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

Mass Spectrometry in Structural and Stereochemical Problems. XXXV.[1] Mass Spectrometric Fragmentation and Hydrogen Transfer Reactions in 16-Keto Steroids. A Novel Synthesis of Side Chain Labeled Cholestanes[2]

BY C. BEARD, J. M. WILSON, H. BUDZIKIEWICZ, AND CARL DJERASSI

RECEIVED JUNE 20, 1963

16α-Hydroxy-$\Delta^{17(20)}$-pregnenes, readily available from Δ^{16}-20-keto steroids, are oxidized to the corresponding α,β-unsaturated ketones and then subjected to 1,4-addition of suitable Grignard reagents. Both C-20 isomeric 16-ketocholestanes can thus be obtained as well as analogs selectively deuterated in positions 22, 23, 24, 25, 26, and 27. Variations in the Grignard reagent lead to differently substituted 17-alkyl-5α-androstan-16-ones. Appropriate deuteration of 16-keto-$\Delta^{17(20)}$-pregnenes prior to Grignard addition affords 16-ketocholestanes labeled at C-21, while C-17 and C-20 labels can be introduced through 17α-bromo and $\Delta^{17(20)}$-unsaturated analogs of 16-ketocholestane. In this manner, a large number of specifically labeled 16-keto steroids have become available for mass spectrometric investigation, and considerable insight has been gained into the nature of the fragmentation and accompanying hydrogen transfer reactions, which occur in the region of the carbonyl function. Particularly noteworthy is the double hydrogen transfer between C-17 and the side chain, the details of which were completely elucidated by deuterium labeling. Certain mass spectrometric features could be correlated with stereochemical features. Thus the generally difficult assignment of stereochemistry at C-20 in cholestanes can be accomplished mass spectrometrically in the 16-keto series by observing the presence (20β) or absence (20α) of an *m/e* 232 peak.

Introduction

The object of the present work is to investigate in further detail the mass spectra of a single class of ketones—the 17β-alkyl-5α-androstan-16-ones (*e.g.*, VII, XIII, XVIII–XXIII). This forms part of a general program[3] to study closely some of the individual steroid ketone types, which have been examined briefly[4] without the use of labeled substrates. We have already indicated in the first paper[4] of this series that a full understanding of the important fragmentation processes requires the use of such labeled substrates, and our recent studies with deuterium-labeled α-[5a] and β-[5b] decalones as well as 11-keto[6] and unsaturated 3-keto[7] steroids represent cases in point. Eventually, this knowledge will lead to the possibility of accurate predictions of the fission paths likely to be taken by a given molecule upon electron bombardment.

The simplest member of the series presently under discussion, namely 5α-androstan-16-one (VIII), as well as the largest one, cholestan-16-one (XXIIIb), have already been examined[4] mass spectrometrically. Selected ketones with side chains of intermediate size were required to investigate the effect of structural changes on the fragmentation pattern, together with suitable deuterium labels for close elucidation of the fission processes and the detection of hydrogen transfers. As shown below, those 5α-androstan-16-ones with C-17 alkyl substituents in the range of 3–8 carbon atoms are of greater interest from the point of view of detailed mass spectrometry than the 17β-methyl (VII) or ethyl (XIII) analogs. The latter do, however, contribute to our over-all conception of bond ruptures among this group of 16-keto steroids.

Initially, our synthetic efforts concentrated on obtaining compounds with (*e.g.*, XIII) and without (*e.g.*, VII) a hydrogen (or deuterium) atom in the γ-position of the carbonyl group, because such a relationship is known[8] to favor hydrogen transfer through a six-membered intermediate. The mass spectra of these two previously undescribed 17β-alkyl-5α-androstan-16-ones when contrasted with those[4] of the unsubstituted parent ketone VIII or of cholestan-16-one (XXIIIa) showed that a more detailed study was in order, and consequently a synthetic program was undertaken to develop a route to selective labeling of the cholestane side chain and to the introduction of side chains of different lengths. The results, described below, are not only necessary as a preamble to the discussion of the mass spectrometric data, but they are of intrinsic interest. Thus, only slight modifications in the general synthetic scheme using radioactive Grignard reagents would lead to a variety of side chain labeled cholestane derivatives of interest for biochemical experimentation.

Synthesis of 17β-Alkyl-16-keto Androstanes and Labeled Analogs.—17β-Methyl-5α-androstan-16-one (VII) was prepared by a route unrelated to that developed subsequently for the higher alkyl substituted androstanes. Conversion of androstan-17-one (I)[9] to the 17α-methyl-17β-hydroxy derivative II with methylmagnesium iodide and acetylation provided 17α-methyl-5α-androstan-17β-ol acetate (III). Pyrolysis of the acetate[10] led to a mixture of exocyclic (IV) and endocyclic (V) olefins, the presence of which was indicated by gas phase chromatography and n.m.r. measurements. Hydration of the mixture by the Brown procedure[11] followed by chromatography provided the desired 17β-methyl-5α-androstan-16α-ol (VI), which was oxidized with the Jones reagent[12] to the required ketone VII.

For the higher, and especially branched, 17β-alkyl substituted 16-keto steroids, an alternate route had to be developed and the following proved to be of wide applicability, utilizing in the first step the hydrazine reduction[13] of α-oxido ketones. Indeed, Huang-Minlon[14] has already reported the hydrazine reduction of

(1) Paper XXXIV: Z. Pelah, J. M. Wilson, M. Ohashi, H. Budzikiewicz, and C. Djerassi, *Tetrahedron*, in press.

(2) Supported by Grants No. A-4257 and CRTY-5061 from the National Institutes of Health of the U. S. Public Health Service.

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

(4) H. Budzikiewicz and C. Djerassi, *J. Am. Chem. Soc.*, **84**, 1430 (1962).

(5) (a) E. Lund, H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *ibid.*, **85**, 941 (1963); (b) *ibid.*, **85**, 1528 (1963).

(6) D. H. Williams, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *ibid.*, **85**, 2091 (1963).

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

(8) F. W. McLafferty, *Anal. Chem.*, **31**, 82 (1959). For further comments, see also F. W. McLafferty, Ed., "Mass Spectrometry of Organic Ions," Academic Press, Inc., New York, N. Y., 1963, Chapter 7.

(9) We are greatly indebted to Dr. Albert Bowers, Syntex, S.A., Mexico City, for the large supply of steroidal starting materials required in this work.

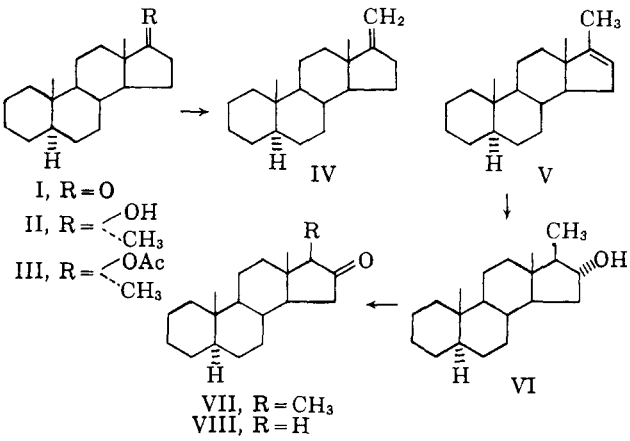
(10) Direct dehydration of the alcohol II with phosphorus oxychloride in pyridine gave a mixture which contained a substantial amount of rearranged product (18-nor-Δ^{13}-17,17-dimethyl-5α-androstene). For pertinent references to such Wagner–Meerwein rearrangements see V. Tortorella, G. Lucente, and A. Romeo, *Ann. chim.* (Rome), **50**, 1198 (1960), and R. Kirdani, R. I. Dorfman, and W. R. Nes, *Steroids*, **1**, 219 (1963).

(11) H. C. Brown "Hydroboration," W. A. Benjamin, Inc., New York, N. Y., 1962; see also S. Wolfe, M. Nussim, Y. Mazur, and F. Sondheimer, *J. Org. Chem.*, **24**, 1034 (1959).

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

(13) P. S. Wharton and D. H. Bohlen, *J. Org. Chem.*, **26**, 3615 (1961); P. S. Wharton, *ibid.*, **26**, 4781 (1961); see also C. Djerassi, D. H. Williams, and B. Berkoz, *ibid.*, **27**, 2205 (1962).

(14) Huang-Minlon and Chung-Tungshun, *Tetrahedron Letters*, 666 (1961).

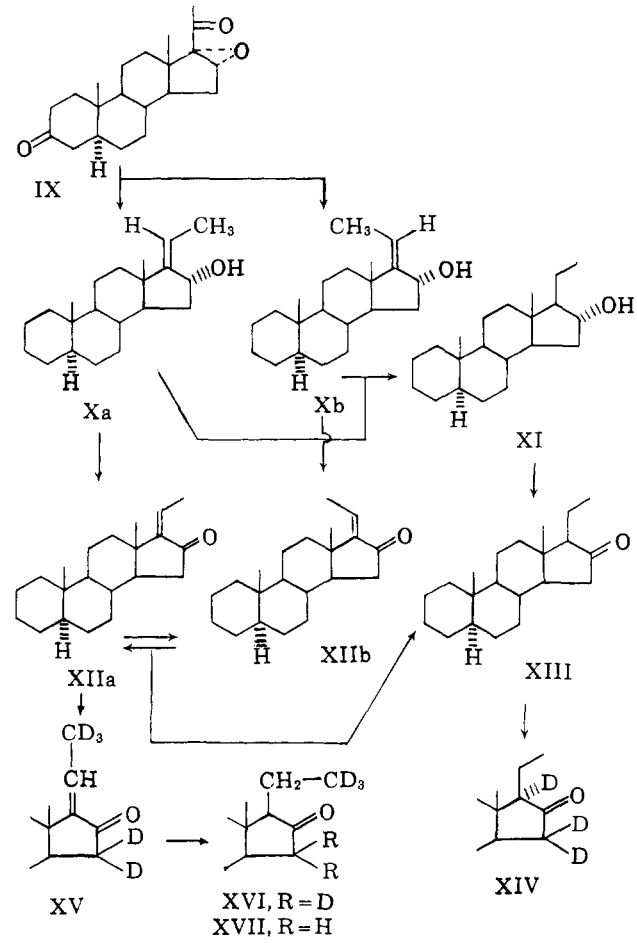


the 16α,17α-oxide of 16-dehydropregnenolone to $\Delta^{5,17(20)}$-pregnadiene-3β,16α-diol and its further oxidation with manganese dioxide to the α,β-unsaturated $\Delta^{17(20)}$-16-ketone. Since for our purposes we did not wish any substituent in ring A, the readily available[9] 16α,17α-oxido-5α-pregnane-3,20-dione (IX)[15] was reduced by the standard Huang-Minlon–Wolff–Kishner reduction, the addition of alkali (not required in the simple hydrazine reduction[13] of oxido ketones) being necessary in this case as the ketone function at C-3 had to be reduced at the same time. The product was shown by chromatography to consist of a resolvable mixture of the two geometric isomers Xa and Xb of $\Delta^{17(20)}$-5α-pregnen-16α-ol, the stereochemical assignments, based on n.m.r. spectrometry, being consistent with the assumption that the isomer with the more hindered hydroxyl group (Xa) would be eluted first. Thus, when the olefinic proton at C-20 is situated *cis* to the hydroxyl group as in isomer Xb, its signal[16] (octet, due to splitting by C-16 and C-21 hydrogens) is found farther downfield (5.57 p.p.m.) than that (5.29 p.p.m.) of the easier eluted isomer Xa. Conversely, the three-proton signal (quartet) of the C-21 methyl hydrogen atoms is found farther downfield (1.77 p.p.m.) in isomer Xa than in Xb (1.73 p.p.m.), because the reverse steric relationship exists between these hydrogens and the hydroxyl function. The above reasoning assumes that the closer the hydroxyl group is to the proton in question, the more unshielded[17] this proton becomes. Further support for these stereochemical assignments comes from a consideration of the coupling constants of the spin–spin splitting of the C-16 hydrogen atom and either the olefinic or C-21 methyl protons. Such splitting is at a maximum when the protons concerned are *trans*[17] oriented. Thus we find for the olefinic proton J = 2 c.p.s. for Xa and 1.5 c.p.s. for Xb, while the coupling constants for the C-21 methyl protons are *ca.* 0.8 in Xb and less than 0.5 c.p.s. in Xa.

That the allylic alcohols Xa and Xb are indeed only geometric isomers is demonstrated by the fact that catalytic hydrogenation of either one afforded the same saturated alcohol, 5α-pregnan-16α-ol (XI), which could be oxidized to 5α-pregnan-16-one (XIII), one of the substrates required for mass spectrometry.

Manganese dioxide oxidation of each pure allylic alcohol Xa or Xb gave the corresponding α,β-unsaturated ketone XIIa or XIIb, which represented the key intermediates in all subsequent syntheses. These two geometric isomers could be interconverted by base or light catalysis,[18] thin layer chromatography indicating an approximately 1:1 composition of the equilibrium mixture. Catalytic hydrogenation of this mixture led to the pure 5α-pregnan-16-one (XIII), which had already been obtained by oxidation of the saturated alcohol XI.

The n.m.r. spectra[19] of the two isomeric α,β-unsaturated ketones completely confirmed the earlier stereochemical assignments performed on the allylic alcohol precursors. Thus the vinyl proton signal in XIIa occurs as a quartet at 5.68 p.p.m., whereas that of XIIb is found at 6.47 p.p.m. This greater deshielding of the vinyl proton in XIIb is consistent[17] with its closer proximity to the carbonyl group. Similarly, examination of the doublet due to the C-21 methyl hydrogen atoms shows that when the latter are *cis* to the 16-keto group, absorption occurs farther downfield, *viz.*, 2.07 p.p.m. in XIIa and 1.83 in XIIb.



Taking advantage of the intermediates described above, it was possible to label 5α-pregnan-16-one (XIII) both in the side chain and in ring D. Standard base-catalyzed exchange of the acidic α-protons at C-15 and C-17 of the ketone XIII by heating with a solution of sodium in heavy water–deuteriomethanol led to 15,15,-17-d_3-5α-pregnan-16-one (XIV). Exchange of the five enolizable hydrogens in the unsaturated $\Delta^{17(20)}$-5α-pregnen-16-one (XIIa) gave a mixture of geometrically isomeric 15,15,21,21,21-d_5-$\Delta^{17(20)}$-5α-pregnen-16-ones (XV), which upon catalytic hydrogenation[20] and base-

(15) A. Ercoli and P. de Ruggeri, *Gazz. chim. ital.*, **84**, 479 (1954).

(16) All n.m.r. signals are expressed as δ-values (c.p.s./60) relative to tetramethylsilane (δ = 0.0).

(17) See L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959, Chapters 6 and 7.

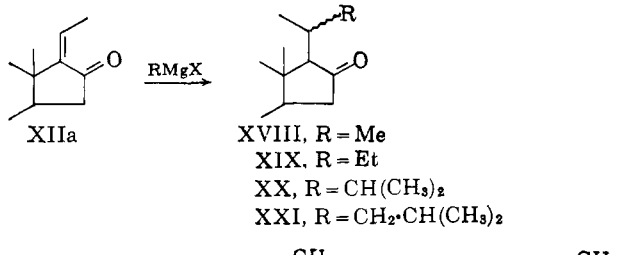
(18) See for instance G. Büchi and N. C. Yang, *Helv. Chim. Acta*, **38**, 1338 (1955). For further discussion see P. de Mayo in "Advances in Organic Chemistry," R. A. Raphael, E. C. Taylor, and H. Wynberg, Ed., Interscience Publishers, Inc., New York, N. Y., 1960, Vol. II, pp. 399–402.

(19) K. S. Brown, Jr., and S. M. Kupchan, *J. Am. Chem. Soc.*, **84**, 4590 (1962), have recently isolated a similar pair of isomeric $\Delta^{17(20)}$-16-keto steroids by degradation of the alkaloid cyclobuxine; their n.m.r. conclusions are in agreement with ours.

(20) Extensive studies in our laboratory (see for instance J. W. Chamber-

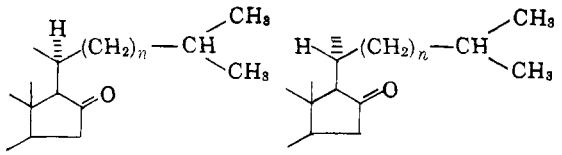
catalyzed back-exchange of the C-15 deuterium atoms of XVI afforded 21,21,21-d_3-5α-pregnan-16-one (XVII) of 92% isotopic purity.

1,4-Addition of Grignard reagents to $\Delta^{17(20)}$-5α-pregnen-16-one (XIIa)[21] in the presence of cuprous chloride usually proceeded in acceptable yield (55–80%) with methyl, ethyl, 3-methylbutyl, and 4-methylpentyl Girgnard reagents to afford the branched ketones XVIII, XIX, XXII, and XXIII. The use of isopropyl[22] and isobutyl Grignard reagents, however, resulted in only very poor yields (<10%) of the desired ketones XX and XXI, accompanied in each instance by approximately 15% of reduction product,[23] namely 5α-pregnan-16-one (XIII). All of these Grignard reactions, with the exception of the one involving methylmagnesium iodide, could give rise to two C-20 epimeric ketones. The presence of two such epimers, in approximately equal amounts,[21] could be demonstrated by thin layer chromatography and by partial separation through column chromatography in the reactions involving the 3-methylbutyl and 4-methylpentyl Grignard reagents. In the latter, the identity of the C-20 epimeric cholestan-16-ones (XXIIIa and XXIIIb) could be established by alternate syntheses (*vide infra*) and the configurations of the next lower homologs (XXIIa and XXIIb) could be related to them by the use of mass spectrometry and thin layer chromatographic R_f values. In the other three ketones, XIX, XX, XXI (R = ethyl, isopropyl, and isobutyl), it could not be established definitely whether the products obtained were mixtures of C-20 epimers or homogeneous.



XIIa

XVIII, R = Me
XIX, R = Et
XX, R = $CH(CH_3)_2$
XXI, R = CH_2·$CH(CH_3)_2$

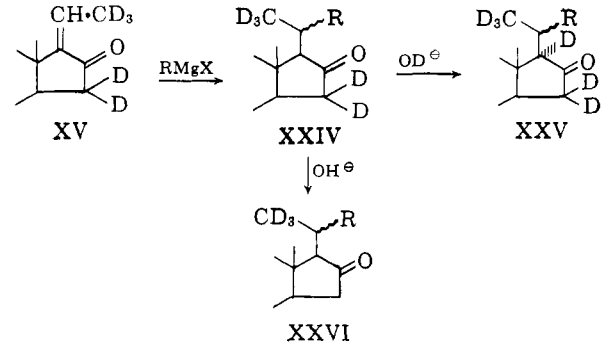


XXIIa, n = 2
XXIIIa, n = 3

XXIIb, n = 2
XXIIIb, n = 3

In any event, they gave only one spot on thin layer chromatography and showed reasonably constant melting points. The isolation of both isomers in the cholestane series (XXIII) suggests this method as a fruitful synthetic alternative to the difficultly accessible[24] 20-isocholestanes (XXIIIa).

By suitable modifications of the above-described 1,4-Grignard additions, it was possible to prepare substances labeled with deuterium in the side chain and in ring D. Thus addition of a Grignard reagent to the equilibrated d_5-unsaturated ketone XV provided the 15,15,21,21,21-d_5-saturated ketones XXIV,[25] which could either be transformed to the d_6 (XXV) or d_3 (XXVI) analogs, depending upon whether further base-catalyzed equilibration was performed with deuterated or nondeuterated solvents. Finally, by using Grignard reagents derived from deuterated alkyl halides, further labeling could be achieved and this was often combined with the above-described exchange-labeling. As an example, there might be cited the reaction of the d_5-unsaturated ketone XV with d_3-methylmagnesium iodide followed by treatment with hydroxide to produce 21,21,21,22,22,22-d_6-5α-20-methylpregnan-16-one (XXVI, R = CD_3).



XV XXIV XXV

XXVI

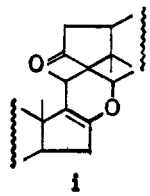
As noted below, the substances which have been studied most closely by mass spectrometry have been the 20α-(XXIIIa) and 20β-(XXIIIb) cholestan-16-ones and a detailed examination of the fission and transfer processes has entailed the preparation of analogs labeled with deuterium in all possible positions in the side chain. This in turn has required the synthesis of 1-bromo-4-methylpentanes labeled in all five different positions, which was accomplished by exposure of the various labeled 4-methylpentanols to the standard hydrobromic acid–sulfuric acid conditions. The introduction of two deuterium atoms each in the 1-, 2-, and 3-positions to provide the labeled alcohols XXVII, XXVIII, and XXIX was straightforward and followed established procedures[26] as outlined in the accompanying flowsheet.

In most cases, the homogeneity of the intermediates was checked by gas phase chromatography and, where applicable, also by mass spectrometry. The most laborious sequence was that involving the formation of the terminally labeled alcohols XXXIV and XXXV, which were needed for the ultimate introduction of deuterium in positions 25, 26, and 27 of the cholestane side chain. The starting material was the known[27] diethyl 3-benzyloxypropylmalonate (XXX, R = H) which was transformed into its sodio derivative (using sodium hydride in tetrahydrofuran) and the deuterated analog XXX (R = D) generated by addition of heavy water. Reduction with lithium aluminum hydride, conversion

lin, Ph.D. Thesis, Stanford University, 1963) have shown that very little isotope scrambling occurs during catalytic hydrogenation or deuteration of α,β-unsaturated ketones, in contrast to olefins or dienes.

(21) Usually this particular (more easily obtainable) geometric isomer was employed; nevertheless, where recognizable, a mixture of C-20 epimeric saturated ketones was still produced.

(22) In this instance, there was also encountered 24% of a dimeric product, the infrared spectrum ($\lambda_{max}^{CHCl_3}$ 5.78 μ) of which indicated the presence of a saturated carbonyl group, while its n.m.r. spectrum established the absence of olefinic protons. Since its molecular weight was shown to be 600, using the recently described mass spectrometric direct-inlet technique (J. F. Lynch, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *Experientia*, **19**, 211 (1963)), its structure may well be i.



i

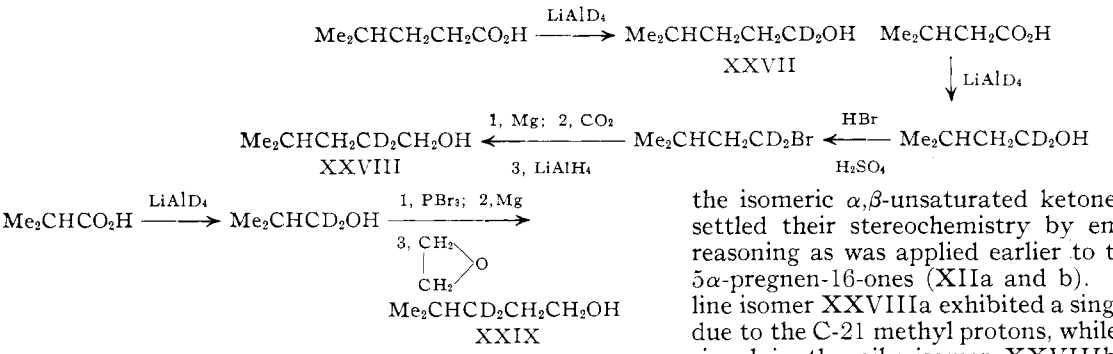
(23) See M. S. Kharasch and O. Reinmuth, "Grignard Reactions of Non-Metallic Substances," Prentice–Hall Inc., New York, N. Y., 1954, p. 197.

(24) G. V. Nair and E. Mosettig, *J. Org. Chem.*, **27**, 4659 (1962), and references cited therein.

(25) The deuterium in the exchangeable C-15 position is fairly stable as demonstrated by its virtually complete retention upon chromatography on activity II neutral alumina containing 3% of water.

(26) See for instance, M. J. Coon and S. Gurin, *J. Biol. Chem.*, **180**, 1159 (1949).

(27) L. C. Cheney and J. R. Piening, *J. Am. Chem. Soc.*, **67**, 2213 (1945).

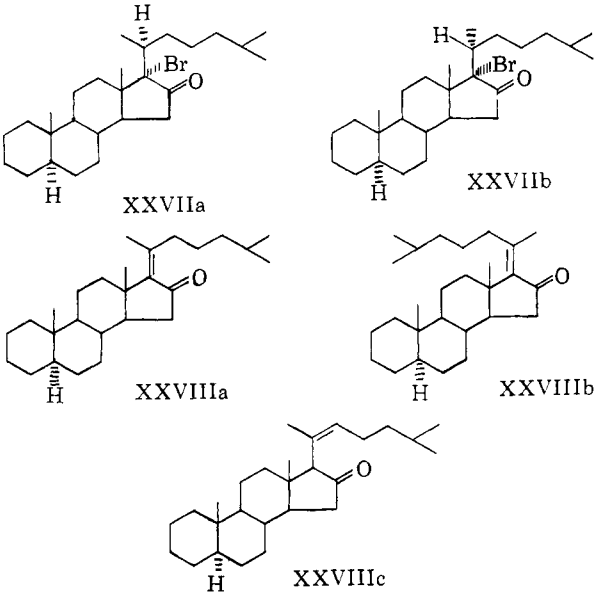


of the resulting diol XXXI (R = D) to the ditosylate XXXII (R = D), followed by a second reduction with lithium aluminum hydride and by lithium–ammonia cleavage of the benzyl ether protecting group, provided the 4-monodeuterio-4-methylpentanol (XXXIV). When the above reaction sequence was repeated with nondeuterated diethyl 3-benzyloxypropylmalonate (XXX, R = H), except that the cleavage of the ditosylate XXXII (R = H) was performed with lithium aluminum deuteride, the terminally labeled d_2-analog XXXV was obtained.

$C_6H_5CH_2O(CH_2)_3CR(CO_2Et)_2 \longrightarrow$
XXX, R = H or D
$C_6H_5CH_2O(CH_2)_3CR(CH_2OH)_2 \longrightarrow$
XXXI, R = H or D
$C_6H_5CH_2O(CH_2)_3CR(CH_2OTs)_2 \longrightarrow$
XXXII, R = H or D
$C_6H_5CH_2O(CH_2)_3CR(CH_2R')_2 \longrightarrow$
XXXIII, R = D, R′ = H or R = H, R′ = D
$\longrightarrow HO(CH_2)_3CR(CH_2R')_2$
XXXIV, R = D, R′ = H
XXXV, R = H, R′ = D

As mentioned earlier, alternative routes to pure 20α- and 20β-cholestan-16-ones (XXIIIa and b) were examined. When the mixture of C-20 epimeric cholestan-16-ones (XXIIIa and b) was brominated in acetic acid solution, the 17α-bromo-20β-cholestan-16-one (XXVIIb) crystallized preferentially from the reaction mixture, while the oily 20α-isomer XXVIIa was isolated by chromatography of the mother liquors. Dehydrobromination of the crystalline bromo ketone XXVIIb with calcium carbonate in dimethylacetamide solution[28] afforded a three-component mixture, from which the crystalline $\Delta^{17(20)}$-cholesten-16-one (XXVIIIa) could be isolated easily by chromatography. The other two products exhibited very close R_f values on thin layer chromatography and were not separated, although their constitution could be defined in terms of the geometric isomer XXVIIIb and the β,γ-unsaturated analog XXVIIIc.

The structure of the crystalline $\Delta^{17(20)}$-cholesten-16-one (XXVIIIa) was established by the following observations. Its ultraviolet and infrared spectra were characteristic of α,β-unsaturated ketones and its molecular weight was determined mass spectrometrically. Irradiation converted it into a mixture of α,β-unsaturated ketones (XXVIIIa and b), which could be separated easily to give the starting ketone XXVIIIa and an oily α,β-unsaturated ketone XXVIIIb, the latter representing another component of the original dehydrobromination mixture. Furthermore, heating of the pure unsaturated ketone XXVIIIa with methanolic aqueous alkali yielded exactly the same picture (three spots) on thin layer chromatography as the crude dehydrobromination product. The n.m.r. spectra of the isomeric α,β-unsaturated ketones XXVIIIa and b settled their stereochemistry by employing the same reasoning as was applied earlier to the isomeric $\Delta^{17(20)}$-5α-pregnen-16-ones (XIIa and b). Thus, the crystalline isomer XXVIIIa exhibited a singlet at 1.88 p.p.m.,[16] due to the C-21 methyl protons, while the corresponding signal in the oily isomer XXVIIIb occurred at 2.14 p.p.m. Both isomers showed infrared absorption bands at 5.90 and 6.21 μ, while their n.m.r. spectra demonstrated the absence of olefinic protons.



However, the infrared spectrum of the mixture of XXVIIIb and the third constituent of the dehydrobromination mixture contained an additional band at 5.79 μ, typical of a saturated ketone, and the n.m.r. spectrum exhibited signals at 1.61, 2.14, and 2.47 p.p.m. due to methyl protons as well as olefinic proton absorption at 5.12 p.p.m. (multiplet). Since the mass spectrometrically determined molecular weight of the mixture showed that both components possessed the molecular weight 384, it follows that the third product produced in the dehydrobromination is the nonconjugated isomer XXVIIIc. Its formation in the base treatment of pure $\Delta^{17(20)}$-cholesten-16-one (XXVIIIa) is mechanistically unexceptional.

Catalytic hydrogenation of the crystalline $\Delta^{17(20)}$-16-ketone XXVIIIa led to 20α-cholestan-16-one (XXIIIa), which was also obtained by zinc dust reduction in acetic acid solution of 17α-bromo-20α-cholestan-16-one (XXVIIa). Similarly, catalytic hydrogenation of the oily $\Delta^{17(20)}$-16-ketone XXVIIIb or zinc dust reduction of 17α-bromo-20β-cholestan-16-one (XXVIIb) provided 20β-cholestan-16-one (XXIIIb). Substitution of these reagents by catalytic deuteration or zinc–deuterioacetic acid made available analogs deuterated at C-17 and C-20. As noted in the Experimental section, lithium–ammonia reduction of $\Delta^{17(20)}$-cholesten-16-one (XXVIIIa) afforded a mixture of the two C-20 isomeric cholestan-16-ones (XXIIIa and b), with the 20β-isomer predominating as judged by thin layer chromatography.

The availability of a series of 17β-alkyl-5α-androstan-16-ones also afforded the opportunity of examining the

(28) G. F. H. Green and A. G. Long, *J. Chem. Soc.*, 2532 (1961).

TABLE I

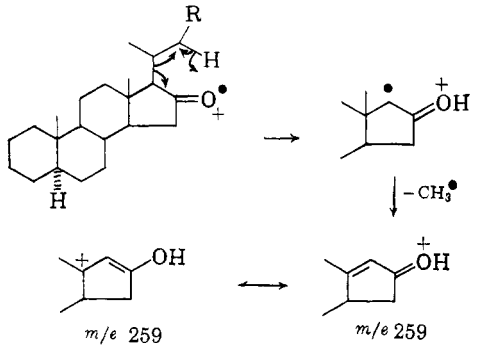
OPTICAL ROTATORY DISPERSION DATA (METHANOL SOLUTION) FOR 17β-ALKYL-5α-ANDROSTAN-16-ONES

Compound	Trough	Peak	Molecular amplitude
5α-Androstan-16-one (VIII)	$[\alpha]_{312.5}$ −4590°	$[\alpha]_{272.5}$ +4200°	−241
17β-Methyl-5α-androstan-16-one (VII)	$[\alpha]_{312}$ −4000°	$[\alpha]_{268}$ +4080°	−233
5α-Pregnan-16-one (XIII)	$[\alpha]_{314}$ −3740°	$[\alpha]_{270}$ +4160°	−239
20-Methyl-5α-pregnan-16-one (XVIII)	$[\alpha]_{314}$ −3600°	$[\alpha]_{268}$ +3770°	−233
20-Ethyl-5α-pregnan-16-one (XIX)	$[\alpha]_{319}$ −3960°	$[\alpha]_{272}$ +3600°	−250
20β-Cholestan-16-one (XXIIIb)	$[\alpha]_{321}$ −2940°	$[\alpha]_{273}$ +3130°	−234
20α-Cholestan-16-one (XXIIIa)	$[\alpha]_{321}$ −3070°	$[\alpha]_{276}$ +3220°	−243
17α-Bromo-20α-cholestan-16-one (XXVIIa)	$[\alpha]_{280}$ −785°	$[\alpha]_{338}$ +636°	+66

effect of a C-17 substituent upon the powerful negative Cotton effect associated[29] with a 16-keto function in a steroid or the corresponding bicyclic analog. *A priori*, the rotatory contribution of the free-rotating alkyl side chain may be appreciable but, as noted in Table I, the molecular amplitude values are roughly the same, thus demonstrating that the asymmetry of the cyclopentanone ring[30] plays the governing role. The sole exception is the last entry (XXVIIa) in Table I, where the expected[31] positive contribution of the 17α-bromine atom becomes dominant.

Discussion of Mass Spectral Fragmentation Processes

Introduction.—As mentioned in the beginning of this article, one of the principal reasons for examining the mass spectra of the present series of 16-keto steroids was to ascertain the validity of the six-membered cyclic transfer process[8] (arrows in XXIX) in 17-alkyl-16-keto androstanes. Such a process, represented below in terms of single electron transfers, would accommodate the existence[4] of a strong *m/e* 259 peak (*e.g.*, Fig. 10).



While such a process has been demonstrated to occur,[8] it does not operate in all cases for which it has been proposed. Thus, although 11-keto steroids seem ideally suited for such a transfer, there is essentially no loss of hydrogen from C-1.[6] Analogous cases involving 1-, 7-, and 15-keto steroids are at present under investigation in this Laboratory. The distance factor may be quite critical for this transfer and in the 17β-alkyl-5α-16-keto androstanes, in which the transfer in question is very important, the minimum distance[32] between a γ-hydrogen on the side chain and the carbonyl oxygen (*ca.* 1.4 Å.) is certainly less than the separation (1.8 Å.) between the nearest (equatorial) hydrogen on C-1 and the carbonyl oxygen in the 11-keto androstanes.

(29) C. Djerassi, W. Closson, and A. E. Lippman, *J. Am. Chem. Soc.*, **78**, 3163 (1956); C. Djerassi, R. Riniker, and B. Riniker, *ibid.*, **78**, 6362 (1956).

(30) See W. Klyne, *Bull. soc. chim. France*, 1396 (1960); *Tetrahedron*, **13**, 29 (1961).

(31) C. Djerassi and W. Klyne, *J. Am. Chem. Soc.*, **79**, 1506 (1957); C. Djerassi, J. Osiecki, R. Riniker, and B. Riniker, *ibid.*, **80**, 1216 (1958).

(32) These approximate distances are based on measurements taken from Dreiding models.

In the case presently under discussion, namely 5α-pregnan-16-one (XIII) and its higher homologs, the *m/e* 259 peak was found by deuterium labeling to involve hydrogen transfer from the γ-position.

Other prominent peaks which are usually present in the mass spectra (*e.g.*, Fig. 10) of these 16-ketones are the M-15, *m/e* 301, *m/e* 216, and *m/e* 217 fragments; the first two of these have been studied in close detail. The strong M-15 peak in the spectrum (Fig. 9 and 10) of cholestan-16-one (XXIII) has been shown not to arise principally from loss of C-21 as was suggested[4] originally, although loss from this position can be important in lower members of the 16-ketone series. The peak at *m/e* 301, which was originally[4] attributed to the expulsion of the isohexyl part of the side chain by fission of the 20–22 bond, has now been proved to have been correctly assigned. Its genesis, however, is due to a very interesting double hydrogen transfer (one in each direction and hence not discoverable in the absence of deuterium labels) and its nature has been completely elucidated.

At the present stage very little can be said about the transfers involved in the formation of the *m/e* 216 and *m/e* 217 fragments, as suitably labeled compounds are not available. Nevertheless, reasonable predictions can be made and the general fission[4] previously assigned is confirmed. There are numerous minor peaks, which vary from compound to compound, for example, at *m/e* 201, 231, 232, 273, 274, and 283, which have been examined more closely where interesting shifts have been observed. A detailed discussion of the mass spectra follows, grouped according to the fragmentation process occurring.

The Molecular Ion.—This is most significant when the number of possibilities for, and likelihood of, cleavage reactions are lowest. Consequently, the relative size of this peak decreases from 5α-androstan-16-one (VIII) in which it is the base peak (Fig. 1), through the higher 17-alkyl-16-ketones to cholestan-16-one (XXIII) (see Fig. 9 and 10). 20-Isopropyl-5α-pregnan-16-one (XX) represents an extreme case where one cleavage is so favorable that the molecular ion intensity is only 0.4% that of the base peak (Fig. 6).

Peak M-15.—This peak is present in all of the spectra measured. In 5α-androstan-16-one (VIII, Fig. 1) it is presumably due to loss of an angular methyl group, while in 17β-methyl-5α-androstan-16-one (VII, Fig. 2) it may involve also some loss of the 17-substituent. This cleavage is, however, less pronounced in this spectrum (Fig. 2) and in that of its 15,15,17-d_3-analog. Similarly, in 5α-pregnan-16-one (XIII) the M-15 peak (*m/e* 287 in Fig. 3) is rather small, and is due in part (33%) to loss of the C-21 methyl group, as shown in Table II by the behavior of the 21,21,21-d_3-analog.

In 20-methyl-5α-pregnan-16-one (XVIII) the M-15 peak (*m/e* 301 in Fig. 4) is of great interest. It is much more intense and almost all of it is due to the loss of C-21 (or C-22), as demonstrated (Table II) by the

TABLE II

SHIFTS OF PRINCIPAL MASS SPECTRAL PEAKS OF 5α-PREGNAN-16-ONE (XIII) AND 20-METHYL-5α-PREGNAN-16-ONE (XVIII) IN THEIR DEUTERATED ANALOGS[a]

Compound	Molecular ion (M^+)	M-15	M-18	*m/e* 259
5α-Pregnan-16-one (XIII, Fig. 3)	302	287	284	259
15,15,17-d_3-5α-Pregnan-16-one	304 5% 305 95%	290	287	260 19% 261 14% 262 67%
21,21,21-d_3-5α-Pregnan-16-one	304 6% 305 92% 306 2%	290 67% 287 33%	286 7% 287 93%	259 *ca.* 20% 260 *ca.* 60% 262 *ca.* 20%
20-Methyl-5α-pregnan-16-one (XVIII, Fig. 4)	316	301	298	259
15,15,17-d_3-20-Methyl-5α-pregnan-16-one	318 3% 319 97%	304	301	261 12% 262 88%
21,21,21-d_3-20-Methyl-5α-pregnan-16-one	318 2% 319 98%	301 44% 304 56%	301 (+ small amt. of 300)	259 60% 260 40%
21,21,21,22,22,22-d_6-20-Methyl-5α-pregnan-16-one	321 4% 322 96%	304 89% 307 11%	303 *ca.* 20% 304 *ca.* 80%	259 11% 260 89%

[a] Wherever possible, the percentage shifts of the peaks are corrected for isotopic contaminants.

shifts to M-18 observed in the 21,21,21-d_3- and 21,21,21,-22,22,22-d_6-derivatives (44 and 89%,[33] respectively).

In 20-ethyl-5α-pregnan-16-one (XIX) the M-15 peak (*m/e* 315 in Fig. 5) is rather small, owing to the more favored production of an M-29 fragment (*m/e* 301) by the same process (see below) which yields M-15 in the 20-methyl (lower) homolog. About 20% of the observed M-15 ion is due to loss of the C-21

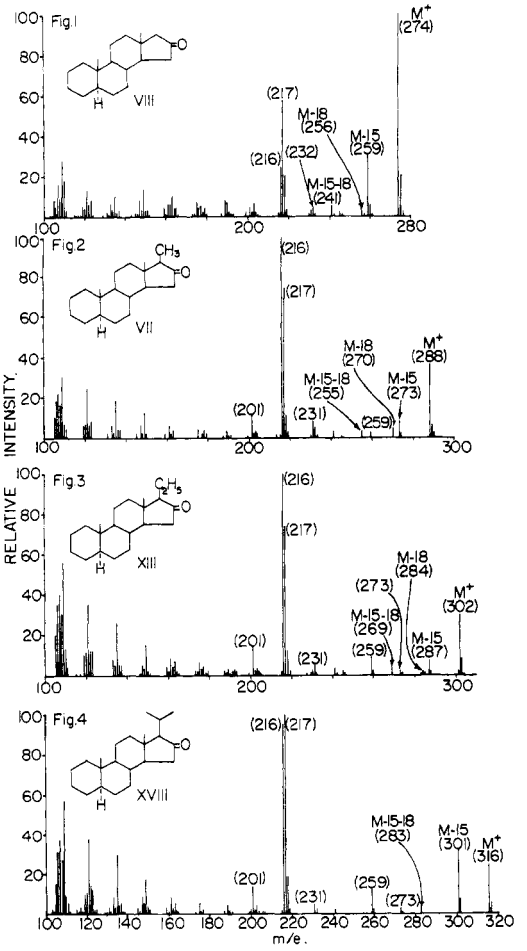


Fig. 1.—Mass spectrum of 5α-androstan-16-one (VIII).
Fig. 2.—Mass spectrum of 17α-methyl-5α-androstan-16-one (VII).
Fig. 3.—Mass spectrum of 5α-pregnan-16-one (XIII).
Fig. 4.—Mass spectrum of 20-methyl-5α-pregnan-16-one (XVIII).

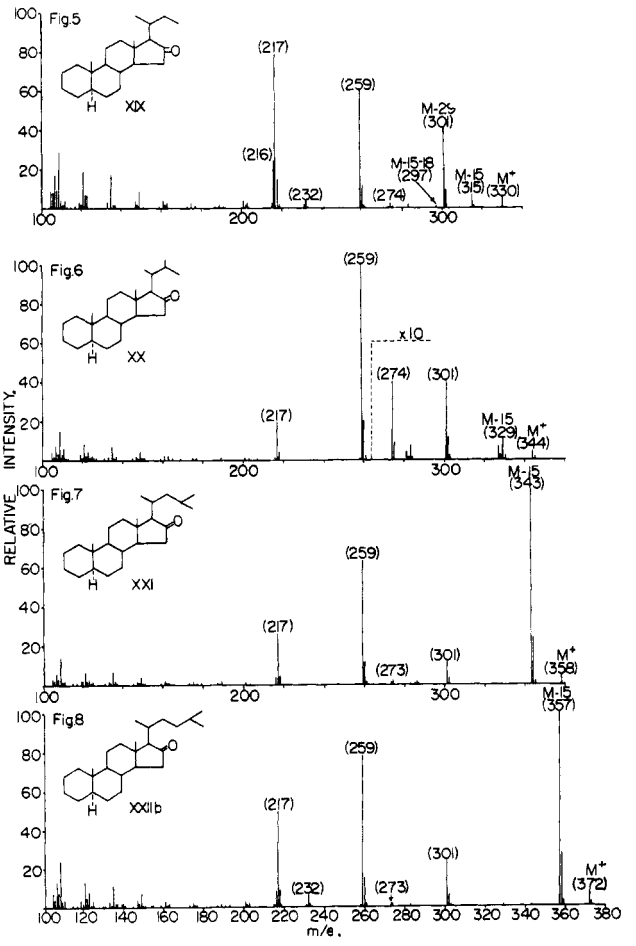


Fig. 5.—Mass spectrum of 20-ethyl-5α-pregnan-16-one (XIX).
Fig. 6.—Mass spectrum of 20-isopropyl-5α-pregnan-16-one (XX).
Fig. 7.—Mass spectrum of 20-isobutyl-5α-pregnan-16-one (XXI).
Fig. 8.—Mass spectrum of 20β-(3-methylbutyl)-5α-pregnan-16-one (XXIIb).

(33) There is not sufficient discrepancy between these two values to indicate the operation of an isotope effect. In any event, the stereochemistry at C-20 is not known for the unsymmetrical 21,21,21-d_3-compound; one C-20 epimer may predominate and, furthermore, rotation may not be completely free.

TABLE III

SHIFTS OF PRINCIPAL MASS SPECTRAL PEAKS OF 20-ETHYL-5α-PREGNAN-16-ONE (XIX) IN ITS DEUTERATED ANALOGS

Compound	Molecular ion (M^+)	*m/e* 315 (M-15)	*m/e* 259 (M-29)	*m/e* 259	*m/e* 232
20-Ethyl-5α-pregnan-16-one (XIX, Fig. 5)	330	315	301	259	232
21,21,21-d_3-20-Ethyl-5α-pregnan-16-one	332 5% 333 95%	315 20% 318 80%	304	259 97% 260 3%	232
15,15,21,21,21-d_5-20-Ethyl-5α-pregnan-16-one	334 9% 335 91%	317 20% 320 80%	306	261 97% 262 3%	234
15,15,17,21,21,21-d_6-20-Ethyl-5α-pregnan-16-one	335 10% 336 90%	318 25% 321 75%	307	262 97% 263 3%	234
21,21,21,22,22,23,23,23-d_8-20-Ethyl-5α-pregnan-16-one	337 7% 338 93%	320 24% 323 76%	304	260	232 *ca.* 20% 233 *ca.* 80%

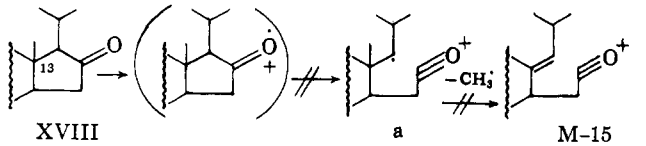
methyl group, as shown (Table III) by the shift of 80% of this *m/e* 315 peak to *m/e* 318 in the 21,21,21-d_3-, 15,15,21,21,21-d_5- and 15,15,17,21,21,21-d_6- analogs. The mass spectrum (Table III) of the 21,21,21,22,22,23,23,23-d_8-compound shows a 24% loss of 18 mass units, indicating that only 4% of the total M-15 ion in the spectrum (Fig. 5) of 20-ethyl-5α-pregnan-16-one (XIX) comes from C-23.

20-Isopropyl-5α-pregnan-16-one (XX) constitutes a special case as mentioned above, and is discussed in the section dealing with the *m/e* 259 peak. The remaining three 20-alkyl-5α-pregnan-16-ones which we have studied, XXI (isobutyl), XXII (isopentyl), and XXIII (isohexyl), show (Fig. 7–10) very strong M-15 peaks, which are usually the most intense ones in the spectrum. While only cholestan-16-one (XXIII) has been compared (Table IV) with deuterium labeled analogs, these closely related lower homologs (XXI, XXII) obviously belong to the same class.

Both 20α- (XXIIIa) and 20β- (XXIIIb) cholestan-16-one have been examined (Fig. 9 and 10) and although there are slight quantitative differences in the M-15 peaks (stronger in 20α-) they behave essentially the same as far as this cleavage is concerned. All of the deuterium labeled cholestan-16-ones here described (Table IV) lose practically all this fragment as M-15, strongly suggesting that it is the C-18 methyl group[34] which is lost (without exchange of hydrogens). Thus 26,27-d_2-cholestan-16-one shows (Table IV) a 2% shift of M-15 to M-16, and 21,21,21-d_3-cholestan-16-one exhibits only a 1% shift of M-15 to M-18.

Metastable peaks are visible in some cases at positions calculated for the transition $M^+ \rightarrow$ M-15; *e.g.*, for 20-methyl-5α-pregnan-16-one (XVIII, Fig. 4) at *m/e* 287.7 (*m/e* 316 → 301), and at *m/e* 357.8 (*m/e* 386 → 371) for cholestan-16-one (XXIII, Fig. 9 and 10). In proposing a mechanism for the loss of a methyl group from these molecules, there are two facts which have to be borne in mind: First the larger loss of methyl from the long side chain 16-ketones (XXI, XXII, XXIII) as compared with the lower alkyl homologs, and, second, the difference in position from which the methyl is lost (C-21 and C-22 from XVIII *vs.* C-18[34] from the higher members).

Considering first 20-methyl-5α-pregnan-16-one (XVIII), if the well documented[5,35] α-cleavage of ketones occurred (see species a), one would expect



XVIII a M-15

(34) The present evidence does not exclude rigorously the C-19 methyl group as a possible contributor.

(35) A. G. Sharkey, J. L. Schultz, and R. A. Friedel, *Anal. Chem.*, **28**, 934 (1956).

the related loss of methyl to be that from C-13, as this C-18 methyl group leaves a tertiary center, rather than C-20 which is secondary

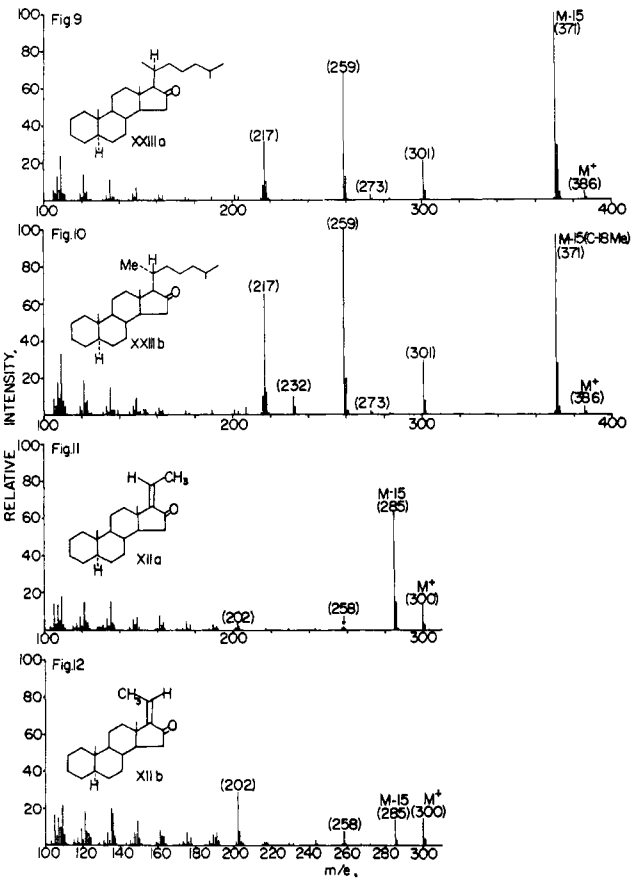


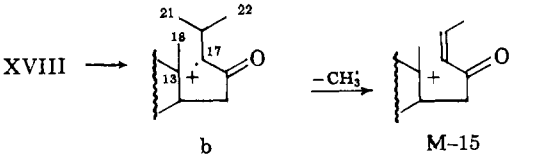
Fig. 9.—Mass spectrum of 20α-cholestan-16-one (XXIIIa).

Fig. 10.—Mass spectrum of 20β-cholestan-16-one (XXIIIb).

Fig. 11.—Mass spectrum of *cis*-$\Delta^{17(20)}$-5α-pregnen-16-one (XIIa).

Fig. 12.—Mass spectrum of *trans*-$\Delta^{17(20)}$-5α-pregnen-16-one (XIIb).

This is not what is observed in the case of XVIII, and alternative β-fission (b) may be invoked



b M-15

Such a 13–17 bond rupture (b) would certainly not be followed by the loss of C-18, but rather C-21 or C-22, as is in fact observed. (The small amount (11%) of methyl lost other than as C-21 or C-22 may well be due to C-18[34] by process a.)

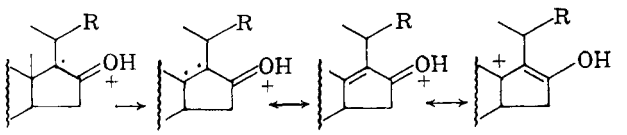
TABLE IV

SHIFTS OF PRINCIPAL MASS SPECTRAL PEAKS OF 20α-(XXIIIa) AND 20β-(XXIIIb) CHOLESTAN-16-ONE IN THEIR DEUTERATED ANALOGS

Compound	Molecular ion (M^+)	M-15	*m/e* 301	*m/e* 259	*m/e* 232[a]
20α-Cholestan-16-one (XXIIIa, Fig. 9)	386	371	301	259	...
20β-Cholestan-16-one (XXIIIb, Fig. 10)	386	371	301	259	232
15,15,17-d_3-20α-Cholestan-16-one	388 8% 389 92%	373 2% 374 98%	303 60–62%[b] 304 38–40%	262	...
15,15,17-d_3-20β-Cholestan-16-one	388 7% 389 93%	373 1% 374 99%	303 63–65%[b] 304 35–38%	262	234
21,21,21-d_3-Cholestan-16-one (mixt. 20α and 20β)	388 5% 389 93% 390 2%	371 1% 374 99%	304	259 97% 260 3%	232
22,22-d_2-Cholestan-16-one (mixt. 20α and 20β)	387 6% 388 88% 389 6%	372 1% 373 99%	301 98% 302 2%	259 13% 260 87%	232
23,23-d_2-20α-Cholestan-16-one	387 5% 388 95%	372 1% 373 99%	301 79% 302 21%	259 99% 260 1%	...
23,23-d_2-20β-Cholestan-16-one	387 5% 388 95%	372 1% 373 99%	301 77% 302 23%	259 99% 260 1%	232 9% 233 91%
24,24-d_2-20α-Cholestan-16-one	387 7% 388 93%	373	301 79% 302 21%	259 99% 260 1%	...
24,24-d_2-20β-Cholestan-16-one	387 7% 388 93%	373	301 79% 302 21%	259 98% 260 2%	232
25-d_1-20α-Cholestan-16-one	386 1% 387 99%	372	301 84% 302 16%	259	...
25-d_1-20β-Cholestan-16-one	386 1% 387 99%	372	301 84% 302 16%	259	232
26,27-d_2-Cholestan-16-one (mixt. 20α and 20β)	387 5% 388 95%	372 2% 373 98%	301	259	232
20-d_1-20α-Cholestan-16-one	[c] ...	371 12% 372 80% 373 8%	302	259 95% 260 5%	...[d]
15,15,17,21,21,21-d_6-Cholestan-16-one (mixt. 20α and 20β)	389 4% 390 2% 391 7% 392 87%	376 2% 377 98%	306 *ca.* 64% 307 *ca.* 36%		
15,15,21,21,21-d_5-Cholestan-16-one (mixt. 20α and 20β)	389 3% 390 6% 391 89% 392 2%		306 >95%		

[a] The *m/e* 232 peak is absent (Fig. 9) in the 20α- series and can be used for stereochemically diagnostic purposes (see footnote *d*). [b] Range given because of unknown distribution of deuterium in d_2-contaminant. [c] Low intensity of mol. ion peak. Assuming no loss of 16 mass units, the M-15 peak is the best guide to isotopic composition. [d] The mass spectrum of the product arising from lithium–ammonia reduction of $\Delta^{17(20)}$-cholesten-16-one (XXVIIIa) exhibited an *m/e* 232 peak, thus demonstrating (see footnote *a*) the presence of the 20β-isomer.

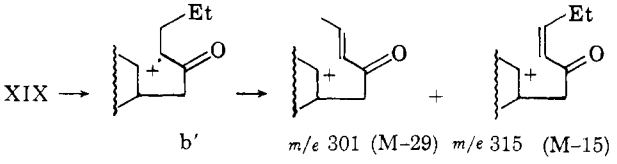
The very large loss (Fig. 7–10) of methyl from cholestan-16-one (XXIII) and its relatives (XXI, XXII) can be explained if the M-15 peak arises from loss of the C-18 methyl function from the β-position (C-13) of the oxonium ion-radical c. The situation is in some ways analogous to that in the radical-ion, produced by cleavage (a) of the 16–17 bond, which was invoked to explain the slight loss of C-18 from XVIII. One would expect the methyl group to be lost from the tertiary center, leaving the favorable resonance-stabilized tertiary allylic carbonium ion



c M-15 (R = isobutyl, isopentyl, isohexyl)

For this process to occur, it is necessary to form the radical c by some hydrogen transfer process, the hydrogen from C-17 going to where the hydrogen on oxygen came from. We present below (see discussion of *m/e* 301 peak) conclusive evidence that such processes do operate in a closely related cleavage, which gives an *m/e* 301 ion from the longer side chain molecules. In theory, such a process could obtain with c (R = ethyl (XIX)), but it appears that the transfer of primary hydrogen (from a methyl group), which this would entail, is not favorable (*vide infra*). Furthermore, it should be recalled that hydrogen transfer from a γ-carbon atom gives rise to an entirely different fragmentation—the *m/e* 259 peak discussed below. The intermediacy of species c, arising from hydrogen transfers not available to the lower homologs (*e.g.* XVIII), for the abundant M-15 ion in XXI–XXIII, explains the lower intensity of this peak in XVIII, where the less favored cleavage β to a ketone (b) must intervene.

Cleavages Giving Rise to *m/e* 301 Peak.—The smallest molecule in the mass spectrum (Fig. 4) of which this peak occurs is 20-methyl-5α-pregnan-20-one (XVIII), where it has been shown to correspond to loss of 15 mass units. The analogous fission (b′) in the 20-ethyl homolog XIX could give rise to M-15 (*m/e* 315) or M-29 (*m/e* 301) peaks

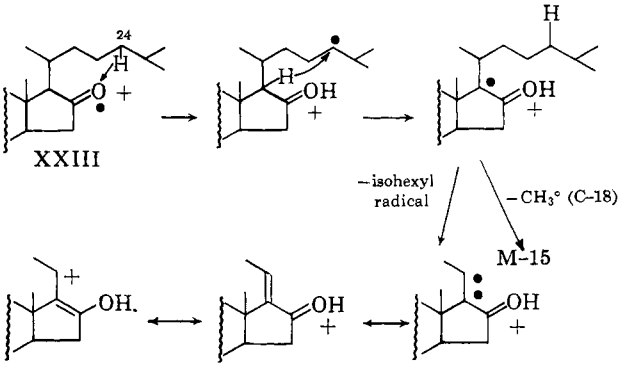


b′ *m/e* 301 (M-29) *m/e* 315 (M-15)

In fact, the bond from C-20 to the more substituted carbon (22) breaks and the dominant cleavage yields the *m/e* 301 ion (Fig. 5). This loss of ethyl occurs without any transfer of hydrogen to or from the group lost, as shown (Table III) by the loss of 34 mass units (*m/e* 304) from the 21,21,21,22,22,23,23,23-d_8-analog and of 29 mass units (*m/e* 304) from the 21,21,21-d_3-derivative. As mentioned above in the discussion of the M-15 peak, the loss of methyl from XIX is derived to the extent of *ca.* 20% from C-21, which means that expulsion of a methyl group from other sources (C-18?) is favored in a 4:1 ratio. In view of the fact that no transfers are occurring in these cleavages, one might expect that the processes operating to give the M-15 (*m/e* 315) and M-29 (*m/e* 301) fragments in XIX (Fig. 5) and the M-15 (*m/e* 301) ion in XVIII (Fig. 4) are completely analogous, as suggested above. In which case, the ratio of M-15 (from loss of C-21 and C-22) to M-15 from other (C-18?) sources (in XVIII) would be approximately the same as the ratio in XIX (Fig. 5) of M-15 from loss of C-21 and *m/e* 301 to M-15 from other sources (very little methyl is lost as C-23). This is true, approximately 11% being lost in both cases as methyl attached to C-20. Implied in this value is the very small loss of C-21 as methyl compared with C-22 and C-23 as ethyl in XIX, and this amounts to approximately 3% as derived from a consideration of the mass spectrum (Table III) of the 21,21,21-d_3-analog.

The only other deuterium labeled 16-ketone studied has been cholestan-16-one (XXIII) and in this compound the *m/e* 301 fragment arises from loss of the isohexyl side chain. This cleavage occurs in part by the mechanism (see b and b′) proposed above for XVIII and XIX; however, approximately 60–65% of the fission involves hydrogen transfers which have been followed (Table IV) by comparison of deuterated analogs. The first evidence of such transfers came from a study of the 15,15,17-d_3-analogs of 20α- and 20β-cholestan-16-one (XXIIIa and XXIIIb). Here it was found (Table IV) that for the 20α- (XXIIIa) isomer, 0.6–0.62 atom of deuterium was transferred during the loss of the isohexyl side chain, *i.e.*, the *m/e* 301 peak of XXIIIa was shifted to the extent of 60–62% to *m/e* 303 and of 38–40% to *m/e* 304 in the 15,15,-17-d_3-analog. The ranges quoted arise because there was some (8%) d_2-contaminant present and the distribution of deuterium in this is unknown. Similarly, the 15,15,17-d_3-20β-isomer showed (Table IV) a transfer of 63–65%. This slight difference in percentage transfer between XXIIIa and XXIIIb seems to be real and could be explained by the greater steric hindrance in bringing the 20α-isomer around to a suitable position for transfer of hydrogen from side chain to carbonyl oxygen (see below).

Comparison (Table IV) of the 15,15,21,21,21-d_5-spectrum with those of the 15,15,17-d_3- and 15,15,17,21,-21,21-d_5- species showed that less than 5% of the transferred deuterium came fr m C-15 and none from C-21; the results in Table IV demonstrate that C-20 is similarly not implicated. There must be a corresponding transfer from the side chain to the steroid nucleus to preserve the observed balance (no net transfer), and examination (Table IV) of the mass spectra of the side chain labeled compounds revealed the sources of this back transfer. For example, 23,23,d_2-XXIIIa and b showed, respectively, 21 and 23% shifts of *m/e* 301 to *m/e* 302. This small difference in percentage transfer was not observed with other positions, and for those deuterated analogs involving low percentage transfers, a mixture of 20α- and 20β-isomers was sometimes used. By observing (Table IV) the shifts in other labeled analogs, the percentage transfers from all of the side positions could be determined as: C-22, 2%; C-23, 23%; C-24, 21%; C-25, 16%; C-26 and C-27, 0%. *The total percentage for these back transfers is 62%, in good agreement with the loss (60–65%) from the nucleus.* We have already invoked one possible description of this transfer process to explain the formation of the M-15 ion in cholestan-16-one (XXIII) and other higher alkyl homologs. Thus, the exchange of hydrogen between positions 17 and 24 can be visualized as



Analogous transfers can be proposed for the other positions, *although it should be noted that various sized (7 → 9) rings are involved in these transfers*, and that removal of a hydrogen atom from C-22 (6-membered ring) triggers a different type of fragmentation, which gives rise to the *m/e* 259 ion.

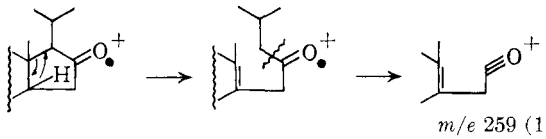
Peak *m/e* 259.—The cyclic mechanism, XXIX,[5] which is outlined in the introduction to this mass spectral Discussion section and which has been proposed[36] for the formation of this fragment, can only operate when there is a hydrogen atom available for transfer from the γ-carbon atom. Thus, although there exists a peak at *m/e* 259 in the spectrum (Fig. 2) of 17-methyl-5α-androstan-16-one (VII), it is extremely small and must be due to at least two other processes, as shown by the approximately equal splitting between *m/e* 259 and 261 in the 15,15,17-d_3-analog.

5α-Pregnan-16-one (XIII) is the first member of the present series which can undergo this cyclic type of transfer, and there is a small but significant peak at *m/e* 259 in the mass spectrum (Fig. 3) of this substance. About 60% of this peak is due to the above-mentioned transfer process, as is demonstrated by the mass spectrum (Table II) of the 21,21,21-d_3-analogs.

In the spectrum (Fig. 4) of the next member of the series, 20-methyl-5α-pregnan-16-one (XVIII), the *m/e* 259 peak is more important and is mostly due to a transfer of the type expected. Thus, in 21,21,21,22,22,-22-d_6-XVIII, 89% of the peak appears (see Table II) at *m/e* 260 and the remaining 11% at *m/e* 259. Similarly, in the 15,15,17-d_3-analogs, 88% remains at *m/e* 262, while 12% is shifted to *m/e* 261. This would indicate that 11–12% of the *m/e* 259 ion owes its genesis

(36) See footnote 19 in ref. 4.

to a fission of the type originally[4] considered, involving loss of C-17 and its attached substituents, together with another hydrogen which may come from C-14

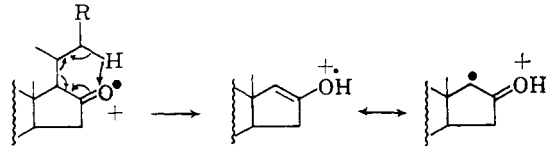


m/e 259 (11%)

There is, however, a slight discrepancy in the fragmentation observed (Table II) with the 21,21,21-d_3-analog where 40% of the peak occurs at *m/e* 260 and 60% at *m/e* 259. The respective percentages should be 44 and 56%, based on the values obtained with the d_0- (XVIII, Fig. 4) and d_6-compounds. Perhaps an isotope effect is operating, or a stereoselective transfer from C-22 in 21,21,21-d_3-XVIII, the stereochemistry at C-20 in this substance being unknown.

20-Ethyl-5α-pregnan-16-one (XIX) shows (Fig. 5) a very strong peak at *m/e* 259, and the large increase in intensity compared with the lower homolog XVIII can be attributed to the presence of secondary hydrogens at C-22. This is shown clearly by the shifts observed (Table III) in suitably labeled analogs: In 21,21,21-d_3-XIX, there is only a 3% shift to *m/e* 260 from 259 and similarly in 15,15,21,21,21-d_5-XIX, a 3% shift to *m/e* 262 is noted with 97% moving to 261. In 15,15,17,21,21,21-d_6-XIX, there is a 3% shift to 263 and 97% to 262, whereas in the 21,21,21,22,22,23,23,-23-d_8-analog the entire peak moves to *m/e* 260. Thus, if the improbable transfer from C-23 is ignored (suitably labeled analogs have been examined in the case of cholestan-16-one to justify this assumption—see Table IV), it can be stated with confidence that the *m/e* 259 peak in XIX arises entirely by the cyclic transfer process proposed[36] and that the hydrogen transferred comes 97% from C-22 and only 3% from C-21. While some stereoselective process may operate,[37a] the most likely explanation is that transfer of a secondary hydrogen atom is much more favorable than transfer of a primary one.[37b]

The very great difference in the ease of transfer between a secondary and a primary hydrogen suggests that a similar difference may be expected between tertiary and secondary hydrogen atoms. For this reason, the mass spectrum (Fig. 6) of 20-isopropyl-5α-pregnan-16-one (XX), has been examined. This material was obtained in extremely poor yield, but the above supposition is borne out very strikingly, as the peak at *m/e* 259 is by far the largest in the spectrum. There is usually a very weak peak at *m/e* 274 in the spectra of those compounds showing *m/e* 259. This can be attributed to the fragment d first formed, before the loss of methyl, and this supposition is supported by the recognition in the spectrum of XX of a small metastable peak at *m/e* 245.5 (*m/e* 274 → 259) corresponding to the loss of methyl from the *m/e* 274 fragment.



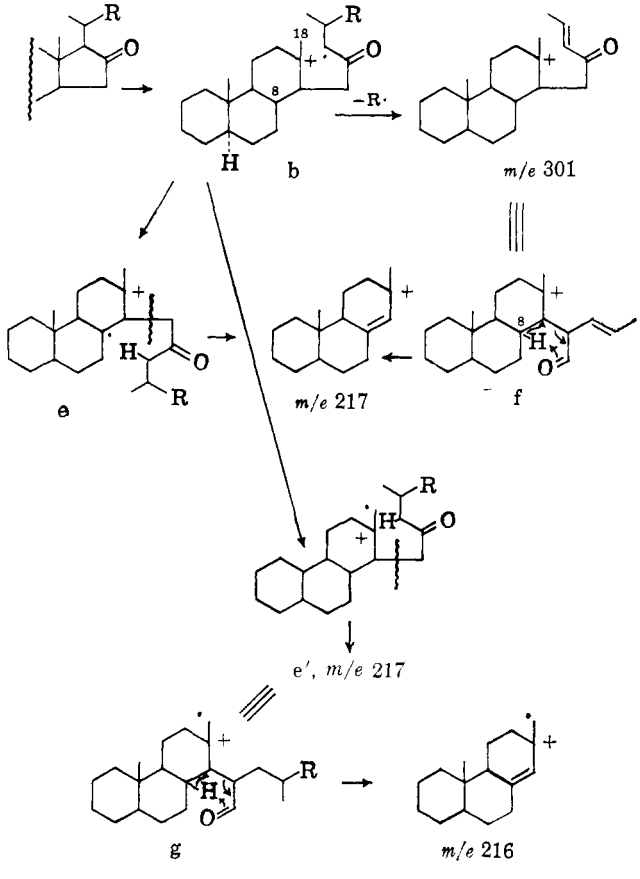
d, *m/e* 274

In 20-ethyl-5α-pregnan-16-one (XIX), which shows (Fig. 5) the *m/e* 274 peak fairly well, it is moved to *m/e* 275 in 21,21,21,22,22,23,23,23-d_8-XIX, to *m/e* 276 in 15,15,21,21,21-d_5-XIX, to *m/e* 277 in 15,15,17,21,21,-21-d_6-XIX, and to *m/e* 274 in 21,21,21-d_3-XIX. These figures do not exclude the possibility of transfer from C-23, but this would seem unlikely in view of previous examples.

In cholestan-16-one (XXIII) itself, there is a transfer (Table IV) of 87% of deuterium from C-22 in the 22,22-d_2-analog, and a transfer of 3% from C-21 in the 21,21,21-d_3-compound, values which agree well with the percentage transfer from C-21 and C-22 observed (Table II) in XVIII. The remainder of the *m/e* 259 ion may arise from more or less random hydrogen transfers from the rest of the side chain.

Peaks *m/e* 216 and *m/e* 217.—These peaks, in varying ratio, are present in the spectra (Fig. 1–10) of all the 16-keto steroids of this series (and many other steroids, after appropriate mass adjustment for substituents), and can readily be identified[4] with the loss of ring D and its substituents, together with one or two hydrogens from the rest of the nucleus. None of the labeled compounds described in this paper have produced spectra showing shifts of these two peaks, so it appears that there is no back transfer occurring.

As far as the relationship between the *m/e* 216 and 217 peaks is concerned, in general it seems that as the intensity of *m/e* 259 increases in the 17-substituted series (*e.g.*, VII → XVIII → XIX → XX) the ratio of intensity of *m/e* 216 to *m/e* 217 decreases. Thus there seems to exist a greater competition between the processes, producing *m/e* 259 and 216 than between those affording *m/e* 259 and 217. In addition, there are metastable peaks visible for $M^+ \rightarrow$ *m/e* 216 in VII (found: *m/e* 163.2) and XIII (found: *m/e* 155.7) and for *m/e* 301 → 217 (found: *m/e* 157.5) in XVIII, XIX, XX, XXI, XXII, and XXIII.

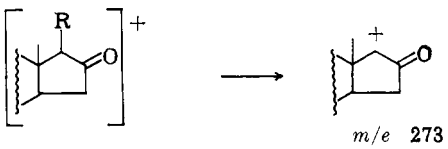


(37) (a) Just as with 21,21,21-d_3-XVIII, the stereochemistry at C-20 in the three 21,21,21-d_3-analogs of XIX (Table III) is unknown. However, the identical selectivity is also observed (Table IV) in the corresponding cholestan-16-ones, where both isomers (XXIIIa and b) are available. (b) A similar conclusion can be reached from the mass spectrum of methyl 2-ethylhexadecanoate (R. Ryhage and E. Stenhagen, *Arkiv Kemi*, **15**, 333 (1960)), where transfer of the γ-hydrogen from a methylene group is greatly favored over that originating from a methyl function.

A general scheme rationalizing these observations involves first breaking the 13–17 bond (b) and then transferring a hydrogen atom from the nucleus to either C-17 or the carbonyl oxygen. Transfer from C-8 to the carbonyl oxygen gives the *m/e* 217 fragment by a cyclic process (f), but transfer of a hydrogen atom (*e.g.*, process e or e′) to C-17 followed by breakage of the 14–15 bond can also yield *m/e* 217. However, if the rearrangement to C-17 occurs followed by a further transfer of another hydrogen atom to oxygen (b → e′ → g), then the *m/e* 216 fragment is produced. The greater competition of the process forming the *m/e* 259 fragment (involving transfer of hydrogen from C-22 to the carbonyl oxygen (see XXIX)) with that producing *m/e* 216 rather than that yielding *m/e* 217 is explained. To form *m/e* 216, transfer to oxygen must occur, but *m/e* 217 can be produced by other processes and it will be noted that the *m/e* 216 peak is most intense in Fig. 1–4, where the *m/e* 259 ion is only of low abundance.

Some of the possibilities are indicated in the above scheme with the most likely transfers originating from C-8, C-12, or C-18.

Miscellaneous Small Peaks.—A very small peak at *m/e* 273 appears in most of the spectra of 17-alkyl-16-ketones, and seems to be due to a loss of the side chain. Its intensity is too small for any possible hydrogen transfers to be clearly defined.

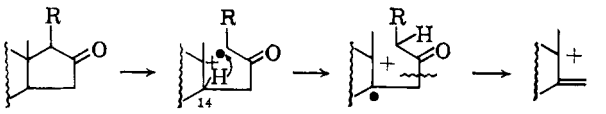


m/e 273

There are various peaks due to loss of 18 mass units from different species. Thus, there are very small M-18 peaks in VII (Fig. 2), XIII (Fig. 3), and VIII (Fig. 1), but not in the higher homologs. Many of the ketones show (*e.g.*, Fig. 1–5) loss of 18 mass units from the M-15 ion. Metastable peaks are visible for many of these eliminations of water. With the limited labels available and owing to the very small abundance of these ions, it is not possible to say which hydrogens are lost. Although they are probably abstracted in a random fashion as has been demonstrated[5] for decalones, certainly they are not, for example, removed cleanly from the α-carbon atoms. A peak at *m/e* 201 appears to a significant extent in the mass spectra of VII (Fig. 2), XIII (Fig. 3), and XVIII (Fig. 4) and its position is unchanged in any of the deuterated compounds. It is probably due to the loss of one methyl group from the A–B–C nucleus, and metastable peaks (at *ca.* *m/e* 188) are visible corresponding to the transition *m/e* 216 → *m/e* 201.

Peaks *m/e* 231, 232, and 233.—These peaks are rather small, but they show interesting shifts in certain deuterated analogs. The fragments *m/e* 232 and 233 seem to be related to each other, but not the *m/e* 231 ion.

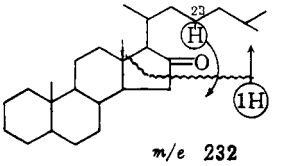
Considering first the *m/e* 231 peak, it is generally far too small to be amenable to quantitative estimation of shifts. In 17β-methyl-5α-androstan-16-one (VII) (Fig. 2) it is due to several processes, as shown by the confusing splitting in the 15,15,17-d_3-analog. As far as can be seen, the *m/e* 231 fragment in the higher homologs, when appreciable (*e.g.*, Fig. 3), seems to come largely by fission of the 13–17 and 15–16 bonds, without transfer of hydrogen from the eliminated portion. Thus in 15,15,17-d_3-XIII and 15,15,21,21,21-d_5-XIII, the peak is located at *m/e* 233. The same situation obtains (Fig. 4) in 20-methyl-5α-pregnan-16-one (XVIII), where the fragment appears at *m/e* 231 in the parent compound and its 21,21,21,22,22,22-d_6-analog, while it is shifted to *m/e* 233 in the 15,15,17-d_3-derivative. With larger side chains, the *m/e* 231 peak almost disappears, but seems to follow the same pattern of shifts as in the lower homologs. A plausible cleavage sequence for producing the *m/e* 231 fragment might be the following which results when the species with a broken 13–17 bond transfers a hydrogen from C-14.



m/e 231

The peaks at *m/e* 232 and 233 are only significant in the higher alkyl homologs XIX (Fig. 5), XXIIb (Fig. 8), XXIIIb (Fig. 10) and move together in the deuterated analogs. The compounds for which the most complete information is available are again the cholestan-16-ones (XXIII). Whereas the "natural" 20β-epimer XXIIIb shows (Fig. 10) peaks at *m/e* 232 and 233, they are essentially absent from the spectrum (Fig. 9) of the 20α-isomer. This is also observed in the spectra of XXIIa and b, the former not showing the *m/e* 232 and 233 peaks, while the 20β-compound does (Fig. 8).

The very selective nature of this fission process, with regard to the stereochemistry at C-20, is unusual, as is the strongly specific hydrogen transfer involved (Table IV). In 20β-cholestan-16-one (XXIIIb), the *m/e* 233 peak is about one-fourth of the size of the *m/e* 232 peak (after correction) and moves with the latter in all the following cleavages. The peak position of the *m/e* 232 ion is unchanged in the 17-d_1-, 20-d_1-, 21,21,21-d_3-, 22,22-d_2-, 24,24-d_2-, 25-d_1-, and 26,27-d_2-analogs. It occurs at *m/e* 234 in the 15,15,21,21,21-d_5-, 15,15,17,21,21,21-d_6- and 15,15,17-d_3-species and at *m/e* 233 (91%) in 23,23-d_2-XXIIIb. These shifts indicate that the hydrogens on C-15 are retained in the *m/e* 232 and 233 fragments, and that all hydrogens on the side chains are lost, except one from C-23, which is transferred. A corresponding back transfer must occur from the nucleus to the fragment which is lost, and this can be represented schematically as



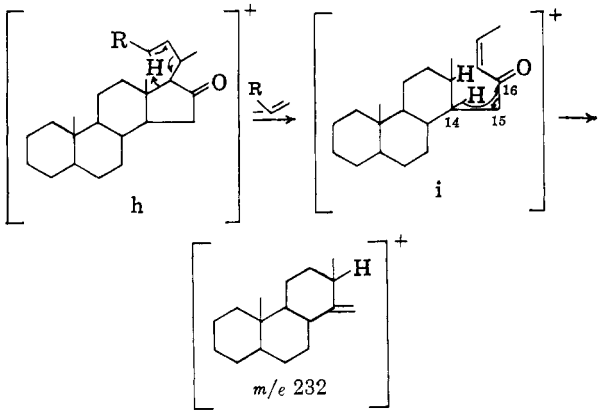
m/e 232

In the formation of the *m/e* 233 peak, the same transfer (to the extent of *ca.* 75%) appears to occur from C-23, only in this case the back transfer from the nucleus presumably is not taking place.

Similar, but not so complete, evidence can be obtained from the deuterated 20-ethyl-5α-pregnan-16-ones (XIX) (Table III). Thus, the *m/e* 232 peak is unmoved in 21,21,21-d_3-XIX, but occurs at *m/e* 234 in 15,15,21,21,21-d_5-XIX and 15,15,17,21,21,21,-d_6-XIX and at *m/e* 233 (80%) in 21,21,21,22,22,23,23,23-d_8-XIX. These labels do not distinguish between transfer from C-22 and C-23, but in view of the behavior of 20β-cholestan-16-one (XXIIIb) one can reasonably assume that the migrating hydrogen originates from C-23.

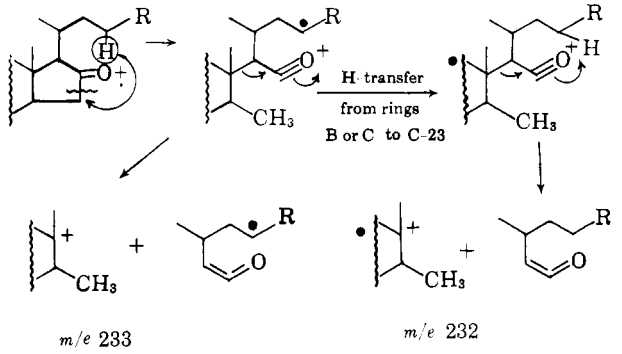
Examination of models shows that the only reasonable positions to which hydrogen can be transferred specifically from C-23 are C-13, 15, and 16. Movement to C-16 does not help matters, because C-16 is

lost in the fission process and the hydrogen would have to be retransferred to the nucleus. A cyclic rearrangement involving a hydrogen shift (h) from C-23 to C-13, followed by transfer (i) of the C-14 hydrogen[38] to C-16 with concomitant fission of the 15–16 bond, could give rise to the *m/e* 232 fragment, while fission of the 15–16 bond before the second transfer (i) would lead to *m/e* 233.



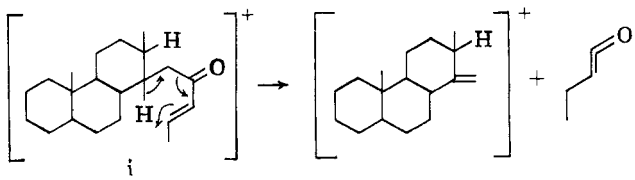
However, one has to bear in mind the sensitivity of the transfer process to the stereochemistry at C-20, and inspection of models does not offer any obvious reasons for the pronounced stereoselectivity in the 20β-series.

The remaining position, C-15, involves a rather unorthodox seven-membered cyclic transition state, and the possible details are by no means established. However, the correct stereochemical preference (20β over 20α) would be predicted from a consideration of the steric hindrance to approach to this carbon atom since there exists greater interference between C-18 and C-21 in the 20α-epimer. The transfer to C-15 would have to occur as a concerted process, as once the D ring is open, free rotation would lead to nonspecific transfers.

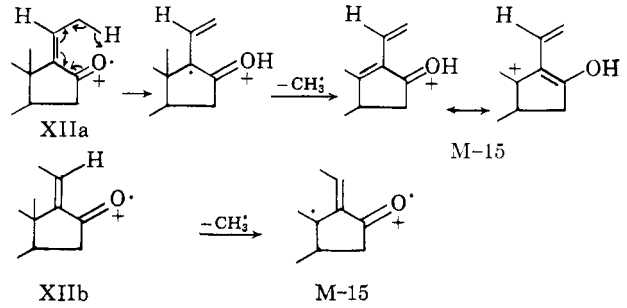


The possibility of encouraging some particular fission process by introducing a suitable tertiary center into the molecule has already been demonstrated in the case of the *m/e* 259 ion (Fig. 6) with XX. Application of this idea to the encouragement of the fission producing the *m/e* 232 species failed. Thus, XXI possesses a tertiary hydrogen atom at C-23, yet its spectrum (Fig. 7) showed essentially no *m/e* 232 peak. Several possible explanations can be offered for this observation; one is that we are dealing with the 20α-isomer (the C-20 stereochemistry of XXI is unknown), which does not show the *m/e* 232 peak. Another is that the two methyl groups at C-23 are causing sufficient hindrance to prevent the transfer, the process being known to exhibit great sensitivity to steric influence. Finally, it may be that for reasons unknown, the tertiary center does not promote the transfer in this case. In this connection, it may be noted that 20-ethyl-5α-pregnan-16-one (XIX) has primary hydrogens at C-23, yet these seem to transfer quite efficiently to give a noticeable *m/e* 232 peak (Fig. 5) in contrast to other comparisons of primary and secondary hydrogen, which we have encountered. Again, a favorable steric effect may be acting in this instance.

(38) The C-14 hydrogen transfer may also be visualized as



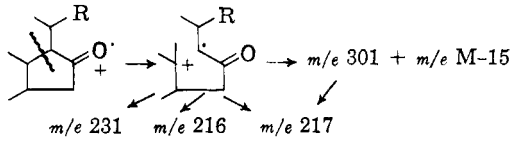
Mass Spectra of *cis*- (XIIa) and *trans*- (XIIb) $\Delta^{17(20)}$ 5α-Pregnen-16-one.—The mass spectra (Fig. 11 and 12) of the geometrically isomeric α,β-unsaturated ketones XIIa and XIIb have been measured and show a surprising difference. Part of this difference can be attributed to a process related to the transfer of hydrogen from C-22 in the saturated compounds. Thus, the M-15 peak in the spectrum (Fig. 12) of XIIb is much smaller than in that (Fig. 11) of XIIa, and this might arise because of the greater loss of methyl to form the ion more stabilized by resonance



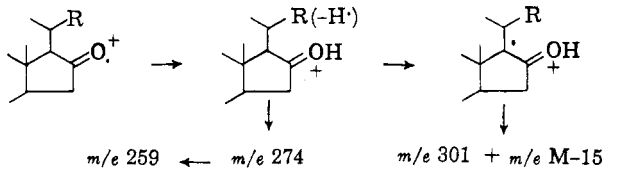
The spectrum (Fig. 12) of XIIb shows two peaks (*m/e* 258 and 202) which are practically absent from that (Fig. 11) of XIIa. The former (M-42) is common in cyclic α,β-unsaturated ketones with no α-substituent[7] and moves to M-44 in the mixture of 15,15,21,21,21-d_5-XIIa and b.

Thus, in agreement with earlier conclusions,[7] it is caused by elimination of C-15 and C-16 as ketene. The *m/e* 202 peak is unchanged in the d_5-analog and probably involves loss of the D ring together with the C-18 methyl group and one hydrogen atom.

Conclusion.—Although the details of rearrangement and fragmentation that emerge from this study seem more complicated than those originally[4] conceived, a closer consideration of the situation suggests a rationale for the formation of most of the major peaks in the diagnostically important higher mass range. Such a unification would not have been possible without the employment of deuterium labeled analogs. Consider, for example, the different processes which can take place following the breakage of a 13–17 bond



or the consequences of transferring a hydrogen atom from the side chain to the carbonyl group



As has been found, the importance of these various possibilities will depend on the nature of the R group in a more or less predictable manner, the principal fragmentation being triggered by the proximity of the carbonyl group and certain rather specific hydrogen transfer processes. The mechanistic importance of uncovering such hydrogen rearrangements can hardly be overemphasized and is well demonstrated in the present study.

Experimental[39]

17α-Methyl-5α-androstan-17β-ol (II).—A solution of 5α-androstan-17-one (I, 2.74 g.)[9] in ether (30 ml.) was added over a period of 10 min. to the Grignard reagent prepared from magnesium (1.22 g.) and methyl iodide (7.1 g.) in ether solution (30 ml.). The mixture was heated under reflux for 8 hr., left overnight at room temperature, and decomposed with ice-cold 2 *N* hydrochloric acid. The crude product, isolated by ether extraction, was recrystallized from hexane, yielding 17α-methyl-5α-androstan-17β-ol (II), m.p. 163–164.5° (2.0 g., 69%).

Anal. Calcd. for $C_{20}H_{34}O$: C, 82.69; H, 11.80. Found: C, 82.50; H, 11.72.

Preparation and Pyrolysis of 17α-Methyl-5α-androstan-17β-ol Acetate (III).—A solution of 17α-methyl-5α-androstan-17β-ol (II, 1.0 g.) in acetic anhydride (10 ml.) was heated under reflux for 4.5 hr., and then evaporated under reduced pressure. The residual material was used directly for pyrolysis to the olefins, but a small sample from one batch was recrystallized twice from ethanol; m.p. 101–102°, $[\alpha]^{26}D$ −3° (*c* 0.65), $\lambda_{max}^{CHCl_3}$ 5.75 and 7.9 μ.

Anal. Calcd. for $C_{22}H_{36}O_2$: C, 79.46; H, 10.92. Found: C, 79.71; H, 11.03.

The crude acetate (1.0 g.) was pyrolyzed for 20 min. at 265° in an open test tube in a metal bath. The product was passed in hexane through a column of activity I neutral alumina (Merck), giving 635 mg. of solid residue. Part of this material was recrystallized from ethanol, whereupon the analytical sample melted at 64–65°, $[\alpha]^{28}D$ +20.5° (*c* 0.86); λ_{max}^{KBr} 3.27, 3.30, 11.44, and 12.72 μ.

Anal. Calcd. for $C_{20}H_{32}$: C, 88.16; H, 11.84. Found: C, 88.05; H, 12.06.

Gas phase chromatography (SE-30 column at 226°) of this material and of the crude product indicated that both were mixtures of two components. The n.m.r. spectrum showed the presence of a methylene group (multiplet at δ 4.62 p.p.m.) and a single vinyl proton (δ 5.26 p.p.m.)

17α-Methyl-5α-androstan-16-one (VII).—A solution of the crude olefin mixture (IV and V, 500 mg.) from the pyrolysis of III and boron trifluoride etherate (1.5 g.) in ether (40 ml.) was treated at room temperature with a solution of lithium aluminum hydride (0.3 g.) in ether (15 ml.). After the addition was complete (30 min.), stirring was continued for a further 1.5 hr., and the excess of diborane decomposed by addition of saturated sodium sulfate solution. Solid sodium sulfate (anhydrous) was added and the solution filtered and evaporated. The residue, in 95% ethanol (20 ml.) containing 0.6 g. of sodium hydroxide, was treated at 25° with 1 ml. of 30% hydrogen peroxide and stirred for 3 hr., then diluted and extracted with ether. Evaporation gave 430 mg. of residual solid, which was freed from any unchanged olefin by rough chromatography on activity II neutral alumina. In this manner, 385 mg. of crude alcohol, m.p. *ca.* 140–160°, was obtained. This was dissolved in acetone and stirred with anhydrous magnesium sulfate and a slight excess of 8 *N* chromic acid added. After 1 hr., solid sodium bicarbonate was added and the mixture filtered and evaporated. The residue was taken up in ether, washed with saturated sodium bicarbonate solution, and then with water. Evaporation of the dried solution gave 330 mg. of solid, which was chromatographed on 30 g. of silica gel (Merck) using benzene, yielding 248 mg. (47%) of the desired ketone VII, m.p. 132–135°. Recrystallization from methanol gave analytically pure material, m.p. 135–136°, $[\alpha]^{26}D$ −158° (*c* 0.86), $\lambda_{max}^{CHCl_3}$ 5.75 μ.

Anal. Calcd. for $C_{20}H_{32}O$: C, 83.27; H, 11.18. Found: C, 83.29; H, 11.08.

(39) All melting points are corrected and were determined in capillary tubes unless otherwise indicated. Rotations were measured in chloroform and n.m.r. spectra in deuteriochloroform solution. We are indebted to Dr. L. J. Durham for these latter measurements and to Mrs. Ruth Records for the optical rotatory dispersion curves, which were obtained with a Nippon Bunko (JASCO) recording spectropolarimeter. Thin layer chromatography (t.l.c.) was carried out on silica gel G (E. Merck, Darmstadt) and the chromatograms developed by heating, after spraying with 2% ceric sulfate in 2 *N* sulfuric acid. Mass spectra were obtained with a Consolidated Electrodynamics Corp. mass spectrometer Model 21-103C, using an all-glass inlet system heated to 200°. The isatron temperature was maintained at 270°, the ionizing voltage at 70 v., and the ionizing current at 50 μa. All microanalyses are due to Messrs. E. Meier and J. Consul.

In another, smaller scale experiment, the hydroboration product was chromatographed more carefully and gave, after recrystallization from methanol and from hexane, 17β-methyl-5α-androstan-16α-ol (VI), m.p. 182.5–183°, $[\alpha]^{25}D$ −15° (*c* 0.4).

Anal. Calcd. for $C_{20}H_{34}O$: C, 82.69; H, 11.80. Found: C, 82.41; H, 11.52.

Oxidation of this alcohol VI (5 mg.) gave the ketone VII (4 mg.) identical in all respects with the previously obtained material.

Exchange of the acidic protons for deuterium was achieved in the usual way by refluxing a solution of the ketone (12 mg.) in deuteriomethanol (1.5 ml.) containing 10% deuterium oxide and 10 mg. of dissolved sodium. After 1 hr. the solution was evaporated under reduced pressure, heavy water added, and the organic material extracted into dry ether. The ethereal solution was washed with heavy water, dried, (magnesium sulfate), and evaporated. Recrystallization of the residue from deuteriomethanol gave 15,15,17-d_3-17β-methyl-5α-androstan-16-one (9 mg.), m.p. 134.5–136°, consisting of 4% d_2- and 96% d_3-species.

***cis*- and *trans*-Δ$^{17(20)}$-5α-Pregnen-16α-ols (Xa and Xb).**—A mixture of 16α,17α-oxido-5α-pregnane-3,20-dione (IX,[9,15] 8.0 g.), hydrazine (16 ml., 95%), and water (8 ml.) in diethylene glycol (120 ml.) was refluxed under nitrogen for 45 min. Potassium hydroxide (8.0 g.) was then added (cautiously) and the reflux temperature raised to 195° by distillation. Refluxing was continued for 1.5 hr., then the mixture was cooled, diluted with water, and extracted four times with chloroform. The combined organic layers were washed, dried, and evaporated, leaving 7.65 g. of a thick yellow oil. This was chromatographed on activity II neutral alumina (540 g.) starting with 50% benzene in hexane and proceeding to 100% benzene. The progress of the elution was conveniently followed by thin layer chromatography using methylene chloride.

First, 680 mg. of oil was eluted, almost into the solvent front; this contained at least four major components and two minor ones as judged by gas chromatography on an SE-30 column operated at 245°.

Second, the alcohol Xa (1.77 g., m.p. 90–95°) was eluted. Recrystallization from hexane gave pure Xa (1.36 g., 18%) which melted at 102–103°, $[\alpha]^{28}D$ +10° (*c* 1.5).

Anal. Calcd. for $C_{21}H_{34}O$: C, 83.38; H, 11.33. Found: C, 83.23; H, 11.51.

After a mixture of Xa and Xb (0.28 g.), there was eluted 1.28 g. of material, m.p. 144–150°. This could not be brought to a satisfactory melting point by recrystallization, but showed only one spot on thin layer chromatography. Apparently some of the saturated alcohol XI (produced by diimide reduction) was present as a contaminant.

Finally, 0.89 g. (12%) of alcohol Xb was obtained, m.p. 140–150°, which on recrystallization from methanol, gave 565 mg. of pure Xb, m.p. 154–156°, $[\alpha]^{28}D$ −12.5° (*c* 2.1).

Anal. Calcd. for $C_{21}H_{34}O$: C, 83.38; H, 11.33. Found: C, 83.31; H, 11.31.

5α-Pregnan-16α-ol (XI).—The allylic alcohol Xa (12 mg.) in ethanol (10 ml.) was hydrogenated for 17 hr. in the presence of 12 mg. of prereduced 2% palladium-on-calcium carbonate. Filtration and evaporation gave 12 mg. of crude product, m.p. 173–174°. This was recrystallized from hexane and gave 9 mg. of pure alcohol XI, m.p. 174.5–175°, $[\alpha]^{25}D$ −12° (*c* 1.6).

Anal. Calcd. for $C_{21}H_{36}O$: C, 82.83; H, 11.92. Found: C, 82.78; H, 12.00.

An identical reduction of the isomeric alcohol Xb gave an equal quantity of material indistinguishable by m.p., mixture m.p., infrared, and thin layer chromatography comparison, from that obtained as described above.

A larger quantity of 5α-pregnan-16α-ol (XI) was obtained by hydrogenation of the intermediate fractions (1.28 g., m.p. 144–150°) from the above chromatography.

5α-Pregnan-16-one (XIII).—5α-Pregnan-16α-ol (XI, 60 mg.) in acetone (10 ml.) was oxidized in the usual way with 8 *N* chromic acid at 25°. This gave 55 mg. (92%) of crude ketone, m.p. 102–103°. An analytical sample, recrystallized from ethanol, melted at 103–103.5°, $[\alpha]^{27}D$ −155° (*c*, 0.92), $\lambda_{max}^{CHCl_3}$ 5.75 μ.

Anal. Calcd. for $C_{21}H_{34}O$: C, 83.38; H, 11.33. Found: C, 83.62; H, 11.39.

Deuterium exchange, performed in the usual way on this ketone (10 mg.), followed by recrystallization from deuteriomethanol, gave 15,15,17-d_3-5α-pregnan-16-one, m.p. 102.5–103° (see Table II).

***cis*- and *trans*-Δ$^{17(20)}$-5α-Pregnen-16-ones (XIIa and XIIb).**—Δ$^{17(20)}$-5α-Pregnen-16α-ol (Xa, m.p. 102–103°, 50 mg.) was dissolved in dry benzene (5 ml.) and the solution stirred with 500 mg. of activated manganese dioxide (obtain from Beacon Chemical Products, Inc., Cambridge, Mass.). This and all subsequent operations were carried out in the dark, as far as possible. After 24 hr., the solution was filtered and evaporated under reduced pressure, leaving 46 mg. (92%) of residue (XIIa, m.p. 144–146°).

Part of this was recrystallized from hexane to give the analytical specimen, which exhibited m.p. 146.5–147.5°; $\lambda_{max}^{cyclohexane}$ 240 and 235 mμ, ε (for both maxima) 9015; $\lambda_{max}^{CHCl_3}$ 5.83 and 6.08 μ.

Anal. Calcd. for $C_{21}H_{32}O$: C, 83.94; H, 10.73. Found: C, 84.24; H, 10.85.

The isomeric $\Delta^{17(20)}$-5α-pregnen-16-one (XIIb) was prepared in a completely analogous fashion from Xb and the crude product (92%) melted at 155–160°. Recrystallization from hexane gave material of m.p. 160–161.5°; $\lambda_{max}^{cyclohexane}$ 236 mμ, ε 9650; $\lambda_{max}^{CHCl_3}$ 5.83 and 6.08 μ.

Anal. Calcd. for $C_{21}H_{32}O$: C, 83.94; H, 10.73. Found: C, 83.70; H, 10.75.

The ketones XIIa and XIIb were both easily isomerized to a mixture of the two by exposure to diffused daylight. After 1 hr., thin layer chromatography indicated that in both cases what was originally a solution of pure ketone in hexane became a mixture containing comparable amounts of both isomers. Similarly, base effected an equilibration of the isomers, presumably *via* the enolate anion with free rotation about the 17–20 bond. The stereochemistry of XIIa and XIIb and the allylic alcohols Xa and Xb, from which they were derived, was established by n.m.r. spectroscopy, as described in the Discussion section.

Deuterated 5α-Pregnan-16-ones (Table II).—The α,β-unsaturated ketone XIIa (20 mg.) was equilibrated in deuteriomethanol containing 10% heavy water and 8 mg. of dissolved sodium. After heating the solution under reflux for 30 min., it was evaporated at 25° under vacuum and heavy water added. Ether extraction gave 20 mg. of crude equilibrated ketone mixture, containing approximately equal quantities of the two geometrical isomers (as judged by thin layer chromatography). The crude product (mol. wt. 305 by mass spectrometry) was hydrogenated at atmospheric pressure in freshly purified ethyl acetate (5 ml.) in the presence of 6 mg. of 10% palladium-on-charcoal for 20 min. This gave **15,15,21,21,21-d_5-5α-pregnan-16-one**, m.p. 95–100°. Part of this material was back exchanged in aqueous methanolic sodium hydroxide solution and the product recrystallized from methanol, yielding **21,21,21-d_3-pregnan-16-one**, m.p. 103–103.5°, which showed infrared C–D absorption at 4.5 μ.

A similar equilibration in nondeuterated solvent, followed by catalytic hydrogenation, gave 5α-pregnan-16-one (XIII), identical in all respects with the sample obtained by oxidation of 5α-pregnan-16α-ol (XI).

Addition of Grignard Reagents to $\Delta^{17(20)}$-5α-Pregnen-16-ones (XII).—The procedure adopted for the 1,4-addition of different Grignard reagents to XII was essentially the same in each case and the following preparation of 21,21,21,22,22,22-d_6-20-methyl-5α-pregnan-16-one is representative.

A solution of 20 mg. of 15,15,21,21,21-d_5-$\Delta^{17(20)}$-5α-pregnen-16-one (mixture of *cis* and *trans* isomers, prepared as described above) in ether (6 ml.) was added to the Grignard solution prepared from d_3-methyl iodide (0.18 mg.) in 3 ml. of ether and containing 6 mg. of cuprous chloride. After refluxing the solution for 5 hr., it was poured onto ice–ammonium chloride solution and the product isolated by ether extraction. The crude material was chromatographed on 15 g. of neutral alumina (activity I alumina deactivated by addition of 3% of water) using 15% benzene in hexane as eluent. In this way, 12 mg. (56%) of 15,15,21,21,21,-22,22,22-d_8-20-methyl-5α-pregnan-16-one was obtained, m.p. 90.5–91°. This material was exchanged in 2 ml. of 90% methanol containing 10 mg. of dissolved sodium (2-hr. reflux). Evaporation and recrystallization of the ether-extracted product from methanol gave pure 21,21,21,22,22,22-d_6-20-methyl-5α-pregnan-16-one, m.p. 91–91.5°.

20-Methyl-5α-pregnan-16-one (XVIII) was prepared in the fashion described for the d_6-analog, except that the deuterium and back-exchange steps were omitted and normal methyl iodide was employed. Again, recrystallization of the product from methanol gave pure XVIII, m.p. 91.5–92.5°, $[\alpha]^{28}D$ −148° (*c* 0.4), $\lambda_{max}^{CHCl_3}$ 5.75 μ.

Anal. Calcd. for $C_{22}H_{36}O$: C, 83.48; H, 11.47. Found: C, 83.75; H, 11.53.

Exchange of the acidic protons for deuterium, in the usual way, gave 15,15,17-d_3-XVIII, m.p. 91.5–92° (see Table II). The 21,21,21-d_3-analog was prepared by the Grignard addition of d_3-methylmagnesium iodide to the undeuterated α,β-unsaturated ketone; m.p. 89–89.5° (see Table II).

20-Ethyl-5α-pregnan-16-one (XIX).—The pure *cis*-ketone XIIa (40 mg.), when treated in the presence of cuprous chloride with Grignard reagent prepared from 406 mg. of ethyl iodide, gave, after chromatography, 30 mg. (82%) of XIX, m.p. 82–83.5°. Recrystallization of this product from methanol yielded pure ketone, m.p. 84–85° (one spot on thin layer chromatography), $[\alpha]^{25}D$ −152° (*c* 0.6).

Anal. Calcd. for $C_{23}H_{38}O$: C, 83.57; H, 11.59. Found: C, 83.71; H, 11.66.

The 15,15,21,21,21-d_5-analog (77%, m.p. 75–76°) of XIX was obtained by reaction of ethylmagnesium iodide with the deuterated α,β-unsaturated ketone XV, followed by chromatography of the crude product on activity II neutral alumina. Back exchange of this material in methanolic sodium hydroxide gave 21,21,21-d_3-20-ethyl-5α-pregnan-16-one, m.p. 76–77°, and exchange in deuterated solvent provided the 15,15,17,21,21,21-d_6 analog, while 21,21,21,22,22,23,23,23-d_8-XIX (m.p. 76–77°) was obtained from the exchanged α,β-unsaturated ketone XV and d_5-ethylmagnesium iodide, followed by back exchange from the acidic 15-position and crystallization. The mass spectrometric details of these deuterated analogs of XIX are collected in Table III.

20-Isopropyl-5α-pregnan-16-one (XX).—The reaction between $\Delta^{17(20)}$-5α-pregnen-16-one (XIIa, 21 mg.) and the Grignard reagent prepared from isopropyl bromide (310 mg.) was conducted in the usual way. Chromatography gave the following products:

First a trace (<1 mg.) of a crystalline solid, m.p. 101–104° after recrystallization from methanol. This material gave one spot on thin layer chromatography and was assigned the structure XX on the basis of its infrared ($\lambda_{max}^{CHCl_3}$ 5.8 μ) and mass spectrum (Fig. 6). Second, 3 mg. of another solid, m.p. 100.5–102° after recrystallization from methanol. The m.p. was not depressed (102–103°) on admixture with 5α-pregnan-16-one (XIII) and the infrared and mass spectra were identical. Third, a highly polar material (5 mg.), m.p. 305–307° after recrystallization from ethanol–benzene ($\lambda_{max}^{CHCl_3}$ 5.78 μ). The mass spectrum obtained by use of a direct inlet system[22] indicated a molecular weight of 600.

20-Isobutyl-5α-pregnan-16-one (XXI).—Chromatography of the product from the reaction between $\Delta^{17(20)}$-5α-pregnen-16-one (XIIa, 30 mg.) and the Grignard reagent prepared from 1-bromo-2-methylpropane (274 mg.) with 10 mg. of cuprous chloride present gave 3 mg. of XXI, m.p. 151–154°, $\lambda_{max}^{CHCl_3}$ 5.77 μ, after recrystallization from methanol. This was followed by 5 mg. of 5α-pregnan-16-one (XIII), m.p. 101–102.5° after recrystallization from methanol.

20α-(XXIIa) and 20β-(XXIIb)(3-Methylbutyl)-5α-pregnan-16-ones.—The 1,4-addition reaction was performed as usual, with 30 mg. of α,β-unsaturated ketone XIIa and the Grignard reagent from 280 mg. of 1-bromo-3-methylbutane, containing 10 mg. of cuprous chloride. Chromatography gave, first, 6 mg. of crystalline 20β-ketone XXIIb, m.p. 107.5–108.5° after recrystallization from methanol, and showing one spot on thin layer chromatography, $\lambda_{max}^{CHCl_3}$ 5.78 μ. This material was followed by a mixture (two close spots on thin layer chromatography) (15 mg.) and then by the 20α-ketone XXIIa (6 mg.), m.p. 76.5–78° after recrystallization from methanol, $\lambda_{max}^{CHCl_3}$ 5.78 μ. A mixture of the two isomers melted below 60°. The mass spectrometrically determined molecular weight of both ketones was 372 and their stereochemical assignment is based on the presence of the *m/e* 232 peak in the spectrum (Fig. 8) of one (XXIIb) but not the other (XXIIa) isomer.

20α-(XXIIIa) and 20β-(XXIIIb) Cholestan-16-ones.—A solution of $\Delta^{17(20)}$-5α-pregnen-16-one (XIIa, 230 mg.) in dry ether (25 ml.) was added to the Grignard solution prepared from 1.32 g. of isohexyl bromide and 200 mg. of magnesium in 25 ml. of ether (containing 25 mg. of dry cuprous chloride). The reaction mixture was heated under reflux for 4 hr. and poured into ice–ammonium chloride solution, and the ether layer separated, washed, and dried. Chromatography on activity II neutral alumina (60 g.), using 10% benzene in hexane, gave 215 mg. (73%) of cholestan-16-one (20α and 20β), m.p. 55–70°, $\lambda_{max}^{CHCl_3}$ 5.79 μ; R.D. in methanol (*c*, 0.1): $[\alpha]_{589}$ −98°, $[\alpha]_{320}$ −3310°, $[\alpha]_{274}$ +3530°, $[\alpha]_{250}$ +2490°.

A separation of the 20α- and 20β-isomers could be achieved by careful chromatography. Thus, 20 mg. of the above mixture when chromatographed on 20 g. of activity II neutral alumina, using 8% benzene in hexane, gave first the 20β-isomer XXIIIb, m.p. 92.5–94° after recrystallization from methanol. A mixture m.p. with pure 20β-cholestan-16-one (described below) was undepressed and both specimens exhibited an identical R_f value on thin layer chromatography. This material was followed by a mixture and finally by the 20α-isomer XXIIIa, m.p. 57–58° (after recrystallization from methanol), which was not depressed by admixture with pure 20α-cholestan-16-one described below.

Deuterated cholestan-16-ones (see Table IV) were obtained by the standard 1,4-addition of deuterated Grignard reagents to XIIa, or, in the case of 15-, 17-, and 21-deuterated analogs, by suitable exchange procedures. The deuterated bromo-4-methylpentanes required to prepare these Grignard solutions were obtained as described below. In most instances the 20α- and 20β-cholestan-16-one analogs were separated for mass spectroscopic examination (by chromatography), as described above for the undeuterated compounds.

1-Bromo-4-methylpentane[40] was conveniently prepared from 4-methylvaleric acid by reduction and conversion of the alcohol into the bromide. 4-Methylvaleric acid (11.6 g.) in dry ether

(40) C. R. Fordyce and J. R. Johnson, *J. Am. Chem. Soc.*, **55**, 3370 (1933).

(150 ml.) was added dropwise with stirring under nitrogen to a solution of lithium aluminum hydride (4.75 g.) in ether (180 ml.). The mixture was heated under reflux for 30 min., then cooled and decomposed by addition of water, followed by dilute sulfuric acid (150 ml., 10%). Crude 4-methylpentanol was isolated from the ether layer and treated directly with a mixture of hydrobromic acid (14.2 ml., 48%) and concentrated sulfuric acid (3.3 ml.) under reflux for 1 hr. The mixture was distilled slowly (1.5 hr.) and more sulfuric acid (0.55 ml. in five portions) added during the distillation. After separating the crude bromide, it was washed with sulfuric acid (2.5 ml.), then taken up in ether, and the ethereal solution washed with dilute sodium bicarbonate solution and water. The distilled product (10.5 g., 64%) boiled at 145–147° (lit.[40] b.p. 142–145°) and showed one peak on gas-phase chromatography (PDEAS column operated at 85°), as did the crude bromide.

1,1-d_2-1-Bromo-4-methylpentane was prepared in the same way as the nondeuterated substance except that lithium aluminum deuteride was used in place of lithium aluminum hydride. Furthermore, in the conversion of the alcohol into the bromide, the addition of sulfuric acid during the distillation was omitted. Thus the alcohol XXVII from 1.16 g. (0.01 mole) of 4-methylvaleric acid was treated with a mixture of 1.5 ml. of 48% hydrobromic acid and 0.4 ml. of concentrated sulfuric acid, and the mixture refluxed for 1.5 hr. The bromide was distilled out over a 30-min. period and separated as before. Redistillation provided 0.6 g. of pure bromide, which was used for all the smaller-scale preparations of deuterated 1-bromo-4-methylpentanes.

2,2-d_2-1-Bromo-4-methylpentane.—Isovaleric acid (5.1 g.) was reduced with lithium aluminum deuteride (2.5 g.) in a total volume of 165 ml. of ether, the solution being heated under reflux for 1 hr. after addition of the acid was completed. Decomposition with water, followed by addition of 10% sulfuric acid, and isolation from the ether layer, gave 4.7 g. of crude alcohol. This was converted into the bromide using a mixture of 48% hydrobromic acid (7.5 ml.) and concentrated sulfuric acid (2 ml.), by heating under reflux for 4 hr. Distillation and separation from the aqueous layer gave crude 1,1-d_2-1-bromo-3 methylbutane, which was purified in the usual way and redistilled. This bromide was transformed into the Grignard reagent (0.8 g. of magnesium in 50 ml. of ether) and treated at −20 to −15° with carbon dioxide (dried by passage through concentrated sulfuric acid). The reaction mixture was then decomposed with sulfuric acid (17 ml., 50%), and the aqueous layer separated and extracted twice more. The combined ether layers were extracted twice with 10% potassium hydroxide (10 ml., 5 ml.) and the aqueous layers combined, washed with ether, and acidified (concentrated hydrochloric acid). Ether extraction gave the crude 2,2-d_2-4-methylvaleric acid, which was reduced with lithium aluminum hydride. The resulting alcohol XXVIII (1.86 g.) was converted into the bromide (2.2 g., pure) as previously described for the nondeuterated material.

3,3-d_2-1-Bromo-4-methylpentane.—Isobutyric acid (4.4 g.) was reduced with lithium aluminum deuteride (2.5 g.) in 165 ml. of ether and the 1,1-d_2-isobutyl alcohol isolated in the usual way by decomposition with water and 10% sulfuric acid. To the crude, dry alcohol was added (below 0°) 1.75 ml. of phosphorus tribromide, with stirring, and the mixture was allowed to warm to room temperature, then stirred for 15 hr., and distilled at 150 mm. The bromide was purified by washing with concentrated sulfuric acid (3 × 0.35 ml.) and dried over potassium carbonate. Distillation through a short Vigreux column gave 2.4 g. of product, b.p. 89–90.5°. The Grignard reagent prepared from this bromide (2.1 g.) and magnesium (0.36 g.) in ether (5 ml.) was treated with ethylene oxide (2.0 g.) in ether (5 ml.) below 10°. After the mixture had warmed up, it was heated under reflux for 30 min., and benzene (8 ml.) added. Distillation was carried out until the temperature of the residual liquid had reached 65°, whereupon refluxing was continued for a further 30 min. The mixture was cooled and decomposed with water and then with dilute sulfuric acid (15 ml. 10%). The organic layer was separated, washed with saturated sodium bicarbonate solution, and dried. Evaporation and distillation gave crude 3,3-d_2-4-methylpentanol (XXIX) which was treated with 4 ml. of 20% sodium hydroxide solution at 100° for 10 min., then separated, and dried (0.7 g.) The bromide (0.52 g.) was prepared from it in the usual way.

4-d_1-1-Bromo-4-methylpentane.—Diethyl 3-benzyloxypropylmalonate (XXX, R = H,[27] 15.4 g.) in dry tetrahydrofuran (20 ml.) was added cautiously to sodium hydride (2.47 g., 50.6%) in 25 ml. of the same solvent. The mixture was heated under reflux for 15 min., cooled, and treated with heavy water (1 ml.) at 5° while cooling. The solvent was removed at room temperature under vacuum and heavy water (20 ml.) added to the residue, which was then extracted into ether. The ethereal solution of XXX (R = D) was washed twice with heavy water, dried over magnesium sulfate, and then added to a solution of lithium aluminum hydride (4.8 g.) in ether (total volume 160 ml.). After heating under reflux for 4 hr., the solution was treated with saturated sodium sulfate solution, boiled briefly, and filtered. The dried filtrate was evaporated and washed three times with hexane to remove the oil originally in the sodium hydride suspension. This left 5.1 g. of the oily diol (XXXI, R = D). Part of this diol (3.92 g.) in dry pyridine (14 ml.) was treated at 8° with *p*-toluenesulfonyl chloride (6.86 g.). The mixture was then stirred at 25° for 16 hr., diluted with a large volume of water, and extracted twice with methylene chloride. The extracts were washed three times with 1 *N* hydrochloric acid, then with dilute sodium bicarbonate and water, and dried. Evaporation gave the crude, oily ditosylate XXXII (R = D, 8.34 g.), which was chromatographed on Florisil (400 g.) using 5% ether in benzene as initial eluent and increasing the ether concentration to 50%. This yielded the purified ditosylate (6.4 g.) as an oil, which gave only one spot on thin layer chromatography (10% ethyl acetate in benzene). Part of this ditosylate (3.8 g.) was reduced overnight at 25° with 1.5 g. of lithium aluminum hydride in 120 ml. of ether, the reaction mixture being decomposed with cooling by addition of water, followed by 10% sulfuric acid. The crude ether XXXIII (R = D, R′ = H) was salted out, extracted into ether, and the solution washed with sodium bicarbonate and dried. Evaporation gave a colorless oil (1.5 g.), containing some polar (alcohol?) contaminant (as evidenced by t.l.c. analysis), and the pure benzyl ether XXXIII (R = D, R′ = H, 1.3 g.) was obtained by passage in 20% ether in pentane through a column of activity II alumina (60 g.). Cleavage of the benzyl ether was effected by addition (in 20 ml. of ether) to a solution of 0.8 g. of lithium in *ca.* 150 ml. of liquid ammonia. The mixture was stirred for 4 hr., decomposed by cautious addition of ammonium chloride, and the ammonia allowed to evaporate. Water and more ether were added and the ether layer separated and dried. Evaporation of the ether left pure 4-d_1-4-methylpentanol (XXXIV, R = D, R′ = H, 0.83 g.) which was converted into the bromide (1.03 g.) in the usual way.

1-Bromo-4-(d_1-methyl)-5d_1-pentane was made as described above for the 4-d_1-1-bromo-4-methylpentane, except that the deuteration of the starting malonate XXX (R = H) was omitted and lithium aluminum deuteride was employed in place of lithium aluminum hydride in the reduction of the ditosylate. Diethyl 3-benzyloxypropylmalonate (XXX, R = H, 10.5 g., 0.034 mole) gave on reduction 6.6 g. of crude diol which was converted into the ditosylate as described above. Again the purified ditosylate remained an oil, but it gave the correct analysis.

Anal. Calcd. for $C_{27}H_{32}O_7S_2$: C, 60.88; H, 6.06. Found: C, 60.73; H, 6.22.

Subsequent steps were carried out exactly as described for the 4-d_1-analog.

20α- (XXVIIa) and 20β- (XXVIIb) 17α-Bromocholestan-16-ones.—The mixture of 20α- and 20β-cholestan-16-ones (193 mg.), prepared as described above, was dissolved in acetic acid (2.5 ml.), and a solution of bromine (80 mg.) in 0.8 ml. of acetic acid was added in six portions over a 15-min. period. After continuing to stir well for a further 5 min., the crude 20β-17α-bromo ketone XXVIIb was filtered and washed with methanol. Recrystallization of this crude product (162 mg., m.p. 118–121°) from ethanol, gave material (106 mg., m.p. 120–123°) showing only one spot on thin layer chromatography. An analytical sample, recrystallized once more from ethanol, melted at 122–124°, $[\alpha]^{23}D$ +24.8° (*c*, 2.1). The n.m.r. spectrum showed no absorption attributable to hydrogen situated on the same carbon as bromine.

Anal. Calcd. for $C_{27}H_{45}BrO$: C, 69.66; H, 9.84; Br, 17.13. Found: C, 69.64; H, 9.74; Br, 17.16.

Chromatography of the evaporated (room temperature, vacuum) mother liquors from the bromination reaction gave an oil exhibiting a single spot on thin layer chromatography of lower R_f value than XXVIIb, and assigned the C-20 isomeric structure XXVIIa.

Zinc Dust Reduction of the Isomeric 17α-Bromocholestan-16-ones (XXVIIa and b).—The pure 20β-isomer XXVIIb (13 mg.) was dissolved in a mixture of ether (2 ml.) and acetic acid (0.5 ml.) and stirred with 45 mg. of zinc dust for 3 hr. The mixture was then filtered, diluted with ether, and the ethereal solution washed with sodium bicarbonate solution and dried. Thin layer chromatography indicated that the product was a mixture of two components and the less polar one (7 mg., m.p. 90.5–91.5°) was isolated by chromatography (10 g. of silica gel, Merck). Recrystallization from methanol gave pure XXIIIb, m.p. 95–96°. Substitution of deuterioacetic acid in the above procedure gave 17d_1-XXIIIb consisting of 52% d_0- and 48% d_1-species. A very similar reduction of the oily bromoketone XXVIIa gave XXIIIa, m.p. 67–68.5°, not depressed on admixture with the catalytic reduction product (m.p. 70–71°), from XXVIIIa described below.

Dehydrobromination of 17α-Bromo-20β-cholestan-16-one (XXVIIb).—The bromoketone XXVIIb (45 mg.) was dissolved in 1 ml. of freshly distilled dimethylacetamide, 45 mg. of calcium carbonate was added, and the mixture was heated under reflux with stirring for 15 min. The solvent was removed under vac-

uum at room temperature, the residue taken up in ether, and the solution washed with 1 *N* hydrochloric acid and water and dried. Evaporation gave 37 mg. of an oil which showed three spots on thin layer chromatography. Chromatography of the mixture on activity II neutral alumina (18 g.) using 5% benzene in hexane separated the first component (16 mg., m.p. 91.5–94°) from the other two products (8 mg.) which were eluted together as an oil. The first component, $\Delta^{17(20)}$-cholestan-16-one (XXVIIIa), was recrystallized quickly from methanol in subdued light, whereupon it melted at 97.5–98°, $\lambda_{max}^{CHCl_3}$ 5.90 and 6.21 μ; $\lambda_{max}^{cyclohexane}$ 250 mμ, ϵ 11,600.

Anal. Calcd. for $C_{27}H_{44}O$: mol. wt., 384.6. Found: mol. wt., 384 (mass spec.).

The oily mixture exhibited a very similar mass spectrum as compared to that of the crystalline isomer XXVIIIa and exhibited $\lambda_{max}^{CHCl_3}$ 5.79, 5.90, and 6.21 μ. Dehydrobromination of a mixture of XXVIIIa and b gave essentially the same results. In order to obtain the other geometric isomer XXVIIIb in a pure state, 46 mg. of XXVIIIa was dissolved in 10 ml. of hexane and the solution exposed (using an aluminum foil reflector) to direct sunlight for 1 hr. The resulting mixture was chromatographed in subdued light on 20 g. of activity II neutral alumina, using 10 and 20% benzene in hexane as eluent. First there was eluted 31 mg. of recovered XXVIIIa, followed by 13 mg. of a homogeneous (thin layer chromatography) oil (XXVIIIb), which exhibited $\lambda_{max}^{CHCl_3}$ 5.90 and 6.21 μ, $\lambda_{max}^{cyclohexane}$ 248 mμ, ϵ 12,100, as well as a mass spectrometrically determined molecular ion peak at *m/e* 384.

Reduction of the Isomeric $\Delta^{17(20)}$-Cholestan-16-ones (XXVIIIa and b).—Pure $\Delta^{17(20)}$-cholestan-16-one (XXVIIIa, 50 mg.) in 15 ml. of cyclohexane was hydrogenated at 22° for 1 hr. in the presence of 25 mg. of 10% palladium-on-charcoal. The solution was filtered and evaporated, leaving a crude product, m.p. 53–56°. Recrystallization from methanol gave pure 20α-cholestan-16-one (XXIIIa), m.p. 58–59°. Sometimes another polymorphic form was obtained, m.p. 70–71°. In either case, the melting point was not depressed when mixed with the sample of XXIIIa prepared from zinc dust reduction of XXVIIa or from chromatography of the 1,4 Grignard addition reaction to XII. In each case, the R_f values (thin layer chromatography) of XXVIIa from the different sources were identical, and not as high as for XXVIIb.

An identical catalytic reduction of the oily α,β-unsaturated ketone (XXVIIIb) gave material, m.p. 89.5–92°, after recrystallization from methanol. Its m.p. was not depressed (91–94°) on admixture with XXIIIb prepared by the zinc dust reduction of XXVIIb (above), and the R_f values of XXIIIb from these sources were identical with each other, but higher than that of XXIIIa. A mixture of XXIIIa and b showed a marked melting point depression (48–63°).

A small-scale lithium in liquid ammonia reduction of XXVIIIa in tetrahydrofuran gave a mixture of XXIIIa and b (as judged by thin layer chromatography), with the 20α-isomer XXIIIb predominating; infrared spectroscopy indicated that there was no unreacted α,β-unsaturated ketone present.

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

Mass Spectrometry in Structural and Stereochemical Problems. XLI.[1] Isotope Effect in Hydrogen Rearrangement Processes: The Mass Spectra of Methyl Butyrate and Its γ-Mono-, Di-, and Trideuterio Analogs

BY D. H. WILLIAMS, H. BUDZIKIEWICZ, AND CARL DJERASSI

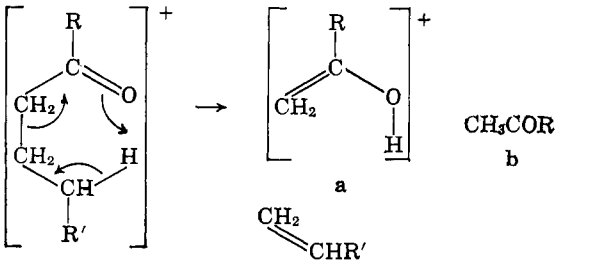
RECEIVED AUGUST 5, 1963

The mass spectra of methyl butyrate and its γ-mono-, di-, and trideuterio analogs have been measured and an isotope effect corresponding to a transfer of 0.88 atom of deuterium per atom of hydrogen in a specific rearrangement process has been observed. This effect is probably also operative in many related γ-hydrogen rearrangements observed in mass spectrometry.

The usefulness of deuterium-labeling of organic molecules for gaining insight into fragmentation and specially rearrangement processes under electron impact has been demonstrated frequently, some recent examples being provided by fatty acids[2] and steroids.[3] In some cases, hydrogen transfer reactions are straightforward and involve the shift of only one specific hydrogen atom, whereupon they can be followed easily in the deuterated analogs. In other instances, one specific fragmentation process involves a complex series of different hydrogen rearrangements (*e.g.*, 11-keto steroids[4]), and for the elucidation of such fragmentations, quantitative measurements of deuterium transferred from different positions is necessary.

It has been shown that deuterium is less easily split off from hydrocarbons than is hydrogen,[5] and that in the loss of water from cyclic ketones[6] or cyclohexanol[1] discrimination against deuterium takes place. No isotope effect is usually taken into consideration for cyclic transition states,[3] mainly because no relevant data are available. It seemed of importance, therefore, to investigate this problem in a specific, well-defined mechanism, which would allow, if possible, wide generalization.

One of the most important fragmentation processes of aliphatic carbonyl compounds (ketones,[7] aldehydes,[8] esters[9]) is cleavage of the carbon–carbon bond β to the carbonyl group accompanied by rearrangement of one hydrogen atom. This outstanding fragmentation



process was soon subject to many investigations. Thus Beynon[10] pointed out that this rearrangement must be energetically very favorable since it can be observed at low energies where hydrogen rearrangement processes are usually not observed. By labeling the carboxyl group of butyric acid with ^{13}C it could be demonstrated[11] that the fragment in question definitely retains this carbon atom. McLafferty[12] was the first to suggest that the genesis of this cleavage product involves a six-membered transition state in which a γ-hydrogen atom is transferred to oxygen. Appearance potential measurements of the rearrangement ion are in agree-

(1) Paper XL: H. Budzikiewicz, Z. Pelah, and C. Djerassi, *Monatsh.*, in press.

(2) N. Dinh-Nguyen, R. Ryhage, S. Ställberg-Stenhagen, and E. Stenhagen, *Arkiv Kemi*, **18**, 393 (1961).

(3) C. Beard, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **86**, 269 (1964), and references cited therein.

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

(5) F. H. Field and J. L. Franklin, "Electron Impact Phenomena," Academic Press, Inc., New York, N. Y., 1957, Chapter 5.

(6) E. Lund, H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 941 (1963).

(7) A. G. Sharkey, J. L. Shultz, and R. A. Friedel, *Anal. Chem.*, **28**, 934 (1956).

(8) J. A. Gilpin and F. W. McLafferty, *ibid.*, **29**, 990 (1957).

(9) R. Ryhage and E. Stenhagen, *Arkiv Kemi*, **13**, 513 (1959).

(10) J. H. Beynon, "Mass Spectrometry and its Application to Organic Chemistry," Elsevier Publishing Co., New York, N. Y., 1960, p. 356.

(11) G. P. Hap and D. W. Stewart, *J. Am. Chem. Soc.*, **74**, 4404 (1952).

(12) F. W. McLafferty, *Anal. Chem.*, **31**, 82 (1959).