203	149
1,1-d_2 (LIV)[c,d]	d_0 6	374	359	262	...	234	220	219	205	151 (90%)
	d_1 17									149 (10%)
	d_2 77									
3α-d_1 (XIII)[d]	d_0 2	373	358	262	260	233	219	218	204	150 (90%)
	d_1 98									149 (10%)
5α-d_1 (LVI)[c]	d_0 9	373	358	262 (60%)	260	233	219	218	204 (85%)	149 (70%)
	d_1 89			263 (40%)					203 (15%)	150 (30%)
	d_2 2									
7,7-d_2 (LIX)[c,d]	d_0 1	374	359	264	261	234	220 (85%)	219 (95%)	205 (85%)	151 (90%)
	d_1 14						219 (15%)	218 (5%)	204 (15%)	149 (10%)
	d_2 85									
8β-d_1 (LII)[d]	d_0 15	373	358	263 (95%)	260	233	219 (91%)	218 (94%)	204 (78%)	149 (80%)
	d_1 83			262 (5%)			218 (9%)	217 (8%)	203 (22%)	150 (20%)
	d_2 2									
14α-d_1 (LII)[d]	d_0 10	373	358	263 (95%)	260	233	219 (90%)	217 (59%)	204 (75%)	150 (60%)
	d_1 90			262 (5%)			218 (10%)	218 (41%)	203 (25%)	149 (40%)
15ζ-d_1 (XLVII)[b,c]	d_0 39	373	358	263	260	233	218	217 (79%)	203	149 (90%)
	d_1 61							218 (21%)		150 (10%)

[a] See footnote *a* in Table I. [b] Atlas CH-4 spectrum with TO-4 ion source. [c] AEI MS-9 spectrum. [d] CEC 21-103C spectrum.

as well as the ratio of the *m/e* 217 and 218 peaks are changed remarkably. Similar differences between the CEC and Atlas spectra of 5α-cholestane have been reported by Spiteller.[34]

In all these spectra the most intense fragmentation products in the higher mass range are the ions of mass 217 and 218 which are due to the expulsion of the side chain together with a C_3H_6 and C_3H_5 moiety. The intensity of these cleavage products is influenced by the size of the side chain, ranging from 2.5% of the total ion current (sum of *m/e* 217 and 218) in androstane[35] (no side chain), 10.6% in pregnane (C_2 side chain, Figure 3), to 13.2% in cholestane (C_8 side chain, Figure 5).

The results from the extensive deuterium labeling experiments in both the pregnane and cholestane series are summarized in Tables I and II, respectively. Comparison of the shifts of the *m/e* 217 and 218 peaks in the spectra of the labeled pregnanes and cholestanes indicate that analogous bond cleavage processes are responsible for the genesis of these ions in both compounds. Furthermore, it became evident that more than one fragmentation path is involved in the formation of the ion of mass 217. However, the virtually complete retention of the d_3-labeled C-18 methyl function in both ions (Table I) clearly excludes major participation of the previously proposed[6-8a] cleavage patterns A and B (see introductory section). The extensive loss of the C-15 labels in the mass 217 ion (Tables I and II) and its complete loss in the mass 218 ion indicate that cleavage pattern C is the most important contributor, this being responsible for 76–79% of the mass 217 ion and for 100% of the mass 218 ion.

Formally, cleavage pattern C, that is, rupture of the 13–17 and 14–15 bonds, can yield the ion of mass 218 directly without any hydrogen transfer. The deuterium-labeling results, however, revealed that the actural bond fissions occur with a highly site-specific, reciprocal hydrogen migration from the 16 and 18 positions. This is shown (Table I) by the retention of more than 80% of a deuterium atom in the mass 218 ion when C-16 is labeled and by the equivalent loss of one deuterium atom in the 18-d_3-labeled sample. (The observed deuterium transfer values, associated with the formation of the *m/e* 217 and 218 ions, are summarized in Table III.) An attractive mechanism for this fragmentation is depicted by the following sequence a → a_1 → c.

Table III. Deuterium Transfers (%)[a] in Ring D Cleavage of Labeled 5α-Pregnanes and 5α-Cholestanes

Labeled positions	*m/e* 217		*m/e* 218	
	Pregnane	Cholestane	Pregnane	Cholestane
1	..	0	...	0
3	0	0	0	0
5	..	0	...	0
7	..	5	...	15
8	6	6	14	9
9	8	..	0	..
11	0	..	0	..
12	3	..	3	..
14	49	59	0	10
15	0	0	0	0
16	0	..	82–89	..
17, 21	0	..	0	..
18	6	..	82	..
19	0	..	0	..
20[b]	0	..	0	..

[a] Estimated total transfers from charge-retaining side (*m/e* 217) 77–87%, (*m/e* 218) 110–125%; to charge-retaining side (*m/e* 217) 0, (*m/e* 218) 82–89%. These values are uncorrected for isotope effects. [b] 5β-Pregnane.

Rupture of the highly substituted 13–17 bond releases the strain inherent to the *trans*-hydrindan system yielding molecular ion a. This ion appears to be of major significance in the fragmentation of side chain bearing steroids and the enhancement of the ring D fragmentation upon increasing the size of the side chain (*vide supra*) is in agreement with the participation of such an intermediate.

(34) M. Spiteller-Friedmann, S. Eggers, and G. Spiteller, *Monatsh.*, **95**, 1740 (1964).

(35) (a) H. Budzikiewicz and C. Djerassi, *J. Amer. Chem. Soc.*, **84**, 1430 (1962); (b) L. Tökés, Ph.D. Dissertation, Stanford University, 1965.

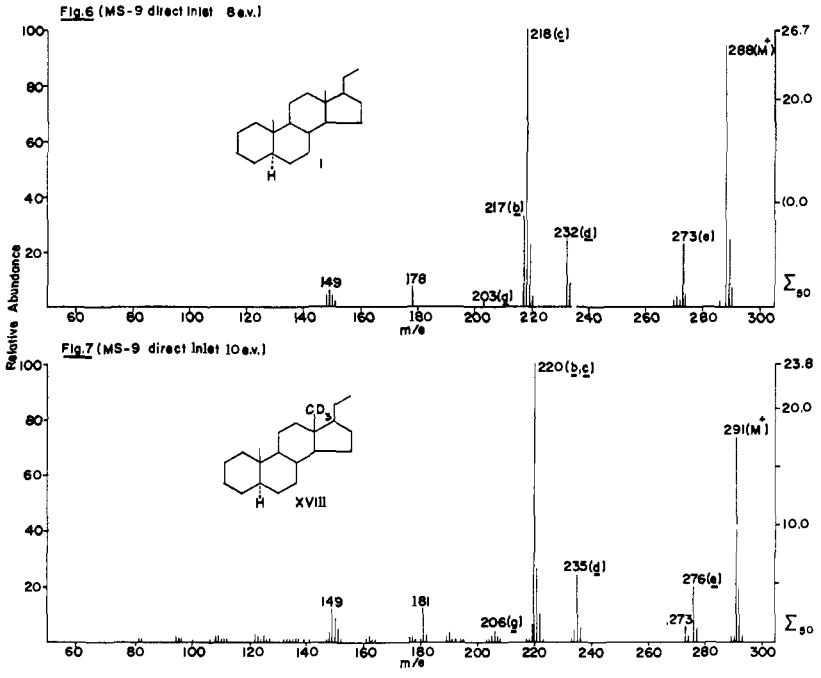
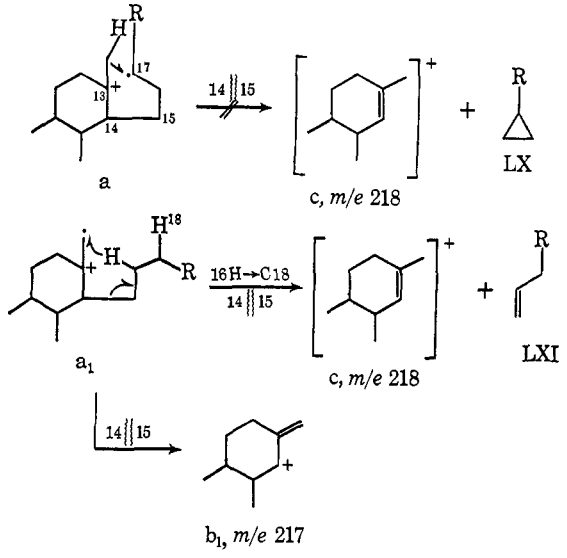


Figure 6. Mass spectrum of 5α-pregnane (I), measured with AEI MS-9 spectrometer at 8 eV.
Figure 7. Mass spectrum of *dl*-5α-pregnane-18,18,18-d_3 (XVIII), measured with AEI MS-9 spectrometer at 10 eV.

The transfer of a hydrogen atom from C-18, which is activated by the adjacent positive charge, relieves the radical site on C-17, yielding an ionized olefin a_1. Abstraction of a hydrogen atom from C-16 in a six-membered transition state to the terminal end of the ionized double bond in a_1 facilitates the cleavage of the 14–15 bond yielding an ionized (c) and a neutral olefin (LXI). Formation of the more stable neutral olefin LXI, instead of the cyclopropyl derivative LX in case of a direct cleavage (a → c), may provide the driving force for what appears to be a mechanistically more elaborate sequence *via* a_1. The nonstereospecific nature of the hydrogen transfer from C-16 (compare the 16α-d_1 and 16,16-d_2 samples in Table I) provides evidence for the participation of a ring D seco intermediate, such as ion a where the stereochemical significance of the 16α-hydrogen atom is lost, excluding the possibility of a multicentered, concerted mechanism prior to fission of the 13–17 bond.[36]



This fragmentation process is apparently an energetically very favorable one since at low electron voltage the *m/e* 218 peak is the most abundant fragment peak in the mass spectrum of pregnane (Figure 6). In the 18-d_3-labeled pregnane XVIII (Figure 7) the majority of this peak is shifted at 10 eV to *m/e* 220, indicating that at low ionizing potentials cleavage by a reciprocal hydrogen transfer is still energetically "cheaper" than the cleavage with a single hydrogen transfer, *i.e.*, *m/e* 217, or without any transfer.

Direct fission of the 14–15 bond in ion a_1, without hydrogen transfer, would yield an allylic carbonium ion b_1 (*m/e* 217). Two additional allylic carbonium ions, b_0 and b_2, can be obtained upon transferring a hydrogen atom from C-8 or C-12,[37] respectively. *A priori* these stabilized carbonium ions b_0, b_1, and b_2 are expected to be the primary candidates for the mass 217 fragment. Nevertheless, possible participation of additional products which would result by transferring a hydrogen atom from other positions which are also sterically accessible to the radical site has to be considered as well. Such potential transfer sites are the 7, 11β,[37] and 14α positions which can be approached by the radical site in molecular ion a to within 1.0, 0.5, and 1.8 Å, respectively.

The deuterium-labeling results (Table III) established that this hydrogen abstraction is a partially random process with one major transfer site, the 14α position which lost 49–59% deuterium label (65–75% of cleavage pattern C after correcting for presence of ion b_4). The

(36) An analogous cleavage mechanism with a reciprocal hydrogen transfer was found to be responsible for the loss of ring A in androstane (see ref 35b).

(37) Ring C has to be "flipped" into its more flexible boat conformation to facilitate the approach of this position by the C-17 radical site. The energy required for this conformational change is small compared to the energy content of the molecule.

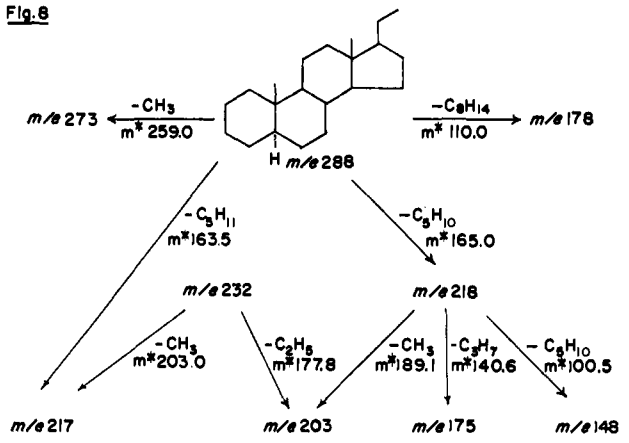


Figure 8. Transitions for which metastable peaks (m*) were observed in the mass spectrum of 5α-pregnane (I). These metastable peaks were shifted by the appropriate values in the spectra of the deuterium-labeled derivatives.

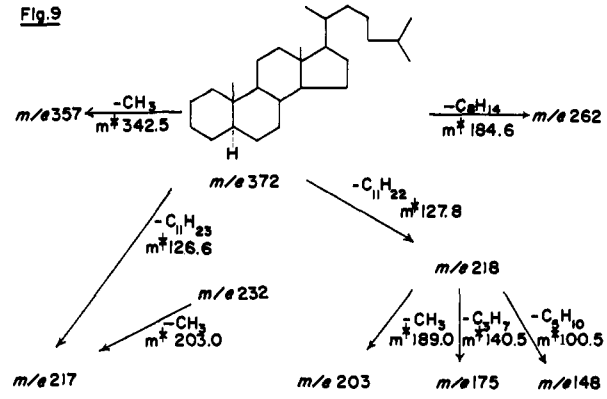
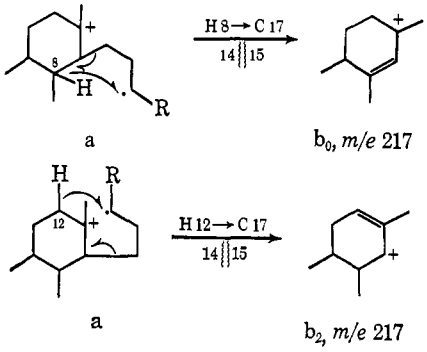


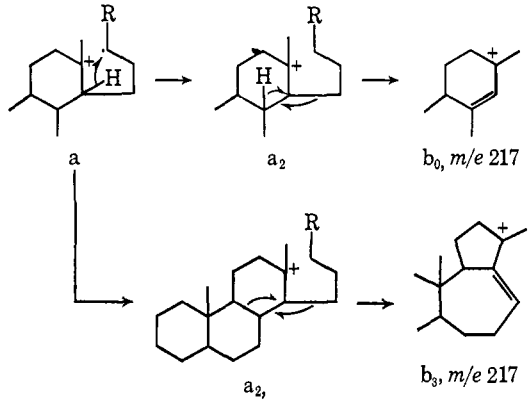
Figure 9. Transitions for which metastable peaks (m*) were observed in the mass spectrum of 5α-cholestane (X). These metastable peaks were shifted by the appropriate values in the spectra of the deuterium-labeled derivatives.

extensive transfer from the 14α position was initially not expected since formally it requires cleavage of two bonds attached to one carbon atom. Usually such an observation may be considered *prima facie* evidence for the participation of a rearrangement step and the following mechanistic explanation is therefore proposed for this cleavage.



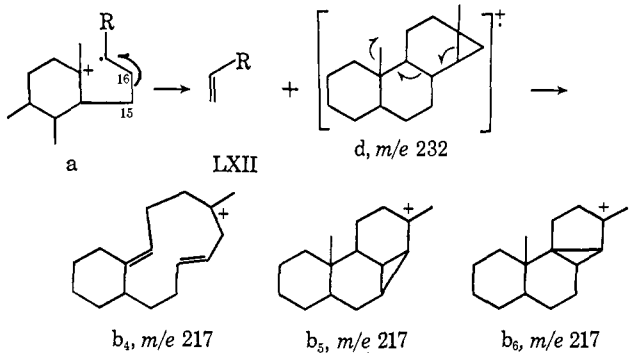
Migration of the activated 14α tertiary hydrogen atom in molecular ion a yields ion radical a_2 which contains the most highly substituted ionized double bond that can be generated in a steroid. In the absence of any energetically favorable cleavages that could lead directly to smaller fragments, a_2 may now undergo either a 1,2 hydrogen shift[38] from C-8, followed by cleavage of the 14–15 bond to give ion b_0, or suffer migration of the 8–9 bond, followed again by fission of the 14–15 bond to provide the allylic ion b_3. Little is known about the nature of this rearrangement although it has been established[39] from related studies in the bicyclic series that the presence of rings A and B is not essential for this step.

The estimated total transfer of 77–87% (Table III) from the charge-retaining side in the formation of the mass 217 ion is in good agreement with the observation that only 76–79% of this ion's origin is based on cleavage pattern C. The other 21–24% is due to the loss of a methyl group (see Figures 8 and 9) from a precursor of mass 232 and, therefore, is not accompanied by hydrogen transfer. The labeling results (Table I) clearly indicate that the C-19 angular methyl function is responsible for 19% out of the total of 21% of the expelled methyl radical. This value together



with the 2% loss of the C-18 methyl group is in excellent agreement with the observation (Table II) that 21% of the deuterium label is retained in 5α-cholestane-15-d_1 (XLVII).

The ion of mass 232, which itself is an energetically favored fragment (see Figure 6) of the steroid skeleton, owes its origin mainly (90% in pregnane and 100% in cholestane) to a partial ring D cleavage (see the appropriate shifts in Tables I and II). Homolysis of the activated 15–16 bond in molecular ion a yields a neutral olefin LXII and ion d (*m/e* 232), which is formally an ionized cyclopropane. Expulsion of the C-19 methyl group from ion d can be explained by



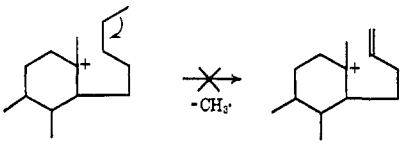
(38) 1,3 shifts of hydrogen are also conceivable (see C. Djerassi in "Advances in Mass Spectrometry," The Institute of Petroleum, London, 1968), but are not depicted for the sake of brevity.

(39) Unpublished observation from this laboratory.

opening of the cyclopropane ring and concomitant fission of the 8–9 and 10–19 bonds yielding ion b_4.

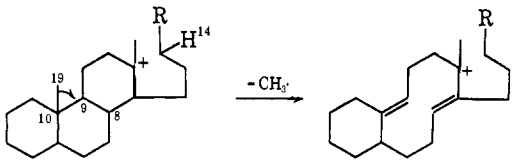
Ions b_5 and b_6, in which the cyclopropyl rings may participate in stabilizing the positive charge,[40] are possible structures for the fragmentation products arising by hydrogen abstraction (see Table III) from C-7 and C-9. The mechanism of the transfer from the 9α position (8%) is obscure since the C-17 radical site has no means of approaching that hydrogen atom in molecular ion a. It is possible, therefore, that this ion is formed by the expulsion of a hydrogen atom from an *m/e* 218 precursor. Actually, such a mechanism has been offered by Spiteller, *et al.*,[34] as a rationalization for the genesis of the mass 217 ion and they cited as support an observed metastable peak for the *m/e* 218 → *m/e* 217 transition in the spectrum of cholestane. Careful analysis of the mass spectra of a number of our labeled and unlabeled pregnane and cholestane samples which were measured on three different mass spectrometers (including the one used by Spiteller[34]) and with some samples employing a logarithmic transfer recorder[41] failed to reproduce the reported metastable peak at *m/e* 216. Instead, we obtained clearly discernible metastable peaks (see Figures 8 and 9) corresponding to the direct formation of both *m/e* 217 and 218 ions from the molecular ion and a smaller one for the transition *m/e* 232 → *m/e* 217. The assignment of these metastable peaks was further confirmed when they showed the appropriate shifts corresponding to the loss, transfer, or retention of the deuterium atoms in the spectra of the labeled samples. In view of these results and the labeling evidence indicating a high fraction of site-specific ejection of hydrogen, it is likely that hydrogen loss proceeds predominantly by a transfer mechanism.[42]

Examination of the mass spectra of the available deuterium labeled samples also provided information concerning the nature of other significant fragment ions. One of the most general features of steroidal spectra is the expulsion of a methyl radical from the molecular ion. In pregnane (Table I) it is now clearly established that the loss of one of the two angular methyl functions (rather than of a ring methylene group with associated hydrogen migration) is the exclusive source of the expelled methyl radical. Fission of the 20–21 bond in molecular ion a to yield olefin e_0 does not take place.



a e_0, *m/e* 273

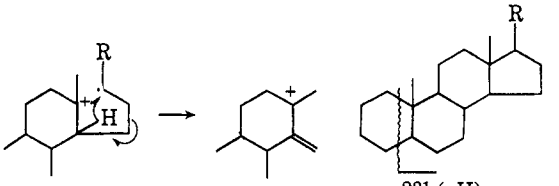
The reason for the enhanced loss of C-19 (80% of C-19 *vs.* 17% of C-18) may be due to various reasons, one of them may be the preferential cleavage of the highly strained 13–17 bond in this molecule, which prevents rupture of a second bond connected to the same C-13 quaternary center. Alternatively, activation of the 10–19 bond by cleavage of the 8–9 linkage in the previously discussed ion a_2 might give rise to e_1—a fragmentation mode analogous to the formation of ion g (*m/e* 203) discussed below.



a_2, e_1, *m/e* 273

The ejection of the side chain in the steroid skeleton is not very significant and is responsible for the small *m/e* 259 peaks in the spectrum of both pregnane and cholestane (Figures 3 and 5). The low intensity of this cleavage is not surprising in view of the strong preference for opening of ring D in these molecules and is completely repressed (see Figure 6) at low voltage.

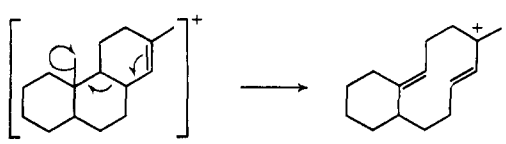
In the mass spectrum (Figures 1–3) of 5α-pregnane the previously discussed *m/e* 232 peak is accompanied by a less intense one at *m/e* 231. The latter reflects the occurrence of several different fragmentation products and according to the labeling results (Table I) about 20% of it is due to a partial ring D cleavage in conjunction with the transfer of a hydrogen atom from the charge-retaining side, mainly (three-fourths of this cleavage pattern) from C-14. A possible mechanism for this cleavage is depicted below by a → f.



a f, *m/e* 231 231 (-H) LXIII

The majority (70%) of this ion is due to a ring A fragmentation (see LXIII), probably analogous to those found in androstane,[35b] but other mechanisms must also be involved in order to explain the partial loss (24%) of C-19.

The small peak at *m/e* 203 (Figures 1–5) is associated with several ions of different origins but the majority of it is due to the loss of a methyl radical from an *m/e* 218 precursor (Figures 8 and 9). According to the labeling results (Table I) expulsion of the C-19 methyl group is responsible for about half of these ions and a possible mechanism for this transformation is illustrated by c → g. Metastable peak analysis (Figures 8 and 9) provided supporting evidence for this transition in both pregnane and cholestane, although it also indicated some loss of an ethyl group from the *m/e* 232 ion. The minor contributors to this peak are of too low intensity for precise analysis.



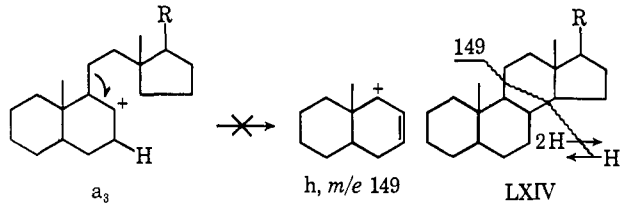
c, *m/e* 218 g, *m/e* 203

(40) F. McLafferty, "Mass Spectrometry of Organic Ions," Academic Press Inc., New York, N. Y., 1963, p 519. For examples in ground-state chemistry, see L. B. Jones and V. K. Jones, *Tetrahedron Lett.*, 1493 (1966), and references cited therein.

(41) R. T. Aplin, H. Budzikiewicz, H. S. Horn, and J. Lederberg, *Anal. Chem.*, 37, 776 (1965).

(42) Evidence is available in the literature that observation of a metastable peak cannot be used as an unequivocal proof for a one-step decomposition process; see, for example, J. Seibl, *Helv. Chim. Acta*, 50, 263 (1967).

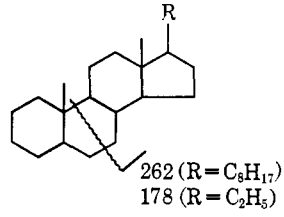
The intense *m/e* 149 peak is a general feature of steroidal hydrocarbon spectra and it has its own history in literature. It is due to the loss of rings C and D together with an extra hydrogen atom from the charge-retaining side. Biemann[11] suggested C-7 as the source of the transferred hydrogen atom as outlined by the following plausible mechanism (see $a_3 \rightarrow$ h).



a_3 h, *m/e* 149 LXIV

Our current labeling results (Tables I and II) confirm that about 90% of the mass 149 ion retained rings A and B, but the previously proposed[11] mechanism ($a_3 \rightarrow$ h) is excluded by the observation that both hydrogens attached to C-7 are retained (see Table II). The surprising loss of 70 and 80% of the deuterium label from C-5 (Table II) and C-8 (Tables I and II), and the retention of 60–65% of the 14α label in both pregnane and cholestane indicate that a very complex mechanism is operating in conjunction with a triple hydrogen transfer (see LXIV). Furthermore, it appears that the participation of the hydrogen transfer is affected by the configuration at C-5 since the partial splitting of the *m/e* 149 peak into *m/e* 151 is the only major difference between the spectra (Figures 3 and 4) of 5α- and 5β-pregnane.

In the mass spectrum of cholestane (Figure 5) there is a peak at *m/e* 262 which is not apparent in the spectrum of pregnane. The loss of ring A labels and complete retention of the C-7 and C-15 labels are indicative of a ring B fragmentation as shown on structure LXV.



LXV

Partial loss of the 8β (5%) and 14α (5%) labels and the retention of 60% of the 5α deuterium atom (Table II) show that part of this cleavage is associated with a reciprocal hydrogen transfer, C-5 being a major transfer site. This cleavage pattern is probably responsible for a part of the *m/e* 178 ion in the spectrum of pregnane (Figures 1–3) and metastable peaks were observed in the spectra of both compounds (see Figures 8 and 9) indicating the direct formation of these fragments from the molecular ion.

The other peaks in these spectra, including the *m/e* 175 and 148 ions cited in Figures 8 and 9, are due to a number of ions from different parts of the molecules making it extremely difficult and in many cases quite meaningless to analyze the deuterium-labeling results.

Experimental Section[43]

Modified Huang-Minlon Reductions. The following general procedure was used for samples ranging from 2 to 50 mg. A mixture of 2 ml of ethylene glycol, 1 ml of *n*-butyl alcohol, 0.5–1.0 ml of 95% hydrazine hydrate, and the ketone was heated under reflux for 1 hr. Upon cooling to about 100°, 150 mg of potassium hydroxide was added, and the heating was continued without a reflux condenser until the temperature reached 200°. After heating under reflux between 200 and 215° for an additional 4 hr, the reaction mixture was cooled and poured into water. Ether extraction, washing with water, and drying over anhydrous magnesium sulfate gave the crude hydrocarbon. The products were purified as follows (the position of the keto function in the starting material is indicated in parentheses).

A. The products were purified by thin layer chromatography (tlc) on 10% silver nitrate containing silica gel H,[16] followed by recrystallization from methanol: **5α-pregnane** (I, from 20-one[17] II) mp 84–86; **5α-cholestane** (X, from 3-one XI), mp 80.5–81.5°; **5α-pregnane-3,3-d_2** (XVII, from the 20-one[17] XIV), mp 84–85°; ***dl*-5α-pregnane-18,18,18-d_3** (XVIII, from 20-one[17] XV), mp 112.5–113.5°; **5α-cholestane-1,1-d_2** (LIV, from 3-one[33] LIII), mp 80.5–81.5°.

B. The products were purified by tlc on silica gel H followed by recrystallization from methanol: **5α-pregnane-16α-d_1** (XIX, from 20-one[17] XVI), mp 84–85°; **5α-pregnane-9α-d_1** (XXIII, from 12-one[18] XXI), mp 83–86°; **cholestane-5α-d_1** (LVI, from 3-one[29b] LV) mp 81–82°.

C. The products were purified by filtration through a small alumina column using hexane as eluent, followed by recrystallization from methanol: **5α-pregnane-8β-d_1** (XXII, from 12-one[18] XX), mp 84–86°.

5β-Pregnane (III). 5β-Pregnan-3β-ol-20-one[44] (IV, 200 mg) was oxidized with chromic acid in acetone solution and the resulting crude 3,20-dione (V) was reduced under modified Huang-Minlon conditions. Purification by procedure A, as described above, gave 109 mg (60%) of **5β-pregnane** (III), mp 83–84° (lit.[45] mp 82–83°).

5α-Pregnan-12-one Ethylene Thioketal (XXVI). Boron trifluoride etherate (0.15 ml) was added to a solution of 5α-pregnan-12-one[18] (XXIV, 23 mg) in 0.15 ml of ethane dithiol. After storing at room temperature for 10 min the reaction mixture was diluted with ether and washed thoroughly with dilute sodium hydroxide solution and then with water. Drying over anhydrous magnesium sulfate and evaporation of the solvent gave 29 mg (100%) of crude mercaptal (XXVI, mp 115–118°). Thin layer chromatography in a mixture of benzene and ether (9:1) followed by recrystallization from methanol provided the analytical sample, mp 119–121°, which lacked carbonyl absorption in the infrared spectrum.

Anal. Calcd for $C_{23}H_{38}S_2$: C, 72.95; H, 10.11. Found: C, 72.64; H, 10.25.

5α-Pregnane-12,12-d_2 (XXVII). Freshly prepared[19] deuterium-containing Raney nickel (from 750 mg of alloy) was added to a solution of 20 mg of crude 5α-pregnan-12-one ethylene thioketal (XXVI) in 3 ml of methanol-0-*d*. The resulting suspension was stirred and heated under reflux for 4 hr, the nickel was then removed by filtration, the solvent was evaporated and the residue was purified by tlc on 10% silver nitrate containing silica gel H in hexane. The olefin-free **5α-pregnane-12,12-d_2** (XXVII, 7 mg, 44%) was recrystallized from methanol, mp 83–86° (undepressed when mixed with unlabeled 5α-pregnane). The isotopic purity is shown in Table I.

5β-Pregnane-20,20-d_2 (IX). A solution of 5β-pregnan-3β-ol-20-one[44] (IV, 110 mg) and *p*-toluenesulfonylhydrazide (110 mg) in 5 ml of methanol, containing one drop of sulfuric acid, was heated under reflux for 2 hr, and then poured into water. The crystalline precipitate after filtration and drying at room temperature and

(43) Melting points (uncorrected) were determined on a Kofler block, and the infrared spectra were measured in chloroform solution, unless otherwise indicated, on a Perkin-Elmer Model 137 infrared spectrophotometer. The optical rotations were measured in chloroform by Mrs. D. Aguilar. A Varian A-60 spectrometer was used for the nmr spectra which were measured in deuteriochloroform solution with tetramethylsilane as internal reference. The mass spectra were measured by Messrs. J. W. Smith and R. G. Ross and by Dr. A. M. Duffield (for the choice of the mass spectrometers employed see Tables I and II). Thin layer chromatographies (tlc) were carried out on silica gel H unless otherwise indicated. The elemental analysis are due to Messrs. E. Meier and J. Consul.

(44) This sample was generously supplied by Syntex S.A., Mexico City.

(45) P. M. Jones and W. Klyne, *J. Chem. Soc.*, 871 (1960).

reduced pressure (0.1 mm) provided 163 mg (92%) of the tosylhydrazone derivative VI, mp 122–120°, ν_{max} 1600 (C=C), 1170 (S=O) cm^{-1} (no carbonyl absorption).

Sodium borodeuteride (150 mg) was added to a solution of the tosylhydrazone VI (150 mg) in 1.5 ml of deuteriomethanol. After heating under reflux for 15 hr, the resulting thick slurry was cooled and extracted with ether. The ether solution was washed and dried and the residue after the evaporation of the solvent was purified by tlc in benzene–ethyl acetate mixture (4:1) yielding 91 mg (96%) of **5β-pregnan-3β-ol-20,20-d_2** (VII), mp 143–144° (lit.[46] mp 141–144° for unlabeled material).

The labeled alcohol VII (45 mg) was oxidized with chromic acid in acetone solution and the crude ketone (VIII, 43 mg) was subjected to a modified Huang-Minlon reduction (see above). The product was purified by filtration through a small alumina column in hexane solution, yielding 27 mg (63%) of **5β-pregnane-20,20-d_2** (IX), mp 81–83°. (For isotopic purity, see Table I.)

5α-Pregnane-11,11-d_2 (XXIX). A solution of 5α-pregnan-12-one-11,11-d_2[18] (XXV, 20 mg) and *p*-toluenesulfonylhydrazide (30 mg) in deuteriomethanol (2 ml) containing one drop of acetyl chloride was heated overnight under reflux, then a few drops of deuterium oxide were added and the reaction mixture was cooled in an ice bath. The crystalline precipitate, after filtration and drying, provided 25 mg (81%) of the tosylhydrazone derivative XXVIII: mp 228–229°; ν_{max}^{KBr} 1640 (C=N), 1600 (C=C), and 1170 cm^{-1} (S=O) (no carbonyl absorption).

Lithium aluminum hydride (40 mg) was added to a solution of the crude tosylhydrazone XXVIII (25 mg) in dry dioxane (1 ml) and the resulting suspension was heated under reflux for 24 hr. The excess hydride was decomposed by the addition of a few drops of water. After cooling, the inorganic salts were removed by filtration, the solvent was evaporated under reduced pressure, and the residue was purified by tlc on 10% silver nitrate containing silica gel H in hexane. The olefin-free **5α-pregnane-11,11-d_2** (XXIX) amounted to 4 mg (26%), mp 82–85° (MeOH).

5α-Pregnane-17α,21,21,21-d_4 (XXXII).[47] A solution of 5α-pregnan-20-one (II, 200 mg) in 25 ml of deuteriomethanol (contaminated with trimethyl borate), containing 0.2 ml of 20% sodium deuterioxide in deuterium oxide, was heated overnight under reflux (sodium borate crystals precipitated and were removed by filtration) and the solvent was then evaporated under reduced pressure. The residue was redissolved in 25 ml of deuteriomethanol and the above procedure was repeated. The residue was dissolved in ether, washed with water, and dried over anhydrous magnesium sulfate. Evaporation of the solvent gave a mixture of the 17β and 17α epimers (200 mg) which were separated by tlc in benzene.

A solution of the 17β isomer XXX (36 mg) and *p*-toluenesulfonylhydrazide (36 mg) in 5 ml of deuteriomethanol, containing two drops of deuterium chloride–acetic acid-0-*d* solution in deuterium oxide, was heated under reflux for 3 hr, followed by removal of the solvent and acid catalysts under reduced pressure (0.1 mm). The residue was dissolved in a mixture of 3 ml of dry dioxane and 2 ml of deuteriomethanol. The volume was then reduced to about 1 ml by evaporating some of the solvent and ether was added. The resulting crystalline precipitate was collected by filtration and dried, yielding 44 mg of the crude tosylhydrazone derivative XXXI.

A solution of the tosylhydrazone XXXI (44 mg) and sodium borodeuteride (44 mg) in 5 ml of dry dioxane (freshly distilled from lithium aluminum hydride) was heated under reflux for 5 hr, followed by the addition of 44 mg of sodium borodeuteride. The heating was continued for 5 hr, whereupon the reaction mixture was diluted with ether. The ether phase was washed and dried and the residue after the evaporation of solvent was purified by tlc on 10% silver nitrate containing silica gel H in hexane, yielding 3 mg of **5α-pregnane-17α,21,21,21-d_4** (XXXII), mp 85–86°. (For isotopic composition see Table I.)

5α-Pregnane-16,16-d_2 (XXXIV). A solution of 5α-pregnan-16-one[48] (XXXIII, 15 mg) in dry dioxane (7 ml) and 10% deuteriosulfuric acid in deuterium oxide (3 ml) was placed into a cathode cell. The electrolysis[23] was carried out under a slow stream of dry nitrogen gas with a constant current of 136 mA until no more starting material could be detected by tlc spot test (8 hr). The reaction mixture was diluted with water and extracted with ether. The residue from the ether phase after washing, drying, and evaporation of the solvent was purified by tlc on 10% silver nitrate containing silica gel H in hexane. Recrystallization from methanol gave 6 mg (42%) of pure **5α-pregnane-16,16-d_2** (XXXIV), mp 82–83°. (For isotopic purity see Table I.)

5α-Pregnane-15,15,17α-d_3 (XXXVI). A sample of 5α-pregnan-16-one-15,15,17α-d_3[48] (XXXV, 25 mg) was reduced electrochemically in a protic solvent medium[23] as described above (see XXXIV). The product was purified by tlc on silica gel GF followed by recrystallization from methanol and yielded 8 mg (34%) of **5α-pregnane-15,15,17α-d_3** (XXXVI), mp 82–83.5°.

Pregn-5-ene-3β,20β-diol-19,19-d_2 (XXXVIII).[25] 3β,20β-Dihydroxypregn-5-en-17-al bistetrahydropyranyl ether[25] (XXXVII, 163 mg) was reduced electrolytically[23] in 49 ml of dry dioxane and 21 ml of 10% deuteriosulfuric acid in deuterium oxide. After 4.5 hr of electrolysis at 200 mA most of the dioxane was evaporated under reduced pressure and the residue extracted with ether. The crude product (101 mg, 97%) upon recrystallization from aqueous ethanol gave pure **pregn-5-ene-3β,20β-diol-19,19-d_2** (XXXVIII): mp 203–205° [lit.[49] mp (for the unlabeled analog) 201.5–203.5° (acetone)]; nmr, 0.78 (C-18, 3 H, s), 1.14 (C-21, 3 H, d), and 5.34 ppm (C-6, 1 H), the C-19 signal was not visible; mass spectra 320 (M^+, 92% d_2).

5α-Pregnane-19,19-d_2 (XLI). A solution of pregn-5-ene-3β,20β-diol-19,19-d_2 (XXXVIII, 28 mg) in glacial acetic acid (6 ml) was hydrogenated at atmospheric pressure and temperature with platinum oxide catalyst. The solution was then diluted with ether, washed with sodium bicarbonate solution and water, and was dried over anhydrous magnesium sulfate. The crystalline residue (XXXIX, 28 mg) was oxidized directly with chromic acid in acetone solution yielding 27 mg (97%) of crystalline 5α-pregnane-3,20-dione-19,19-d_2 (XL).

In a separate experiment the corresponding, unlabeled pregn-5-ene-3β,20β-diol under these conditions gave pure 5α-pregnane-3β,20β-diol, mp 194–196° (lit.[49] mp 194.5–195.5°), and 5α-pregnane-3,20-dione, mp 199–201° (lit.[50] mp 200–201°).

The labeled dione XL (24 mg) was reduced under modified Huang-Minlon conditions (see above) and the crude product (20 mg) was filtered through a short alumina column (1 g, activity I) using hexane as eluent. Recrystallization from methanol gave 12 mg (55%) of pure **5α-pregnane-19,19-d_2** (XLI), mp 83–84°.

5α-Pregn-*cis*-17(20)-ene-14α-d_1 (XLIII).[51] 5α-Androstan-17-one-14α-d_1[26] (XLII, 110 mg) in dry tetrahydrofuran (3 ml) was added to a solution of ethyl triphenylphosphonium iodide (3 g) in dry dimethyl sulfoxide (8 ml). The mixture was stirred at room temperature in an atmosphere of nitrogen and a solution of potassium *t*-butoxide (0.81 g) in dry dimethyl sulfoxide (5 ml) was added rapidly. After stirring at room temperature for 20 hr the mixture was heated to 45–55° for 1 hr, cooled, and poured onto ice. Extraction with hexane, washing, drying, evaporation of the hexane, and crystallization from methanol gave 79 mg (69%) of **5α-pregn-*cis*-17(20)-ene-14α-d_1** (XLIII): mp 87–90°; mass spectra 287 (M^+).

A sample of unlabeled 5α-androstan-17-one (500 mg) under identical conditions gave 485 mg (93%) of pure **5α-pregn-*cis*-17(20)-ene**: mp 91–92.5° (MeOH); $[\alpha]_D$ +18.8°; nmr 0.80 (C-19), 0.86 (C-18), and 5.15 ppm (C-20).

Anal. Calcd for $C_{21}H_{34}$: C, 88.04, H, 11.96. Found: C, 88.07, H, 12.06.

5α-Pregnane-14α-d_1 (XLIV). **5α-Pregn-*cis*-17(20)-ene-14α-d_1** (XLIII, 12 mg) in glacial acetic acid was hydrogenated at atmospheric pressure with 25 mg of platinum oxide catalyst. The solution was then diluted with water and extracted with hexane. Washing with sodium bicarbonate solution, drying and evaporation of the hexane gave 9 mg (75%) of **5α-pregnane-14α-d_1** (XLIV), mp 83–84° (MeOH).

5α-Cholestane-14α-d_1 (XLVI). To a solution of 5α-cholest-14-ene[28] (XLV, 100 mg) in dry tetrahydrofuran (15 ml), containing boron trifluoride etherate (0.5 g), a lithium aluminum deuteride (60 mg) solution in tetrahydrofuran (15 ml) was added dropwise over a period of 30 min. The reaction mixture was kept under an atmosphere of nitrogen, stirred and cooled in an ice–salt bath. After 1 hr the cooling bath was removed and the stirring was continued at room temperature for 2 hr. The cooling was resumed in

(46) J. H. Pierce, H. C. Richards, C. W. Shoppee, R. J. Stephenson, and G. H. R. Summers, *J. Chem. Soc.*, 694 (1955).

(47) The preparation of this compound was carried out by Dr. Hugo J. Monteiro of this laboratory.

(48) C. Beard, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *J. Amer. Chem. Soc.*, **86**, 269 (1964).

(49) W. Klyne and E. Miller, *J. Chem. Soc.*, 1972 (1950).

(50) D. H. R. Barton and J. D. Cox, *ibid.*, 783 (1948).

(51) For analogous Wittig reactions, see A. M. Krubiner and E. P. Oliveto, *J. Org. Chem.*, **31**, 24 (1966); G. Drefahl, K. Ponsold, and H. Schick, *Ber.*, **98**, 604 (1965).

a Dry Ice–acetone bath and freshly distilled propionic acid (0.5 ml) was added cautiously. After warming to room temperature, most of the tetrahydrofuran was evaporated under reduced pressure. Propionic acid (10 ml) was added, the reaction mixture was heated under reflux for 17 hr and then poured onto ice. The residue from extraction with ether, washing with sodium bicarbonate solution, drying, and evaporation of the ether was chromatographed on alumina (activity 1). The crude product (68 mg) upon elution with petroleum ether was further purified by tlc on 10% silver nitrate containing silica gel H in hexane, yielding 49 mg (49%) of olefin free **5α-cholestane-14α-d_1** (XLVI), mp 74–76°. (For isotopic purity see Table II).

Cholestane-15ζ-d_1 (XLVII). 5α-Cholest-14-ene[28] (XLV, 95 mg) was hydroborated and then hydrolyzed in the same manner as described above (see XLVI) except that lithium aluminum hydride and propionic acid-0-*d* were used in place of lithium aluminum deuteride and propionic acid. Analogous work-up and purification gave 23 mg (24%) of olefin-free **5α-cholestane-15α-d_1** (XLVII), mp 79–80.5° (MeOH).

5α-Cholest-7-en-3β-ol-6-one (XLIX).[52] 5α-Cholest-7-ene-3β,6α-diol[31] (XLVIII, 1 g) in chloroform (100 ml) was treated with manganese dioxide (14 g). After stirring at room temperature for 22 hr the excess reagent was removed by filtration and the chloroform was evaporated. The residue was chromatographed on an alumina column (activity 2). Elution with chloroform gave some 3,6-dione, followed by the desired **5α-cholest-7-en-3β-ol-6-one** (XLIX): yield 400 mg (40%); mp 196–197° (petroleum ether–$CHCl_3$); λ_{max} 246.5 mμ (log ε 4.10); ν_{max} 3570 (OH), 1650, and 1605 cm^{-1} (CO–C=C); $[\alpha]D$ −3.63° (*c* 1.1).

Anal. Calcd for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07. Found: C, 80.99; H, 10.96.

5α-Cholestane-3,6-dione-8β-d_1 (LI).[53] Approximately 5 ml of deuterioammonia was generated by the addition of 14 ml of deuterium oxide to a stirred suspension of magnesium nitride (20 g) in mineral oil (30 ml). The deuterioammonia was collected directly in the reaction flask (equipped with a Dry Ice trap) at −79° after passing through a trap at 0°. While keeping it at Dry Ice–isopropyl alcohol bath temperature, lithium wire (10 mg) was added, followed by a solution of 5α-cholest-7-en-3β-ol-6-one (XLIX, 50 mg) in 4 ml of anhydrous tetrahydrofuran. The reaction mixture was stirred for 20 min, the bath was then removed and the ammonia was allowed to boil under reflux for 40 min. A saturated solution of ammonium chloride in tetrahydrofuran was then added until the deep blue color disappeared and the ammonia was allowed to evaporate. Ether was added and the organic layer was washed with dilute hydrochloric acid and sodium bicarbonate solutions and finally with water. Drying and evaporation of the solvent gave a semicrystalline residue (47 mg) which was dissolved in acetone and oxidized with chromic acid. After the usual work-up, the product was dissolved in methanol (20 ml) containing 0.2 g of sodium hydroxide and heated under reflux for 15 min to exchange any deuterium introduced at C-7. According to tlc spot test, the product was contaminated with some hydroxy ketone and, therefore, the oxidation step was repeated. The resulting product was purified by tlc in methylene chloride giving 45 mg (90%) of diketone. Recrystallization from methanol gave 13 mg of **5α-cholestane-3,6-dione-8β-d_1** (LI): mp 160–164° (lit.[21] mp 169–171°); ν_{max} 1700 cm^{-1} (C=O).

5α-Cholestane-8β-d_1 (LII).[53] The 5α-cholestane-3,6-dione-8β-d_1 (LI, 40 mg) was reduced under modified Huang-Minlon conditions (see above) and purified by filtration through a small alumina column (0.5 g, activity 2) using petroleum ether as eluent. The crude product (16 mg, 44%) upon recrystallization from methanol–ether gave **5α-cholestane-8β-d_1** (LII, mp 72–75°).

5α-Cholestane-7,7-d_2 (LIX).[54] A solution of 5α-cholestan-7-one (LVII, 0.5 g) and *p*-toluenesulfonyl hydrazide (0.5 g) in methanol (100 ml) containing five drops of hydrochloric acid was heated under reflux for 1 hr. The hydrazone began to precipitate immediately. After cooling, dilute hydrochloric acid was added and the suspension was extracted with ether. Washing, drying, and evaporation of the ether gave 0.65 g (91%) of the tosylhydrazone derivative LVIII, mp 212–212.5° dec.

Anal. Calcd for $C_{34}H_{54}N_2O_2S$: N, 5.05; S, 5.72. Found: N, 5.37; S, 5.91.

The tosylhydrazone LVIII (100 mg) was dissolved in deuteriomethanol (10 ml) and sodium borodeuteride (100 mg) was added. After heating under reflux for 4 hr, the reaction mixture was cooled, dilute hydrochloric acid was added, and the product was extracted with ether. Washing, drying, and evaporation of the solvent gave 59 mg (88%) crude product which was contaminated with some olefin. Purification by tlc on 10% silver nitrate containing silica gel H in hexane, followed by recrystallization from methanol, gave pure **5α-cholestane-7,7-d_2** (LIX), mp 80–81°. (For isotopic composition see Table II.)

(52) This experiment was performed by Dr. R. H. Shapiro of this laboratory.

(53) This reaction was carried out by Dr. Erich Blossey in this laboratory.

(54) This reaction was carried out by Dr. Zvi Pelah in this laboratory

Electron Spin Resonance Spectra of Sulfone-Containing Aromatic Anion Radicals. Inductive Effects of the Sulfone Residue

M. M. Urberg and Carl Tenpas

Contribution from the Department of Chemistry, Hope College, Holland, Michigan 49423. *Received April 25, 1968*

Abstract: The effect of the sulfone dipole on esr spectra of aromatic anion radicals containing a sulfone residue is examined. This effect is approximated by increasing the electronegativity of the α-carbon atom of the aromatic ring, *i.e.*, setting $\alpha = \alpha_C + K\beta_{CC}$. For $K = 1.05$, modified Hückel calculations predict spin distributions in good agreement with esr results for diphenyl sulfone, dibenzothiophene sulfone, and thianthrene tetroxide anion radicals without postulating "d orbital" conjugation.

In the last few years several papers have appeared discussing d orbital effects on the esr spectra of sulfone-containing aromatic anion radicals.[1-3] Using the basic d orbital sulfone model proposed by Moffitt[4] and Koch and Moffitt,[5] Vincow[1] was able to calculate a theoretical spin density distribution for thioxanthone sulfone anion radical (I) which agreed well with experi-

(1) G. Vincow, *J. Chem. Phys.*, 37, 2484 (1962).
(2) E. T. Kaiser, M. M. Urberg, and D. H. Eargle, Jr., *J. Am. Chem. Soc.*, 88, 1037 (1966).
(3) R. Gerdil and E. A. C. Lucken, *Mol. Phys.*, 9, 529 (1965).
(4) W. E. Moffitt, *Proc. Roy. Soc.* (London), A200, 409 (1950).
(5) H. P. Koch and W. E. Moffitt, *Tran. Faraday Soc.*, 47 (1951).