Mass Spectrometry in Structural and Stereochemical Problems. CXXVII.[1] Synthesis and Fragmentation Behavior of Deuterium-Labeled 20-Keto Steroids[2]

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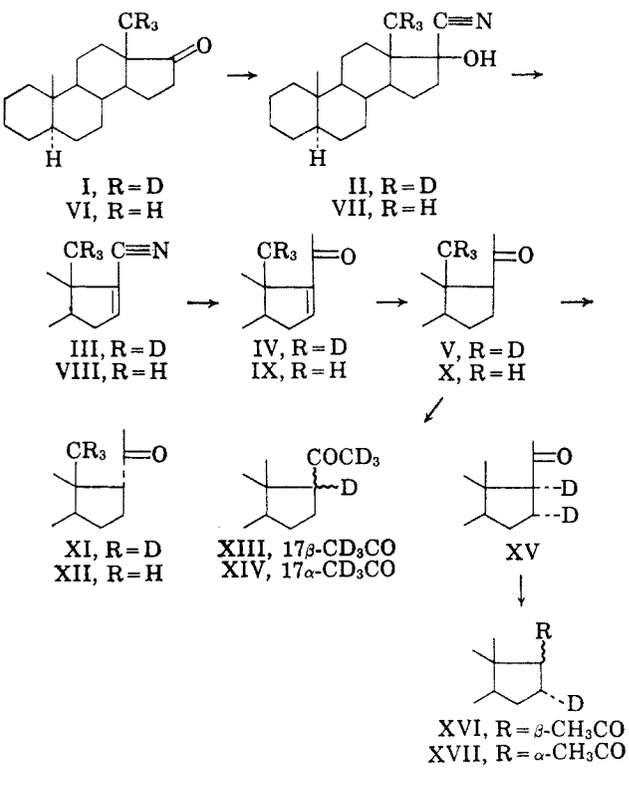
The mass spectra of 5α-pregnan-20-one and its 17α epimer are very nearly identical. The deuterium label at C-12 is not removed in the formation of *m/e* 217. This finding is in accord with the minimum interatomic distance requirement for McLafferty rearrangements. Complete retention of label at C-9 and C-18 is also observed but label at C-16, C-17, and C-21 is lost completely while label at C-8 is removed to the extent of 40%. These results indicate that at least a portion of the *m/e* 217 peak originates by transfer of hydrogen from C-8 to the radical site (C-17) followed or accompanied by the fission of the C-14,15 bond. The genesis of each of the significant peaks in the high mass range of the mass spectrum of 5α-pregnan-20-one is discussed.

Just as in the preceding report, which dealt with the mass spectra of 17-keto steroids,[1] the problem of principal interest in the study of the 20-keto steroids was that centering on the nature of the hydrogen migrations accompanying fragmentation. Upon examining the mass spectra of several polysubstituted pregnanes,[4] Peterson pointed out that the loss of ring D (carbon atoms 15, 16, and 17) along with the attached side chain and an extra hydrogen atom from the charge retaining moiety, is a characteristic cleavage process of pregnan-20-ones lacking oxygen-containing functions at C-17. The deuterium labeling studies reported herein were undertaken to provide information pertaining to the nature of this migration and the other fragmentation characteristics of 20-keto steroids. Such a study is particularly relevant because of the large number of biochemically significant steroid hormones and metabolites possessing this structural feature.

Synthesis of 20-Keto Steroids.—One of the prime candidates from which hydrogen migration was suspected was the 18-angular methyl group. To check this possibility the C-18 deuterated pregnan-20-one was prepared. The initial attempt to achieve this synthesis involved functionalization of C-18 and subsequent conversion to a deuterated methyl group.[5] However, this method led to unexpected difficulties and was eventually abandoned for the conversion of the appropriately labeled 5α-androstan-17-one[1] to the labeled 5α-pregnan-20-one. Thus the conversion of *dl*-18,18,18-d_3-5α-androstan-17-one (I) to *dl*-18-d_3-5α-pregnan-20-one (V) was effected by a well-known scheme[6] involving the sequence of steps shown (I → V). In the same manner 5α-androstan-17-one (VI) was converted to 5α-pregnan-20-one (X). By alkaline equilibration of V and X and separation of the isomers by thin layer chromatography, small amounts of 5α,17α-pregnan-20-one (XII) and *dl*-18-d_3-5α,17α-pregnan-20-one (XI) were obtained. By effecting equilibration in deuteriomethanol, an epimeric mixture of 17,21,21,21-d_4-17β- (XIII) and 17,21,21,21-d_4-17α,5α-pregnan-20-one (XIV) was obtained.

The preparation of 16α,17α-d_2-5α-pregnan-20-one (XV) was achieved by catalytic deuteration of Δ[16]-5α-pregnen-20-one (IX). The low isotopic purity of the product (4% d_0, 23% d_1, 47% d_2, 15% d_3, and 11% d_4) was largely due to deuterium incorporation at the C-21 α position since base-catalyzed back exchange of the 17α-deuterium atom effected simultaneous epimerization at C_{17} and produced 17β- (XVI) and 17α,16α-d_1-5α-pregnan-20-one (XVII), which displayed an improved isotopic purity of 21% d_0, 78% d_1, and only 1% d_2 (see Scheme I).

SCHEME I



The key intermediate in the preparation of the important 12,12-d_2-, 8β-d_1-, and 9α-d_1-5α-pregnan-20-ones was the previously described[7] 5α-pregnane-12,20-dione (XVIII). Selective thioketalization of the dione was achieved by using an equimolar amount of ethanedithiol in dilute acetic acid solution. That C-12 monothioketal formation had occurred was based on the known[8] preference for such ketalization in the 12,20-dione system. This premise was confirmed subse-

(1) Paper CXXVI: L. Tökés, R. T. LaLonde, and C. Djerassi, *J. Org. Chem.*, **32**, 1012 (1967).

(2) Financial assistance by the National Institutes of Health (Grants No. CA-07195 and AM-04257) of the U. S. Public Health Service is gratefully acknowledged.

(3) Recipient of a National Institutes of Health Special Fellowship, 1965–1966 while on leave from the College of Forestry, Syracuse University.

(4) L. Peterson, *Anal. Chem.*, **34**, 1781 (1962).

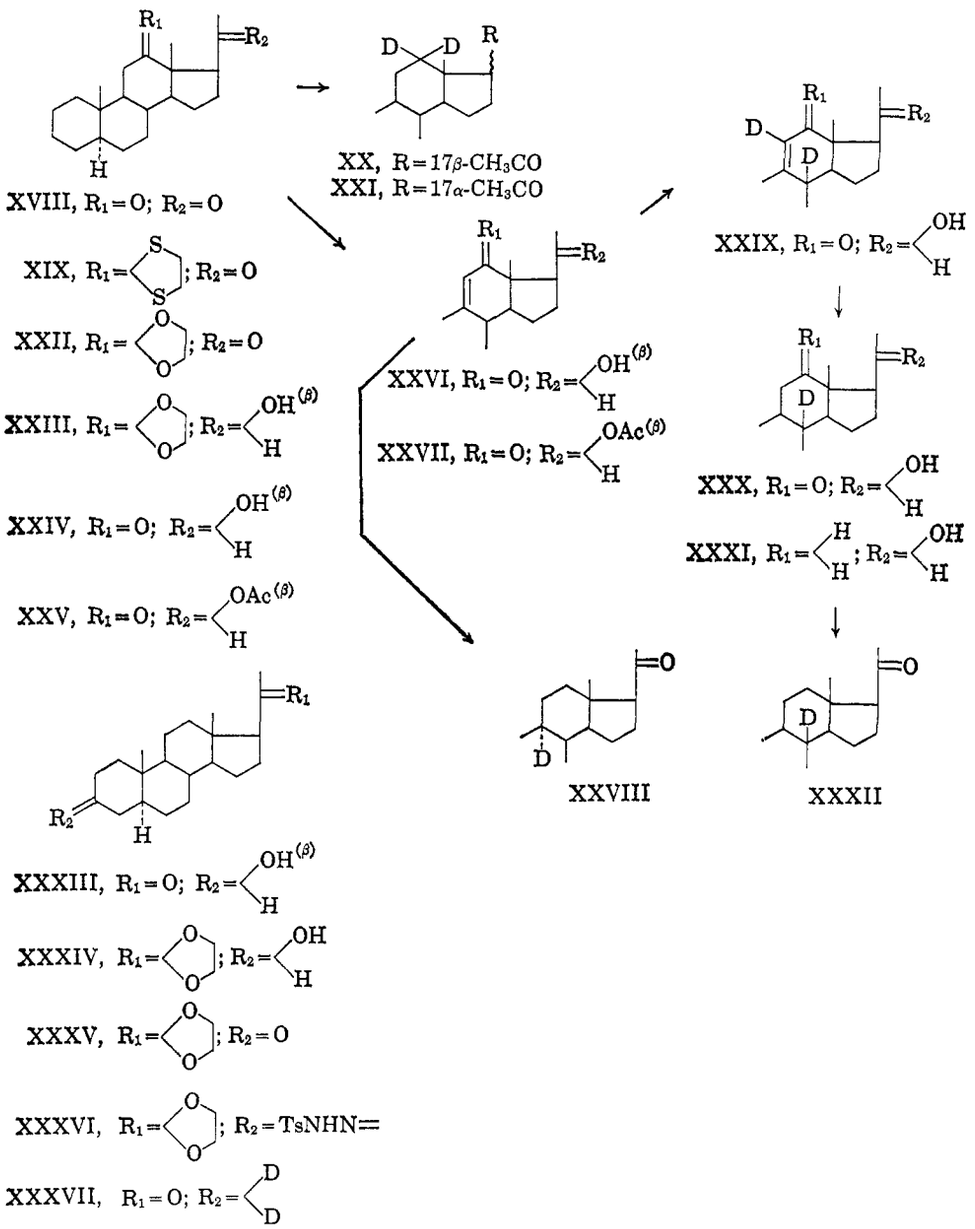
(5) We acknowledge the attempt by Dr. P. Laur of this laboratory to prepare the labeled angular methyl group in the described manner.

(6) C. W. Shoppee, "Chemistry of the Steroids," 2nd ed, Butterworth Inc., Washington, D. C., 1964, p 180.

(7) C. Djerassi and L. Tökés, *J. Am. Chem. Soc.*, **88**, 536 (1966).

(8) D. N. Kirk, P. K. Patel, and V. Petrow, *J. Chem. Soc.*, 1046 (1957).

SCHEME II



quently by the successful conversion of thioketal XIX to 12,12-d_2-5α,17β- (XX) and 17α-pregnan-20-one (XXI), a conversion effected with deuterated Raney nickel[9] followed by reoxidation[10] of the resulting mixture of C-20 alcohols and base epimerization at C-17.

Introduction of deuterium at C-8 and C-9 was accomplished through the intermediate 20β-hydroxy-$\Delta^{9(11)}$-5α-pregnen-12-one (XXVI) and its acetate (XXVII). Conversion of the 12,20-dione into the known[7] C-12 monoketal (XXII) and subsequent reduction of the free keto group with sodium borohydride in methanol gave the 20β-hydroxy ketal (XXIII)[11] which on hydrolysis afforded 20β-hydroxy-5α-pregnan-12-one (XXIV). Treatment of XXIV with acetic anhydride–pyridine for 24 hr at 60° resulted only in partial conversion to the 20β-acetate (XXV). This acetylation was deemed necessary in order to prevent the reoxidation of the 20β-hydroxy function to the 12,20-dione during the selenium dioxide dehydrogenation to the desired α,β-unsaturated ketone (XXVI). However, it was found later that the 20β-hydroxy 12-ketone could be dehydrogenated directly to the α,β-unsaturated keto alcohol (XXVI) in 85% yield. The difficult acetylation of the 20β-hydroxyl group in the related 3α,20β-dihydroxy-$\Delta^{9(11)}$-5α-pregnen-12-one has been noted recently.[13] This behavior was attributed to the sterically hindered conformation in which the 20β- (but not the 20α-) hydroxyl group exists, and the strong intramolecular hydrogen bonding of this hydroxyl group with the $\Delta^{9(11)}$-12-keto function. Since a Dreiding model illustrates that somewhat the same steric situation exists in the saturated ketone (XXIV), the diffi-

(9) D. H. Williams, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 2091 (1963); see also C. Djerassi and D. H. Williams, *J. Chem. Soc.*, 4046 (1963).

(10) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *ibid.*, 39 (1946).

(11) The β configuration at C-20 was assigned on the basis of the reported[8] preference for the formation of the 20β-ols when 20-ketones are reduced with sodium borohydrate. This assignment was confirmed by the positive shift in molecular rotation ($[\phi]_D$ +122°) on acetylation, a result in accord with the well-established patterns[12] of 20β-ols.

(12) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, Chapter 19.

(13) J. C. Grivas, *J. Org. Chem.*, **31**, 1349 (1966).

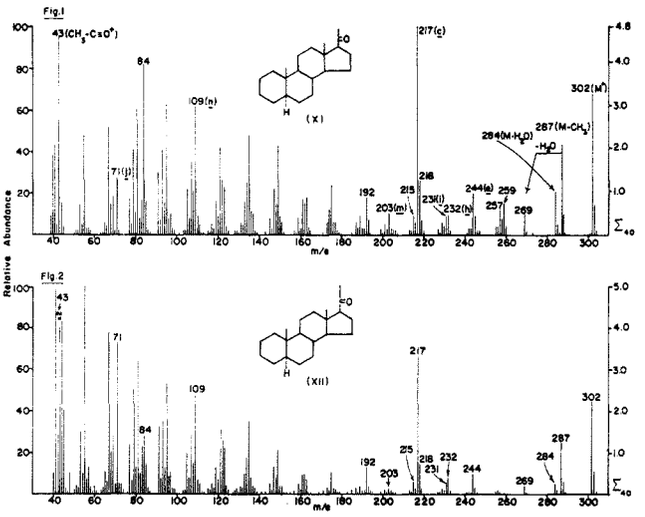


Figure 1.—Mass spectrum of 5α-pregnan-20-one (X).
Figure 2.—Mass spectrum of 5α,17α-pregnan-20-one (XII).

culty of acetylation as well as the inertness of the 20β-hydroxyl group in the dehydrogenation step also can be rationalized on the grounds of steric hindrance and intramolecular hydrogen bonding in both the saturated and α,β-unsaturated ketones.

Catalytic deuteration of the α,β-unsaturated ketone (XXVI), back exchange of deuterium at C-11, reduction of the C-12 keto group by a modified Wolff–Kishner procedure,[14] and Jones oxidation[10] afforded 9α-d_1-5α-pregnan-20-one (XXVIII). Treatment of the α,β-unsaturated ketone (XXVI) with sodium deuteroxide in refluxing deuterium oxide–deuteriomethanol effected deuteration at C-8 and C-11. The resulting α,β-unsaturated ketone (XXIX) was hydrogenated catalytically and subjected to base exchange to remove deuterium at C-11 and thereby gave the saturated, deuterated ketone (XXX) which in turn was reduced by the modified Wolff–Kishner procedure[14] to the deuterated 20β-ol (XXXI). Jones oxidation[10] of XXXI gave the 8β-d_1-5α-pregnan-20-one (XXXII) of very high isotopic purity (see Table I and Scheme II).

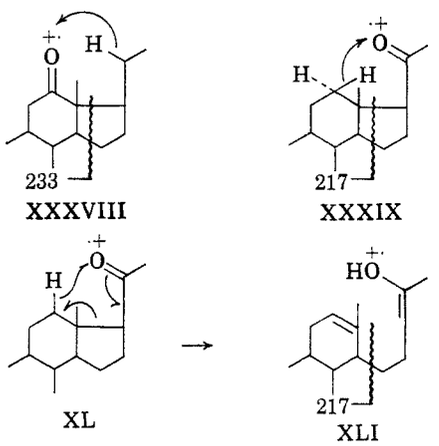
Incorporation of two deuterium atoms at C-3 was effected in the following manner. Conversion of 5α-pregnan-3β-ol-20-one (XXXIII) into its ethylene ketal derivative XXXIV and subsequent chromium trioxide oxidation in pyridine, were carried out according to the procedures of Schütt and Tamm[15] and produced 5α-pregnane-3,20-dione 20-monoethylene ketal (XXXV). Lithium aluminum deuteride treatment[16] of the tosylhydrazone derivative (XXXVI) followed by acid hydrolysis of the C-20 ethylene ketal function gave 3,3-d_2-5α-pregnan-20-one (XXXVII). The isotopic purity of all the deuterium-labeled compounds is given in Tables I and II.

Attempts to prepare a C-15-labeled pregnan-20-one were abortive. Acid-catalyzed exchange[17] of Δ[16]-5α-pregnen-20-one (IX) in deuteriomethanol produced only the 21,21,21-d_3-labeled analog. Likewise, treatment of IX with sodium deuteroxide in O-deuterio-*t*-butyl alcohol produced no enolization at C-15.[18] An attempt to introduce deuterium into the C-14 and C-15 positions of 5α-pregnan-20-one by catalytic deuteration of Δ[14(15)]-5α-pregnen-20-ol resulted in extensive deuterium scrambling and, therefore, the labeled steroid could not be used to obtain meaningful mass spectral data.

(14) Huang-Minlon, *J. Am. Chem. Soc.*, **68**, 2487 (1946).
(15) W. Schütt and Ch. Tamm, *Helv. Chim. Acta*, **41**, 1730 (1958).
(16) M. Fischer, Z. Pelah, D. H. Williams, and C. Djerassi, *Chem. Ber.*, **98**, 3236 (1965).
(17) S. K. Malhatra and H. J. Ringold, *J. Am. Chem. Soc.*, **85**, 1538 (1963).

Discussion of the Mass Spectra

As both Figure 1 and Figure 2 illustrate, the ion of mass 217 corresponding to the loss of ring D (carbon atoms 15, 16, and 17), along with the attached side chain and an extra hydrogen atom from the charge retaining moiety, is the most intense and characteristic feature in the high-mass region of the spectra of 5α-pregnan-20-one (X) and its 17α epimer (XII). We become particularly interested in this process after having established[7] that the ring-D cleavage of 5α-pregnan-12-one (XXXVIII) is accompanied by a specific hydrogen migration from C-20. The question then arose whether the reverse hydrogen transfer process (XXXIX) is responsible for the generation of the *m/e* 217 species in the fragmentation of the 5α-pregnan-20-ones (Figures 1 and 2).



The 12,12-d_2-labeled 17β and 17α isomers were prepared in order to answer this question. The fact that no appreciable hydrogen transfer from C-12 is involved (Tables I and II) in the formation of *m/e* 217 in either compound excludes the possibility of a McLafferty[19] rearrangement operating in this cleavage (XL → XLI). This observation is in agreement with the reported interatomic distance requirement[20,21] (the O → H distance in the transition state should be between, or less than, 1.5–1.8 A) of the transfer since the minimum interatomic distance (judged on Dreiding models) between the carbonyl oxygen and the 12β hydrogen is about 3 A in the 17β epimer and about 2 A to the 12α hydrogen in its 17α counterpart.

The fission of ring D and loss of a hydrogen atom in most steroids has been rationalized[22] through the intervention of a molecular ion of type a. A contributing factor in the preferential fission of the 13,17 bond is

(18) See L. Tökés and C. Djerassi, *Steroids*, **6**, 493 (1965).
(19) F. W. McLafferty, *Anal. Chem.*, **31**, 82 (1959).
(20) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 2, Holden-Day, Inc., San Francisco, Calif., 1964, Chapter 20.
(21) C. Djerassi, G. von Mutzenbecher, J. Fajkos, D. H. Williams, and H. Budzikiewicz, *J. Am. Chem. Soc.*, **87**, 817 (1965).
(22) See ref 7, p 539, for discussions and references.

TABLE I: SHIFTS[a] OF PRINCIPAL MASS SPECTRAL PEAKS OF DEUTERATED ANALOGS OF 5α-PREGNAN-20-ONE (X)

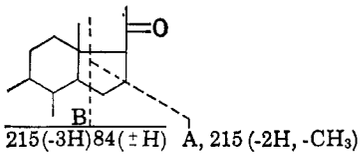
5α-Pregnan-20-one	Isotopic purity (%)	M+	M − CH_3	M − H_2O (%)	M − (H_2O + CH_3) (%)	M − C_2H_3O	M − C_2H_5O	M − C_3H_6O (%)	M − C_4H_6O	M − C_4H_7O	M − C_5H_9O (%)	M − $C_5H_{11}O$ (%)	M − $C_6H_{11}O$ (%)	M − C_8H_{14}	M − $C_{13}H_{21}O$	M − $C_{16}H_{26}$ (%)	M − $C_{17}H_{27}$ (%)
d_0 (X)	...	302	287	284	269	259	257	244	232	231	217	215	203	192	109	84	71
8β-d_1	d_0 (3)	303	288	285	270	260		244 (30)	233		217 (40)	215[c] (40)	204[b]	193	109	84	71
(XXXII)	d_1 (94)						258	245 (70)		232[b]	218 (60)	216 (60)					
	d_2 (3)																
9α-d_1	d_0 (32)	303	288	285	270[b]	260	258	245	233		218	216[b]	204[b]	193	109	84	71
(XXVIII)	d_1 (68)									232[b]							
16α-d_1 (XVI)	d_0 (21)	303	288	285	270	260	258[b]	245[b]	232	231	217	215	203 (∼80)	193	109	(85 + 84)[c]	72
	d_1 (78)												204 (∼20)				
	d_2 (1)																
3,3-d_2	d_1 (6)	304	289	286	271	261[b]	259[b]	246	234[b]	233[b]	219	217	205	192	111 (∼2/3)	84	71
(XXXVII)	d_2 (94)														109 (∼1/3)		
12,12-d_2 (XX)	d_0 (1)	304	289	285 (15)	270 (15)	261	259[b]	246	234[b]	233[b]	219	217[b]	205[b]	194	109	84 (70)	71
	d_1 (21)			286 (85)	271 (85)											85 (30)	
	d_2 (69)																
	d_3 (9)																
16α,17α-d_2	d_0 (4)	304	289	286	271	261[b]	259[b]	245[b]	232	231	217	215	203[b]	194	109	(86 + 85)[c]	73
(XV)	d_1 (23)																
	d_2 (47)																
	d_3 (15)																
	d_4 (11)																
18-d_3 (V)	d_2 (2)	305	290	285 (18)	272 (60) +	262[c]	260[c]	244 (25)	235	234	220	218	206 (70)[c]	195	109 (∼2/3)	84 (70)	71 (85)
	d_3 (98)			286 (36)	269–271			247 (75)							112 (∼1/3)	85 (30)	72 (15)
				287 (46)													
17,21,21,21-d_4	d_2 (4)	306	291	288	273	260	258	244 (75)	232	231	217	215	203	196	109	88	75
(XIII)	d_3 (21)							245 (∼25)									
	d_4 (75)																

[a] Reported shifts are corrected for isotopic impurity as well as ^{13}C contributions and are greater than 90% unless indicated otherwise. [b] Mainly at indicated m/e value, but exact calculation was impossible because of low intensity of peak or isotopic contaminants. [c] Mainly at the indicated values but other peaks are apparent also.

TABLE II.—SHIFTS[a] OF SOME PRINCIPAL PEAKS (m/e) IN THE MASS SPECTRA OF DEUTERATED 5α,17α-PREGNAN-20-ONES

Compd	Isotopic purity (%)	M+	M − 15	M − 18 (%)	M − 33	M − 58	M − 70	M − 71	M − 85	M − 87	M − 110	M − 193	M − 218 (%)	M − 231
16α-d_1 (XVII)	d_0 (21)	303	288	285	270	245	232	231	217	215	193	109	85[b]	72
	d_1 (78)													
	d_2 (1)													
12,12-d_2 (XXI)	d_0 (1)	304	289	286 (85)	271[b]	246	234[b]	...	219	...	194	109[c]	...	71
	d_1 (21)			285 (15)										
	d_2 (69)													
	d_3 (9)													
18-d_3 (XI)	d_2 (2)	305	290	287[b]	272[b]	247	235[b]	234[b]	220	218	195[b]	109 (∼2/3)	84 (∼80)[c]	71
	d_3 (98)											112 (∼1/3)	85 (∼20)	
17,21,21,21-d_3 (XIV)	d_2 (4)	306	291	288	273	244	232	231	217	215	216	109	88	75
	d_3 (21)													
	d_4 (75)													

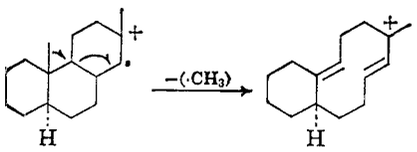
[a] See footnote *a* in Table I. [b] See footnote *b* in Table I. [c] See footnote *c* in Table I.



XLIII

carbon fragment, which are in agreement with these observations but deuterium labeling of C-15 (and C-19 if C-15 is retained) would be required to differentiate between them.

The small *m/e* 203 peak in Figure 1 is surrounded by several other ones preventing accurate determination of the shift values in the labeled spectra. It is apparent (Table I), however, that the majority of this ion is due to a ring-D fragmentation which is most probably analogous to the same peak in 5α-pregnane. The observed partial loss of the deuterium labels from C-18 and 20% retention of the 16α-d_1 label, are reminiscent of those found[23] in 5α-pregnane and, therefore, it is possible that a major part of this ion is formed by a two-step process, the *m/e* 218 intermediate (l) yielding m (*m/e* 203).



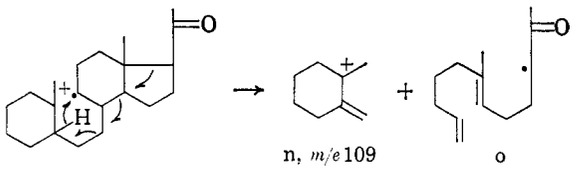
l, *m/e* 218 m, *m/e* 203

The intensity of this *m/e* 203 peak is significantly diminished in Figure 2, which is in good agreement with the relative intensity (8% *vs.* 4%) of the *m/e* 218 species in Figures 1 and 2. The observed small metastable peak in an AEI MS-9 spectrum of 5α-pregnan-20-one at *m/e* 189.0 ($203^2/218$ = 189.0) provides further supporting evidence for this sequence.

In the high mass range of Figures 1 and 2 there is one more peak, at *m/e* 192, which has no counterpart in the mass spectrum of 5α-pregnane. This ion retained at least 90% of the labels in rings C and D and in the 18-methyl function, while expulsion of ring A is indicated by the loss of the C-3 label (see Tables I and II). The oxygen-atom content of this ion is confirmed by high-resolution analysis. On the basis of these observations, it is presumed that a ring-B cleavage (fission of the 6,7 and 9,10 bonds) is responsible for the formation of this ion, as indicated before in formula XLII. In the absence of any deuterium-labeling evidence in ring B, mechanistic interpretations of this cleavage would involve too much speculation, since it is possible that this fragmentation is associated with a reciprocal hydrogen transfer, as is the fission of the 6,7 and 9,10 bonds in 11-ketoandrostane[9] and the α,β-unsaturated 3-ketoandrostenes.[25]

This ring-B cleavage can also occur with the transfer of one hydrogen atom, probably from the activated 5α-tertiary position, in which case the charge is retained on the ring-A fragment, yielding an allylically stabilized tertiary carbonium ion (n, *m/e* 109) and the stabilized neutral fragment (o). Consideration of the deuterium labeling results (Tables I and II) indicates that about

(25) R. H. Shapiro and C. Djerassi, *J. Am. Chem. Soc.*, **86**, 2825 (1964).



n, *m/e* 109 o

two-thirds of the *m/e* 109 ion in Figures 1 and 2, which retained the C-3 label and lost all the other labels, may be represented by formula n.

In addition to the previously discussed ions of mass 43, 71, and 109, there is one more peak (*m/e* 84) in the low mass range of Figures 1 and 2 which is not apparent in the mass spectrum of 5α-pregnane.[23] The empirical formula of this ion (C_5H_8O) was established by high-resolution analysis and the deuterium-labeling results (Table I) confirm the retention of C-17 and C-21. Interpretation of these results are summarized in formula XLIII. The charged fragment most likely includes C-15 since C-18, which serves as a substituent label for C-13, is lost. Therefore, the only obvious candidate for the remaining carbon atom of the five-carbon fragment of mass 84 is C-15. The loss of approximately one-half of the 16α-*d* label can be rationalized in terms of a reciprocal hydrogen transfer, the elimination of hydrogen from the charged moiety being site-specific (from C-16), and the gain from the neutral moiety being random.

Experimental Section[26]

5α-Androstan-17-one Cyanohydrin (VII).—Acetic acid (1.3 ml) was added dropwise during a time interval of 40 min to a stirred and cooled (10°) mixture of 5α-androstan-17-one (200 mg, mp 121–122°), 1.3 g of potassium cyanide, and 8 ml of ethanol. After the addition stirring was continued for 1 hr at 10° and for 3 hr at room temperature, then the reaction mixture was diluted with water. A crystalline precipitate formed which was removed by filtration, washed repeatedly with 2% acetic acid solution, and then dried under reduced pressure (0.1 mm) at room temperature. The resulting crude 5α-androstan-17-one cyanohydrin (VII) amounted to 211 mg (96%, mp 141–146°), which on recrystallization afforded the analytical sample: mp 142–151°, ν_{max} 3570, 3365 (OH), and 2210 cm^{-1} (C≡N), no carbonyl absorption. *Anal.* Calcd for $C_{20}H_{31}NO$: C, 79.67; H, 10.37. Found: C, 79.92; H, 10.33.

18,18,18-d_3-*dl*-5α-Androstan-17-one Cyanohydrin (II).—The racemic, labeled ketone, 18,18,18-d_3-5α-androstan-17-one (I, 71 mg) was converted into its cyanohydrin derivative II in a manner analogous to that described above. The resulting crude product (75 mg; 96%, mp 146–156°), on recrystallization from methanol, provided the pure 18-d_3-*dl*-5α-androstan-17-one cyanohydrin (II, mp 156–165°), exhibiting the same infrared absorption features as the unlabeled analog VII (R = H) in addition to the C–D absorptions.

17-Cyano-Δ^{16}-5α-androstene (VIII).—A solution of 187 mg of the crude cyanohydrin VII and 0.3 ml of freshly distilled phosphorus oxychloride in 6 ml of dry pyridine was heated in a

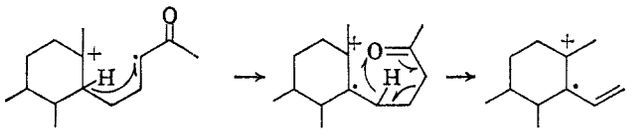
(26) Melting points (uncorrected) were determined on the Kofler block. Optical rotations and infrared spectra were measured in chloroform solution and ultraviolet absorption spectra were determined in ethanol and methanol. The optical rotatory dispersion measurements, performed by Mrs. Ruth Records, were obtained with a Durrum-JASCO Model ORD-5 spectropolarimeter. Thin layer chromatography (tlc) was performed on silica gels H, G, and GF_{254} (E. Merck, A. G. Darmstadt). Spots or bands were developed with ultraviolet light, iodine vapor, or spraying with a 2% ceric sulfate solution, 2 *N* in sulfuric acid, and heating for optimum development of colored spots. Mass spectra were determined, except where indicated, with a C.E.C. Model 21-103 C mass spectrometer using an all-glass inlet system heated to 200° with the isatron temperature maintained at 270° while the ionizing energy was kept at 70 ev and the ionizing current at 50 μa. Mass spectra were determined by Messrs. John Smith and Nelson Garcia. All microanalyses were by Messrs. E. Meier and J. Consul.

The diagnostically more important cleavage processes of 5α-pregnan-20-one elucidated by deuterium labeling are summarized in formula XLII. The complex group of peaks at *m/e* 255–260 in Figure 1 represents an interesting but obscure case. The two major ions (*m/e* 257 and 259) are formed by losing a neutral fragment of 45 and 43 mass units, respectively, from the molecular ion. The only possible empirical formula for the former neutral fragment is C_2H_5O, and high-resolution analysis showed only traces of an oxygen-containing contaminant in the M − 43 ion. This implies that cleavage of the 17,20 bond is involved in the formation of both the ion associated with no hydrogen transfer (*m/e* 259) and the ion associated with a double hydrogen transfer (*m/e* 257) from undetermined positions.

The most interesting aspect of this cleavage is that apparently it is influenced by the configuration at C-17 since the lack of these two ions in Figure 2 is one of the major differences between the 17β and 17α isomers.

Loss of the side chain in the formation of both of these ions is confirmed by the deuterium labeling results (Table I) and intensive rearrangements are likely to be involved in these fragmentations.

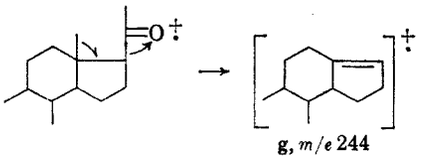
The *m/e* 244 ion, according to high-resolution analysis, is composed of $C_{18}H_{28}$. The deuterium-labeling results show that C-21 is lost entirely, while only parts of C-17 (75%), C-8 (30%), and C-18 (25%) are lost, indicating that more than one mechanism is involved in the genesis of this ion. The major portion (75%) of this fragmentation, that in which C-17 is expelled, is very likely accompanied by a double hydrogen transfer from the charge-retaining side, since a rearrangement must be invoked to account for the formal cleavage of two bonds at C-17. It is possible that at least 40% of one of these two hydrogens is transferred from C-8 for this major portion (75%) of the fragmentation under discussion. A plausible path involving this hydrogen atom is depicted by the transformation of molecular ion a′ to b′ which in turn yields the homoallylically stabilized ion (e) by way of a McLafferty hydrogen transfer.[19] A possible competing process, which would involve the yet unlabeled 14α and 15 positions as the most likely positions for hydrogen abstraction, is depicted by the sequence a′ → f.



a′ f, *m/e* 244

The presence of a metastable peak in an AEI MS-9 spectrum of 5α-pregnan-20-one at *m/e* 197.1 ($244^2/302 = 197.1$) is consistent with the two fragmentation paths proposed to rationalize the loss of C-17.

Genesis of the minor (25%) portion, in which C-17 is retained at the expense of C-18, can be envisaged as the loss of the side chain together with the 18-angular methyl function to yield "ionized olefin" g (*m/e* 244).

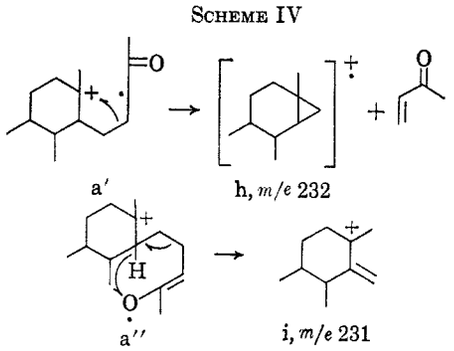


g, *m/e* 244

No metastable peaks were detectable at either *m/e* 230 ($244^2/259 = 229.9$) or at *m/e* 207 ($244^2/287 = 207.4$) which would be indicative of stepwise fragmentations.

In the complex *m/e* 227–232 peak group only the two main ions (*m/e* 231 and 232) were analyzed. It is apparent from the deuterium-labeling results that both ions are formed by the expulsion of a neutral fragment which involves carbon atoms 16, 17, 20, and 21, accompanied by no (*m/e* 232) or by a single hydrogen transfer (*m/e* 231) from the charge retaining moiety. These cleavages are analogous to those responsible for the majority of the *m/e* 232, and for a small part of the *m/e* 231 peaks in the mass spectrum of 5α-pregnane.[23] Therefore, the same types of processes (a′ → h and a″ → i) are proposed for fragmentation of both pregnane and pregnan-20-ones (see Scheme IV).

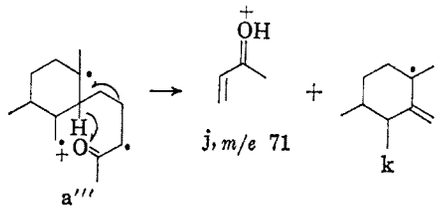
SCHEME IV



a′ h, *m/e* 232

a″ i, *m/e* 231

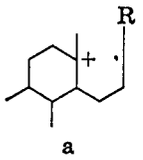
An appealing aspect of the latter cleavage is that it can occur also with charge retention on the oxygen-containing side. According to high-resolution analysis the *m/e* 71 ion in Figure 1, which is negligibly small in the mass spectrum of 5α-pregnane,[23] is due to a C_4H_7O fragment, in which all the labels on C-16, C-17, and C-21 are retained (Tables I and II). Its genesis can be explained by invoking a hydrogen transfer from the still unlabeled 14α position in molecular ion a‴, yielding a molecule of protonated methyl vinyl ketone j (*m/e* 71) and a neutral analog of ion i, the allylically stabilized radical (k).



a‴ j, *m/e* 71 k

The observed increased facility of these partial ring-D cleavages (13,17 and 15,16 bond fissions) in the presence of a 16-methyl substituent[4] is precisely what would be expected according to the mechanisms proposed here since the rupture of the 15,16 bond in molecular ions a–a‴ would be enhanced by the higher degree of substitution at C-16.

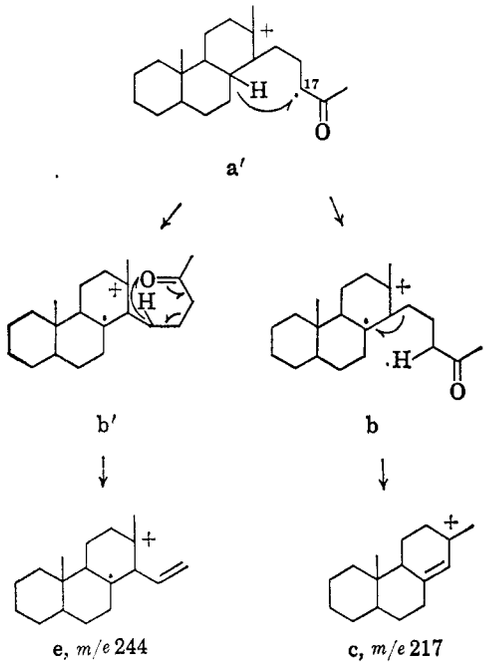
Besides the previously mentioned *m/e* 217 and 218 peaks there is also a small one at *m/e* 215 which is absent in the mass spectrum of 5α-pregnane.[23] The only possible empirical formula for the expelled neutral fragment of 85 mass units is C_5H_9O and the deuterium labeling results (Tables I and II) confirmed the loss of carbon atoms 16, 17, and 21. There are two possible combinations (indicated in formula XLIII) for losing a five-



a

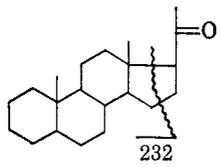
the release of the steric strain inherent in the *trans*-hydrindan system. However, formation of such an ion (a, R = CH_3CO) would be enhanced in the 20-keto steroid since the free radical at C-17 would be stabilized by the C-20 carbonyl function. Such a molecular ion (a′) is the apparent intermediate in the genesis of a number of ions in the mass spectra of both 20-ketopregnane epimers X and XII. Therefore, it is not surprising to find that Figures 1 and 2 show a great deal of similarity since the original configurational differences at C-17 is destroyed in molecular ion a′. Hydrogen transfer to the radical site of ion a′ from any of a number of neighboring positions appeared possible but, an inspection of Table I (and Table II) reveals complete retention of label at C-9 and C-18, as well as the more distant site, C-3. The label on C-8 is lost to a significantly large extent (40%) and on this basis, cleavage of 14,15 bond (ion b) with the formation of an allylically stabilized tertiary carbonium ion (c) is proposed as one of the processes operative in this important fragmentation (see Scheme III). The loss of

SCHEME III



ring D labels on C-16, C-17, and C-21 is consistent with the assigned cleavage pattern. Possibly the observed transfer from C-8 (and the other labeled positions as well) as determined by the extent of deuterium removal (40%) is less than the real transfer as a result of an isotope effect.

A plausible competing cleavage pattern which would account for part or all of the 60% retention of label at C-8 would be a two-step process involving loss of a methyl radical (likely C-19, since C-18 is retained) from an *m/e* 232 ion intermediate (d). Inspection of an AEI MS-9 spectrum of 5α-pregnan-20-one reveals the presence of a very small metastable peak at *m/e* 203.0



d

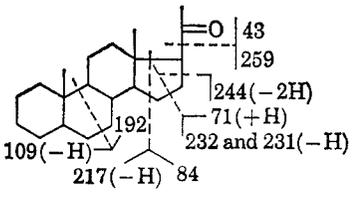
($217^2/232$ = 203.0) which may indicate participation of such a two-step fragmentation since the obtained metastable peak *m/e* 156.0 ($217^2/302$ = 155.9) corresponding to the direct formation from the molecular ion was small as well.

The hydrogen-transfer process associated with ring-D cleavage (*m/e* 217) of 5α-pregnane has been found to be a random one.[23] On the other hand, as has already been noted, the hydrogen transfer associated with the formation of the 233 ion (XXXVIII) from the 12-ketone is more selective. Additional observations point to other dissimilarities of the two fragmentations. The ring-D cleavage in pregnane occurs both with a single (*m/e* 217) and with a reciprocal hydrogen transfer (*m/e* 218)[23] while in 17α- and 17β-pregnan-20-ones the genuine *m/e* 218 peaks free of ^{13}C isotopic contribution from the *m/e* 217 peak, are of only 4 and 8% relative intensity, respectively (Figures 1 and 2). Thus in spite of a close resemblance of key intermediates in ring-D cleavages of both hydrocarbon and ketone it is clear that the nature of the transition state in the fragmentation of the ketone is affected by the presence of the carbonyl function at C-20, although the hydrocarbon-type random transfer with a large isotope effect is probably also operative.

Consideration of the deuterium labeling results (Tables I and II) for some of the other features in the mass spectra of 17β- and 17α-pregnan-20-one (Figures 1 and 2) also reveals the presence of a number of other interesting ions. Since most of these ions are analogous in the mass spectra of both 17α and 17β epimers (with the exception of relative intensity differences), only those ions in Figure 2 will be mentioned which show definite deviation.

The common, though diagnostically limited, feature of steroidal ketones, the loss of a molecule of water[20,24] from M^+, as well as from the $M - CH_3$ ion is associated with random hydrogen abstraction and needs no discussion. It is interesting to note, however, that a significant amount of double hydrogen loss (18%) was observed from the C-18 angular methyl group.

The elimination of a methyl radical from the molecular ion (*m/e* 287) is also apparent in the mass spectra of most steroids.[20] In the pregnan-20-one molecule there are three methyl functions (C-18, C-19, and C-21) which could participate in this fragmentation, but the deuterium labeling results show that only the C-19 methyl function is ejected.



XLII

(23) L. Tökés, Ph.D. Thesis, Stanford University, 1965.
(24) H. Budzikiewicz and C. Djerassi, *J. Am. Chem. Soc.*, **84**, 1430 (1962).

sealed tube at 150° for 2 hr.[27] The resulting dark solution was poured onto a mixture of ice and hydrochloric acid and was extracted with ether. The ether phase was washed with dilute hydrochloric acid solution, which removed all the colored contaminants, and then was washed with bicarbonate solution and water, and dried over magnesium sulfate, and the ether was evaporated. The crude product (175 mg, 99%, mp 129–141°), after two recrystallizations from methanol provided the analytical sample of 17-cyano-Δ^{16}-5α-androstene (VIII): mp 153–154°, $[\alpha]^{27}D$ +21.7° (*c* 0.92), λ_{max} 217 mμ (ϵ 8630), ν_{max} 2210 (C≡N), 1585 and 995 cm^{-1} (C=C). *Anal.* Calcd for $C_{20}H_{29}N$: C, 84.74; H, 10.31. Found: C, 84.60; H, 10.23.

18,18,18-d_3-*dl*-17-Cyano-Δ^{16}-5α-androstene (III).—The dehydration of 70 mg of crude, racemic cyanohydrin II was accomplished by the same procedure as described above for the unlabeled analog, affording 62 mg (96%) of crude product, mp 112–126°. The pure 18-d_3-*dl*-17-cyano-Δ^{16}-androstene (III) was obtained by two recrystallizations from methanol and exhibited mp 132–134°; ν_{max} 2210 (C≡N), 1590, 955 (C=C), and 2065 cm^{-1} (CD); and a molecular ion of *m/e* 286 in its mass spectrum.

Δ^{16}-5α-Pregnen-20-one (IX).—The conversion of the crude cyanide (VIII, 154 mg) into the α,β-unsaturated ketone (IX) was achieved by treatment with methylmagnesium bromide, followed by acid hydrolysis, according to the procedure reported by Butenandt and Schmidt-Thomé[27] for the preparation of $\Delta^{5,16}$-pregnadien-3α-ol-20-one. The semicrystalline residue, thus obtained, was subjected to tlc purification in benzene (owing to the low R_f value of the product in benzene the chromatogram was developed twice before elution) yielding 125 mg (77%) crude product (mp 140–148°) which upon two recrystallizations from methanol gave the pure Δ^{16}-5α-pregnen-20-one (IX): mp 158.5–159.5°, λ_{max} 240 mμ (lit.[28] 155–157°, 240 mμ), ν_{max} 1665 (conjugated C=O) and 1590 cm^{-1} (C=C).

18,18,18-d_3-*dl*-Δ^{16}-5α-Pregnen-20-one (IV).—The racemic α,β-unsaturated ketone (IV) was prepared as described for IX starting with 59 mg of the crude cyanide (III). The crude product (obtained by tlc purification) amounted to 42 mg (67%, mp 121–129°) and recrystallization from methanol gave the pure 18-d_3-*dl*-Δ^{16}-5α-pregnen-20-one (IV): mp 136–137° (the melting point did not change on further recrystallization from methanol); λ_{max} 240 mμ (ϵ 10,300); ν_{max} 1665 (conjugated C=O), 1595 (C=C), 2220 and 2065 cm^{-1} (CD). This product exhibited the correct molecular ion (*m/e* 303) and fragmentation pattern in its mass spectrum.

5α-Pregnan-20-one (X).—The crude Δ^{16}-5α-pregnen-20-one (IX, 100 mg) in methanol solution was hydrogenated for 1 hr at room temperature and atmospheric pressure in the presence of 20 mg of 5% palladized charcoal. The catalyst was removed by filtration through a small column (100 mg) of alumina and the methanol was evaporated under reduced pressure. The residue was redissolved in benzene and the solution was filtered to remove some inorganic salt, and on evaporation of the solvent 95 mg of crude product was obtained, mp 129–135°. The pure 5α-pregnan-20-one (X, mp 136–138°, lit.[29] 136–137° and 136–139°) was prepared by recrystallization from methanol.

18,18,18-d_3-*dl*-5α-Pregnan-20-one (V).—The crude 18-d_3-*dl*-Δ^{16}-5α-pregnen-20-one (IV, 35 mg) was hydrogenated as described above for the unlabeled analog (IX). The crude residue from the benzene solution (29 mg, mp 125–143°) provided the pure sample of 18-d_3-*dl*-5α-pregnan-20-one (V, mp 149–151°) after two recrystallizations from methanol. The melting point of this sample did not change on further recrystallization and it exhibited ν_{max} 1700 (C=O), 2220, 2210, and 2050 cm^{-1} (CD), and *m/e* 305 for the molecular ion and the correct fragmentation pattern in its mass spectrum.

5α,17α-Pregnan-20-one (XII).—A solution of 5α-pregnan-20-one (X, 50 mg) in 10 ml of ethanol containing 2 drops of 20% sodium hydroxide solution was heated under reflux for 4 hr. After cooling the reaction mixture was diluted with ether which was washed with water and dried over magnesium sulfate, and the ether was evaporated. The resulting 49 mg of crystalline epimeric mixture was separated by tlc in benzene (the chromatogram was developed twice before elution) and thereby afforded 38 mg (79%) of the 17β epimer (X, mp 136–138°), and 10 mg (21%) of 5α,17α-pregnan-20-one (XII, mp 103–103.5°) (from methanol, lit.[30] 99–100°).

18,18,18-d_3-*dl*-5α,17α-Pregnan-20-one (XI).—A sample (5 mg) of the crude 18-d_3-*dl*-5α-pregnan-20-one (V) was heated under reflux for 4 hr in a solution of 2 ml of ethanol containing 2 drops of 20% sodium hydroxide solution. The reaction mixture was diluted with ether. The ether solution was washed with water to neutrality and dried over anhydrous magnesium sulfate. Evaporation of the ether produced an epimeric mixture (5 mg) which was separated by tlc in benzene (the chromatogram was developed twice before elution) yielding 3.5 mg of starting material (mp 143–148°) and 0.8 mg of 18-d_3-*dl*-5α,17α-pregnan-20-one (XI, mp 90–114°) which exhibited the expected fragmentation pattern and a molecular ion of *m/e* 305 in its mass spectrum.

-17β- (XIII) and 17,21,21,21-d_4-17α,5α-Pregnan-20-one (XIV).—A solution of 5α-pregnan-20-one (X, 15 mg) in 2 ml of deuteriomethanol was saturated with 20% sodium deuterioxide in deuterium oxide and heated under reflux for 24 hr. After cooling the reaction mixture was diluted with ether, washed rapidly with ice cold water, and subsequently, with additional water to neutrality. The ether solution was dried over magnesium sulfate. Evaporation of the ether produced an epimeric mixture which was separated as described above for the unlabeled analogs, yielding 9.5 mg (63%) of 17α,21,21,21-d_4-5α,17β-pregnan-20-one (XIII, mp 137–139°, recrystallized from MeOD), and 3 mg (20%) of 17β,21,21,21-d_4-5α,17α-pregnan-20-one (XIV), mp 98–103°. Both epimers exhibited the same isotope composition (4% d_2, 21% d_3, and 75% d_4) in the mass spectra.

16α,17α-d_2-5α-Pregnan-20-one (XV).—A solution of Δ^{16}-5α-pregnen-20-one (IX, 10 mg) in 15 ml of methylcyclohexane was stirred in an atmosphere of deuterium gas at room temperature and atmospheric pressure in the presence of 10 mg of 5% palladized charcoal. After 1 hr of deuteration the catalyst was removed by filtration and the solvent was evaporated at reduced pressure. The residue (10 mg) on recrystallization from acetonitrile provided a sample of 16α,17α-d_2-5α-pregnan-20-one (XV), mp 136–138°, isotope composition being 4% d_0, 23% d_1, 47% d_2, 15% d_3, and 11% d_4.

-17β- (XVI) and 16α-d_1-5α,17α-Pregnan-20-one (XVII).—The crude 16α,17α-d_2-5α-pregnan-20-one (XV, 6 mg) was dissolved in 5 ml of methanol containing a few drops of 20% sodium hydroxide solution and was heated under reflux for 36 hr. After cooling, the solution was diluted with ether. The ether solution was washed with water to neutrality then dried over magnesium sulfate. Evaporation of the ether gave an epimeric mixture which was separated by tlc in benzene. Obtained was 5 mg of 16α-d_1-5α-pregnan-20-one (XVI) which on recrystallization from methanol showed mp 136–138°. The 16α-d_1-5α,17α-pregnan-20-one (XVII) amounted to 1 mg and after recrystallization from methanol exhibited mp 100–103°. Both epimers showed the same isotope composition: 21% d_0, 78% d_1, and 1% d_2.

5α-Pregnane-12,20-dione 12-Monoethylene Thioketal (XIX).—5α-Pregnane-12,20-dione[7] (XVIII, 97 mg, 0.306 mmole) was dissolved in 1.2 ml of acetic acid containing 28.8 mg (0.306 mmole) of ethanedithiol, and then 30 mg of *p*-toluenesulfonic acid monohydrate was added to it. After storing at room temperature for 3 hr the reaction mixture was diluted with ether and the resulting solution was washed repeatedly with sodium hydroxide solution and then with water to neutrality. After drying, evaporation of ether gave the crude product (114 mg), which upon three successive recrystallizations from methanol provided 32 mg of analytically pure 5α-pregnane-12,20-dione 12-monoethylene thioketal (XIX): mp 151–152.5°; $[\alpha]^{28}D$ +109° (*c* 0.75); ν_{max} 1710 (C=O) cm^{-1}; ORD (3757) in methanol (*c* 0.13) $[\Phi]_{312}^{peak}$ +4550°, $[\Phi]_{263}^{trough}$ −7110°. *Anal.* Calcd for $C_{23}H_{36}OS_2$: C, 70.35; H, 9.25. Found: C, 70.23; H, 9.29.

The residue from the collected recrystallization mother liquors was purified by tlc in benzene. Thereby an additional 72 mg (87% total yield) of the monomercaptal (XIX, mp 145–148°) was isolated.

-17β- (XX) and 12,12-d_2-17α,5α-Pregnan-20-one (XXI).—A solution of the monothioketal derivative (XIX, 15 mg) in 2.5 ml of deuteriomethanol was treated with freshly prepared[31] deuterium-containing Raney nickel (W-7) from 500 mg of Raney

(27) A. Butenandt and J. Schmidt-Thomé, *Ber.*, **71**, 1487 (1938); **72**, 182 (1939).

(28) O. Mancera, G. Rosenkranz, and C. Djerassi, *J. Org. Chem.*, **16**, 192 (1951).

(29) J. Romo, M. Romero, C. Djerassi, and G. Rosenkranz, *J. Am. Chem. Soc.*, **73**, 1528 (1951); Ch. Meystre and K. Miescher, *Helv. Chim. Acta*, **28**, 1497 (1945).

(30) Dr. P. Laur, unpublished results from this laboratory.

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

nickel alloy and the resulting suspension was stirred and heated under reflux for 3 hr. The nickel was then removed by filtration and the solvent was evaporated under reduced pressure. The crystalline residue (12 mg), thus obtained, was oxidized under Jones conditions[10] and the resulting ketone was dissolved in methanol containing 2 drops of 20% sodium hydroxide solution. After heating under reflux for 4 hr the solution was diluted with ether. The ether solution was washed with water to neutrality and dried over magnesium sulfate. Evaporation of the ether gave the crude epimeric mixture (9.5 mg) which was separated by tlc with benzene (the chromatogram was developed twice before elution of the samples) into 4.5 mg of pure 12,12-d_2-5α-pregnan-20-one (XX, mp 138–139°, from methanol, and undepressed upon admixture with an authentic unlabeled sample), and 0.8 mg of 12,12-d_2-5α,17α-pregnan-20-one (XXI, mp 86–98°). Both epimers exhibited the same isotope composition (1% d_0, 21% d_1, 69% d_2, and 9% d_3) in their mass spectra.

20β-Hydroxy-5α-pregnan-12-one (XXIV).—A 400-mg sample of 12-ethylenedioxy-5α-pregnan-20-one (XXII) in 100 ml of methanol containing also 356 mg of sodium hydroxide and 220 mg of sodium borohydride was allowed to stand at room temperature for 45 hr. The volume of solution was reduced to one-fourth of the original and 200 ml of water was added. The resulting mixture was extracted with ether and the combined extracts were dried. Evaporation of the ether gave 410 mg of crude material whose infrared spectrum revealed no carbonyl absorption. When recrystallized from methanol, the **12-ethylenedioxy-5α-pregnan-20β-ol** (XXIII), exhibited mp 122–124°. The conversion of the C-12-ketal to the corresponding ketone was accomplished by heating the ketal in dilute, aqueous acetic acid solution. The crude material after recrystallization from methanol afforded 288 mg of the 20β-hydroxy ketone (XXIV) which exhibited mp 169–171°, $[\alpha]^{25}D$ +97.5° (*c* 1.0), $\nu_{max}^{CHCl_3}$ 3330 and 1695 cm^{-1}, *m/e* 318 (M$^+$). *Anal.* Calcd for $C_{21}H_{34}O_2$: C, 79.19; H, 10.76. Found: C, 78.96; H, 10.64.

20β-Acetoxy-5α-pregnan-12-one (XXV).—A solution of 288 mg of the 20β-hydroxy ketone (XXIV), in 10 ml of anhydrous pyridine and 8 ml of acetic anhydride was warmed to 65° for 1 hr and then allowed to stand at room temperature overnight. The reaction mixture was poured into crushed ice and the resulting suspension was acidified to pH 1. The solid was collected, washed several times with 5% hydrochloric acid, then with water, and air dried. Its infrared spectrum, while showing the presence of acetate (1736 and 1238 cm^{-1}) also revealed by the absorption at 3330 cm^{-1} that a considerable amount of alcohol had not been acetylated. Unconverted alcohol persisted after treating the above described mixture of acetate and alcohol with acetic anhydride–pyridine for 24 hr at 60°. Therefore, a portion of the mixture (30 mg) was separated into its pure components by careful preparative tlc. Obtained was 23 mg of 20β-acetoxy ketone XXV which had mp 178–179°, $[\alpha]^{25}D$ +120° (*c* 1.15), *m/e* 360 (M$^+$). *Anal.* Calcd for $C_{23}H_{36}O_3$: C, 76.62; H, 10.06. Found: C, 76.32; H, 10.03.

20β-Acetoxy- (XXVII) and 20β-Hydroxy-$\Delta^{9(11)}$-5α-pregnen-12-one (XXVI).—A solution of 118 mg of 20β-acetoxy-5α-pregnan-12-one [XXV, containing some 20β-hydroxy-5α-pregnan-12-one (XXIV)] and 79 mg of sublimed selenium dioxide in 4 ml of acetic acid (6×10^{-4} *M* in hydrochloric acid) was heated under reflux for 16 hr. After cooling the selenium was removed by filtration and the bulk of the acetic acid was distilled at reduced pressure. The residue in ether solution was washed with 2 *N* sodium hydroxide then with water. Evaporation of the ether gave 105 mg of a two-component mixture from which the desired acetoxy α,β-unsaturated ketone (XXVII, higher R_f value) was obtained (60 mg) by preparative tlc and two recrystallizations from methanol. This material exhibited the following physical properties: mp 157–159°; $[\alpha]^{25}D$ +77.8° (*c* 1.00); $\nu_{max}^{CHCl_3}$ 1730, 1681, 1605, 1238 cm^{-1}; λ_{max}^{MeOH} 240 mμ (ϵ 36,000); *m/e* 358. *Anal.* Calcd for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 77.28; H, 9.75.

The second component (lower R_f value) separated by tlc amounted to 5 mg and proved to be identical in all respects with 20β-hydroxy-$\Delta^{9(11)}$-5α-pregnen-12-one (XXVI), the major product obtained by treating the 20β-hydroxy ketone (XXIV, 55 mg) in 5 ml of refluxing acetic acid (6×10^{-4} *M* in hydrochloric acid) for 8 hr with 50 mg of sublimed selenium dioxide. The infrared spectrum of the crude product revealed absorption at 1703 (weak, ketone) 1656 (conjugated ketone) and 1603 cm^{-1} (olefin). Analytical tlc showed two spots both of which were ultraviolet absorbing. The more mobile of the two spots was the smaller and presumably represents the product resulting from oxidation of the 20β-hydroxyl group and responsible for the 1703-cm^{-1} absorption in the spectrum of crude product. The less mobile of the two bands on a preparative plate yielded 49 mg of the 20β-hydroxy α,β-unsaturated ketone (XXVI), which when recrystallized once from methanol exhibited mp 169–171°; $[\alpha]^{25}D$ +92.6 (*c* 1.00); $\nu_{max}^{CHCl_3}$ 3322, 1656, 1603 cm^{-1}; ORD in methanol (*c* 0.25) $[\Phi]_{335}^{trough}$ −1380°, $[\Phi]_{290}^{inflection}$ +11,250°, $[\Phi]_{265}^{peak}$ +15,156°. *Anal.* Calcd for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19. Found: C, 79.92; H, 10.19.

9α-d_1-5α-Pregnan-20-one (XXVIII).—A 30-mg sample of 20β-hydroxy-$\Delta^{9(11)}$-5α-pregnen-12-one (XXVI) in 10 ml of methylcyclohexane was added to a suspension of 60 mg of 10% palladized charcoal (predeuterated). After stirring under an atmosphere of deuterium for 2 hr the catalyst and solvent were removed. The resulting white, crystalline solid (27 mg) produced no absorption above 210 mμ. This material was subject to back exchange (sodium hydroxide in methanol heated to reflux 19 hr under nitrogen), purification by tlc, and then reduction by the modified Wolff–Kishner method[14] according to the following procedure. A mixture of 19 mg of the labeled 20β-hydroxypregnan-12-one, 2 ml of ethylene glycol, 1.5 ml of isobutyl alcohol, and 0.75 ml of hydrazine hydrate (95%) was heated under reflux (about 120°) under nitrogen for 1.5 hr. The reaction mixture was cooled to about 80° and 170 mg of potassium hydroxide was added. The resulting mixture was heated without a reflux condenser in a Wood's metal bath until the temperature reached 200°; after subsequent heating under reflux in an atmosphere of nitrogen for 4 hr the reaction mixture was again cooled and subsequently poured into water. The resulting mixture was extracted with ether and the combined ether extracts were in turn washed to neutrality with water, and dried over magnesium sulfate. Evaporation of the ether and tlc purification gave 15 mg of crystalline, labeled pregnanol which had mp 135–138°, *m/e* 305 (M$^+$) and whose tlc behavior was identical with that of 5α-pregnan-20β-ol. Jones oxidation[10] of a 14-mg sample of the labeled alcohol produced 13 mg of 9α-d_1-5α-pregnan-20-one (XXVIII, mp 133–134°) showing identical tlc and infrared spectral properties (with the exception of bands owing to the presence of deuterium) with 5α-pregnan-20-one. The isotopic purity is given in Table I.

8β-d_1-5α-Pregnan-20-one (XXXII).—Ten drops of 20% solution of sodium deuterioxide in deuterium oxide was added to a solution of 95 mg of mixture of 20β-hydroxy-(XXVI) and 20β-acetoxy-$\Delta^{9(11)}$-5α-pregnen-12-one (XXVII) in 25 ml of deuteriomethanol. This mixture was made homogeneous by the careful addition of additional deuterium oxide and deuteriomethanol. The resulting, clear solution was heated under reflux under nitrogen for 8 days. After processing the solution in the normal manner, 68 mg of deuterium-containing material (XXIX) was obtained. This was hydrogenated over palladized charcoal. The resulting white, crystalline solid showed no conjugated carbonyl absorption in the infrared spectrum and its tlc behavior was identical with that of 20β-hydroxy-5α-pregnan-12-one (XXIV). Back exchange of deuterium at C-11 (see preparation of XXVIII) to give labeled hydroxypregnanone XXX, followed by Wolf–Kishner reduction[14] (as in the preparation of XXVIII) of 43 mg, afforded 39 mg of labeled pregnanol (XXXI, mp 136–138°) whose tlc and infrared spectral properties were identical with those of 5α-pregnan-20β-ol. Jones oxidation[10] of 39 mg of XXXI gave 35 mg of 8β-d_1-5α-pregnan-20-one (XXXII, mp 131–132°) whose isotopic purity is given in Table I.

5α-Pregnane-3,20-dione 20-Monoethylene Ketal (XXXV).—The conversion of 5α-pregnan-3β-ol-20-one[32,33] (XXXIII, 500 mg, mp 192–195°) into its ethylene ketal derivative (XXXIV) and the subsequent oxidation of the 3-hydroxy function were carried out according to the procedure reported by Schütt and Tamm.[15] The resulting crude product (504 mg), was recrystallized from methanol containing 1 drop of pyridine and thereby provided 360 mg of pure **5α-pregnane-3,20-dione 20-monoethylene ketal** (XXXV), mp 187–189° (lit.[15] 188–190°). Further concentration of the mother liquor deposited an additional 32 mg of the pure product XXXV, mp 186–188° (60% total over-all yield).

3,3-d_2-5α-Pregnan-20-one (XXXVII).—The monoethylene ketal derivative (XXXV, 110 mg) and *p*-toluenesulfonylhydrazide

(32) This compound was supplied by Syntex, S. A., Mexico City.

(33) A. Butenandt and L. Mamoli, *Ber.*, **68**, 1847 (1935); Ch. Meystre and K. Miescher, *Helv. Chim. Acta*, **29**, 33 (1946).

(110 mg) were dissolved in 3 ml of methanol. The solution was heated for a few minutes whereupon a heavy, crystalline precipitate appeared. The resulting suspension was cooled in an ice bath, the crystals were collected by filtration, washed well with cold methanol, and dried at room temperature under reduced pressure (1.0 mm). The resulting tosylhydrazone derivative (XXXVI, (132 mg, 82%, mp 149–158° dec) contained traces of a ketonic contaminant according to its infrared spectrum.

A slurry of the crude tosylhydrazone (XXXVI, 90 mg) and lithium aluminum deuteride (90 mg) in dry dioxane (3 ml, reagent grade) was heated under reflux for 2 hr. After this period, the excess deuteride was decomposed with a few drops of deuterium oxide and the mixture was heated again for 5 min. The crystalline residue which was obtained, upon removal of the inorganic salts by filtration and evaporation of the dioxane under reduced pressure, was dissolved in methanol which contained a few drops of dilute hydrochloric acid. After heating under reflux for 1 hr the methanol was evaporated under reduced pressure, the residue was dissolved in ether, and the ether solution was washed with water and dilute sodium bicarbonate solution and dried over magnesium sulfate. Evaporation of the ether gave the crude product which was purified by tlc with benzene, yielding 28 mg (55%) of pure **3,3-d_2-5α-pregnan-20-one** (XXXVII), mp 136–138°; the isotope composition was 6% d_1 and 94% d_2.

Registry No.—VII, 7704-78-1; X, 848-62-4; XXXII, 7733-99-5; XXVIII, 7721-36-0; XVI, 7704-79-2; XXXVII, 7704-80-5; XX, 7704-81-6; XV, 7704-82-7; V, 7704-83-8; XIII, 7704-84-9; XVII, 7704-85-0; XXI, 7721-37-1; XI, 7704-86-1; XIV, 7704-87-2; II, 7721-38-2; VIII, 7704-88-3; III, 7721-39-3; IX, 3752-04-3; IV, 7704-89-4; XII, 7704-90-7; XIX, 7718-52-7; XXIV, 7704-91-8; XXIII, 7738-86-5; XXV, 7704-92-9; XXVII, 7704-93-6; XXVI, 7704-94-1; XXXV, 1452-23-9; XXXVI, 7721-40-6.

C-19 Functional Steroids. XI.[1a] Some Reactions of C-19 Aldehydes[1b,c]

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Degradation of 19-oximino steroidal 5,6-chlorohydrins gave 6,19-hemiacetals which with zinc in acetic acid furnished Δ^5-19 aldehydes in the androstane series. Reduction of the double bond (Pd–H_2) in alcoholic solution gave the 5α-steroidal 19-alkyl acetal. These compounds were hydrolyzed and oxidized to form 3,19-lactones. 19-Oxo-5α-androstane-3β,17β-diol diacetate on ultraviolet irradiation in ethyl acetate in the presence of oxygen gave 5α-androstane-3β,10β,17β-triol 3,17-diacetate.

During the preparation of derivatives of **17**[2] for biological evaluation[3] reactions of steroidal C-19 aldehydes were studied. These results are now described. Treatment of **1**[4] and **2**[5] with nitrous acid gave **3** and **4**, respectively. These compounds were mixtures of C-19 anomers as shown by their nmr spectra. After deuteration of the hydroxyl hydrogen in **3** to obviate spin splitting of the C-19–H resonance, two signals from this proton at 5.30 and 5.78 ppm in a ratio of approximately 75:25 were seen, and two C-18–H signals at 0.80 and 0.85 in the same ratio were observed. The two high-field peaks of each pair clearly arise from the (19*S*)-hydroxy anomer (Figure 1) in which relative to the (19*R*)-hydroxy anomer the C-19–H is more distant from the deshielding C-3 oxygen function and C-18 is more distant from the deshielding hydroxy group. A similar situation prevails in the case of **4**, in which the C-19 and C-18 peaks occur at 5.36 and 0.88 ppm, respectively, in the (19*S*) hydroxy isomer, and at 5.83 and 0.93 for the (19*R*) isomer. (See Scheme I.)

Treatment of **3** and **4** with zinc in acetic acid resulted in elimination of the elements of hypochlorous acid and gave directly **5**[5] and **7**,[6,7] respectively. The over-all yield of the aldehyde from the oxime was approximately 70% in both cases, making this an especially good way for obtaining Δ^5-C-19 aldehydes. Saponification of **5** and **7** gave **6**[5] and **8**,[7] respectively. Reduction of **6** with palladium and hydrogen in methanol solution readily gave acetal **9**. This material had mp 154–156°, but showed two spots on tlc and glpc. The nmr spectrum showed two C-19–H bands in 1:1 ratio at 4.75 and 4.95 ppm and two methoxyl peaks at 3.40 and 3.42 ppm. The C-18 resonance occurred at 0.73 and 0.75 ppm. Unlike the situation in 6,19-hemiacetals **3** and **4**, it is not possible in this case to determine the sterically favored C-19 isomer from an inspection of models, nor is it possible to assign the individual nmr peaks to one or the other isomer. Even when the reduction was performed in isopropyl alcohol solution, two isomers corresponding to **10** were obtained, as shown by C-19–H resonance at 4.91 and 5.13 ppm in the ratio 2:1. The minor component was obtained in pure form after chromatography and recrystallization, and it was found that the low-field C-19 resonance and the high-field C-18 resonance are produced by this isomer.

By contrast to 6,19-hemiacetals **3** and **4**, in which ring B is in a stable chair, ring A must form a boat in **9** and **10**. Therefore, the hydrolysis of **9** and **10** gives open aldehyde **14**, rather than the cyclic hemiacetal, as the sole isolable product.

(1) (a) Paper X: M. E. Wolff and T. Morioka, *J. Org. Chem.*, **30**, 2553 (1965). (b) This investigation was supported in part by a Public Health Service research grant (AM-05016) from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service. (c) A preliminary account of portions of this work has been presented: M. E. Wolff and S.-Y. Cheng, *Tetrahedron Letters*, 2507 (1966). (d) From the Ph.D. Thesis of S.-Y. Cheng, University of California at San Francisco, 1966.

(2) This study was initiated in this laboratory by Dr. Timothy Jen, who prepared compounds **16** and **17**: T. Jen, Ph.D. Thesis, University of California at San Francisco, 1963, pp 46–47, 70–71, 80–81.

(3) Evaluation of **16** for its ability to antagonize the myotrophic–androgenic effect of testosterone propionate showed that it had slight activity when measured by the method of L. O. Randall and J. J. Selitto, *Endocrinology*, **62**, 693 (1958). However, compounds **18**, **19**, **20**, and **21** were inactive in this test. All tests were conducted using ten times as much antagonist as testosterone propionate, and were performed at Endocrine Laboratories, Madison, Wis.

(4) T. Jen and M. E. Wolff, *J. Med. Pharm. Chem.*, **5**, 876 (1962); *J. Med. Chem.*, **6**, 726 (1963).

(5) T. Jen and M. E. Wolff, *J. Org. Chem.*, **28**, 1573 (1963).

(6) K. Tanabe, R. Takasaki, K. Sakai, R. Hayashi, and Y. Morisawa, *Chem. Pharm. Bull.* (Tokyo), **10**, 1126 (1962).

(7) O. Halpern, I. Delfin, L. Magaña, and A. Bowers, *J. Org. Chem.*, **31**, 693 (1966).