deacylation followed by rapid rearrangement. Verification of this hypothesis must await detailed kinetic and product studies in regions of intermediate acidity and salt concentrations.

Registry No.—*N*-Acetylhydrazobenzene, 22293-38-5; 2,3,4,5,6,2′,3′,4′,5′,6′-decadeuterio-*N*-acetylhydrazobenzene, 36358-15-3; *N*-acetylbenzidine, 3366-61-8; benzidine, 92-87-5.

Electron Impact Induced Stereospecific Hydrocarbon Fragmentations. Mass Spectrometric Determination of the Configuration at C-5 in Steroidal Hydrocarbons[1]

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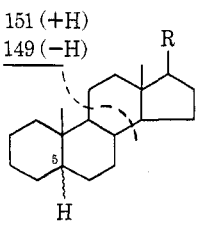
The mass spectra of C-17 side chain bearing steroidal hydrocarbons exhibit stereospecific fragmentation reactions which are diagnostic for the configuration at C-5. The resulting fragment ions are most probably derived from a D-seco molecular ion which is formed by the facile cleavage of the 13–17 bond in the presence of the C-17 side chain. Consequently, these stereospecific reactions are absent in the spectra of C-5 epimeric androstanes which lack the side chain. The cleavage patterns and fragmentation mechanisms of these reactions were studied with the aid of substituent and deuterium labeling techniques and metastable peak analysis. This study lead to the discovery of a site-specific hydrogen transfer from C-12 in association with the formation of the diagnostically important *m/e* 151 ion. The synthesis of the various labeled compounds is described.

Initial recognition of mass spectroscopy as an indispensable tool in structure elucidation originates from hydrocarbon chemistry. Its value in two-dimensional structure elucidation is well established, but generally in this field mass spectroscopy provides very little steric information. In a recent review of the stereoisomeric effect on the mass spectra of hydrocarbons, Meyerson and Weitkamp[2] concluded that the spectra of hydrocarbon stereoisomers, unlike those of many functional group containing species, show no clear evidence of stereospecific reactions. Similarly, careful analysis of the mass spectra of the various 1-methyl- and 2-methyldecalins by the same authors revealed only very modest differences between these stereoisomers.[3] Correlation does exist, for example, between the relative intensity of the $M^+ - CH_3$ fragment ions and the relative stability of the molecules, but these spectral variations are very sensitive to experimental conditions, and they are meaningful only in comparative studies.

Extraction of the maximum amount of structural information (including stereochemistry) from the mass spectrum of a compound is especially important in gc–mass spectroscopy, which can be used to analyze submilligram amounts of complex mixtures. The structure elucidation of the components in such mixtures depends largely on the interpretation of the fragmentation patterns. The importance of reliable interpretations is, therefore, obvious in fields such as natural product chemistry and biological research, where scarcity of the material, or complexity of the mixture, often preclude the application of other physical or chemical techniques.

During the course of earlier detailed examination of the fragmentation mechanisms of steroidal hydrocarbons,[4,5] it was noted that the C-5 epimeric preganes (III and IV) exhibit a significant difference in the relative intensities of the *m/e* 149 and 151 ions.[4] To test for the generality of this fragmentation as a diagnostic feature for the stereochemistry at the A/B ring junction, we examined the mass spectra of the three most important C-5 epimeric hydrocarbon pairs, androstanes (I and II, Figures 1 and 2), pregnanes (III and IV, Figures 3 and 4), and cholestanes (V and VI, Figures 5 and 6) under various experimental conditions. It was found that the characteristic features in the *m/e* 147–153 region of the spectrum provide unequivocal differentiation between the 5α and 5β epimers of C-17 side chain bearing hydrocarbons (see Figures 3–6). This mass range is dominated by an intense *m/e* 149 ion in the spectra of 5α steroids while two significant peaks (*m/e* 149 and 151) are characteristic for the 5β epimers. This difference, however, is not apparent in the mass spectra of 5α- and 5β-androstanes (I and II), which consist of the tetracyclic nucleus only without any side chain at C-17 (compare Figures 1 and 2).



I, 5α; R = H
II, 5β; R = H
III, 5α; R = C_2H_5
IV, 5β; R = C_2H_5
V, 5α; R = C_8H_{17}
VI, 5β; R = C_8H_{17}

The stereoisomeric effect on the fragmentations of these hydrocarbons is of considerable practical as well as theoretical interest. Its practical significance has been clearly demonstrated[6] by the recent studies of the constituents of Green River shale and California petro-

(1) Presented in part at the Pacific Conference on Chemistry and Spectroscopy, Anaheim, Calif., Oct 1971.

(2) S. Meyerson and A. W. Weitkamp, *Org. Mass Spectrom.*, **1**, 659 (1968).

(3) S. Meyerson and A. W. Weitkamp, *ibid.*, **2**, 603 (1969).

(4) L. Tökés, G. Jones, and C. Djerassi, *J. Amer. Chem. Soc.*, **90**, 5465 (1968).

(5) L. Tökés and C. Djerassi, *ibid.*, **91**, 5017 (1969).

(6) (a) E. J. Gallegos, *Anal. Chem.*, **43**, 1151 (1971); (b) W. K. Seifert, E. J. Gallegos, and R. M. Teeter, *J. Amer. Chem. Soc.*, **94**, 5880 (1972).

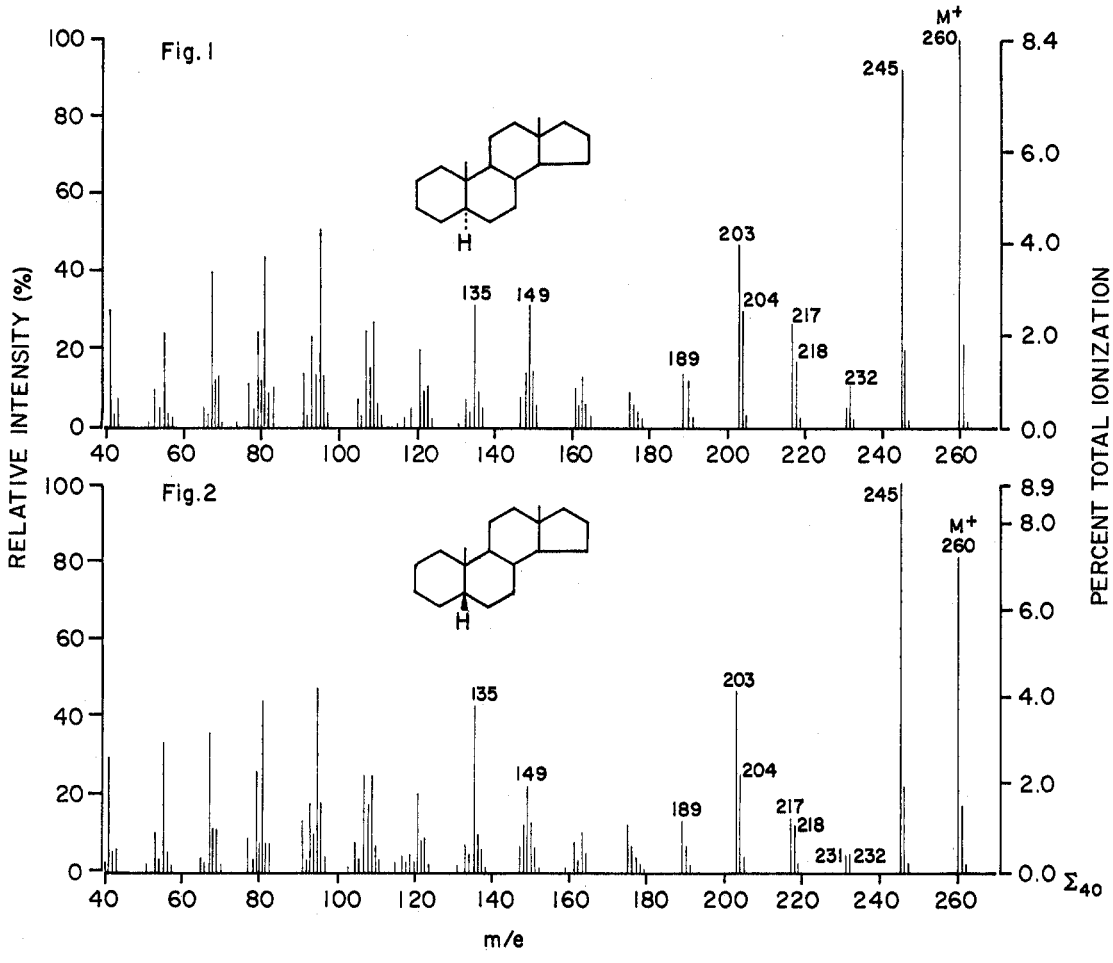


Figure 1.—Mass spectrum of 5α-androstane (I), measured at 70 eV.
Figure 2.—Mass spectrum of 5β-androstane (II), measured at 70 eV.

leum. The same characteristic differences were found in the spectra of C-5 epimeric cholestanes, cholanes, and bisnorcholanes and this observation was applied to the structure elucidation of a variety of steroidal hydrocarbon constituents. The generality of this stereoisomeric effect and its fragmentation mechanism, however, have not been established.

This mass spectrometric stereochemical information may also find an important application in the carbon-skeleton analysis[7] of steroidal compounds. This technique involves stripping of all functional groups and saturation of double and triple bonds by catalytic hydrogenation in less than a milligram of sample, followed by gas chromatographic separation and mass spectrometric analysis of the resulting hydrocarbons. If suitable catalytic systems can be developed to avoid epimerization of the asymmetric centers, the application of this technique could be extended to stereochemical studies in addition to skeletal size determination.

A priori it is reasonable to assume that the same cleavage is responsible for the formation of both *m/e* 149 and 151 ions and the mass difference is then due to different directions of the net hydrogen transfer. Precise understanding of the genesis of the *m/e* 151 ion is essential, however, to establish the criteria and limitations of the formation of this ion when its presence or absence is utilized for stereochemical assignment of unknown steroids.

The *m/e* 149 ion is a general feature of steroidal hydrocarbon spectra. Various mechanisms have been proposed[8] for the formation of this ion in the 5α series, but the true complexity of this cleavage was uncovered only as a result of extensive deuterium-labeling work.[4] In 5α-androstane this ion is generated by different cleavage patterns, yielding a complex mixture of fragments,[5] while in 5α-pregnane and 5α-cholestane about 90% of it originates from rings A and B, by the net ruptures of the 8–14 and 9–11 bonds[4] (see I–VI). This major cleavage pattern requires the loss of an extra hydrogen from the charge-retaining fragment. The labeling results, however, revealed three hydrogen transfers in association with this cleavage in relatively high degree of site specificity. Two of them (5α H and 8β H) originate from the charge-retaining side and one (14α H) from the expelled neutral fragment.[4] The lower and upper range of the transfer values, depending on the extent of deuterium loss or retention due to fragment ions (~10%) which do not originate from rings A and B, are summarized on VII.[9] The interpretation of these results is further complicated by the possible

(7) This technique has been recently reviewed by M. Beroza, *Accounts Chem. Res.*, **3**, 33 (1970).

(8) K. Biemann, "Mass Spectrometry," McGraw-Hill, New York, N. Y., 1962, p 345; H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. I, Holden-Day, San Francisco, Calif., 1963, p 96.

(9) It should be noted that this cleavage pattern cannot be established unequivocally on the basis of deuterium-labeling evidence alone. The loss of a single deuterium from a tertiary position can be interpreted in terms of either deuterium transfer or as expulsion of that CD unit. The complex results indicated on VII could also be interpreted as shown below, although

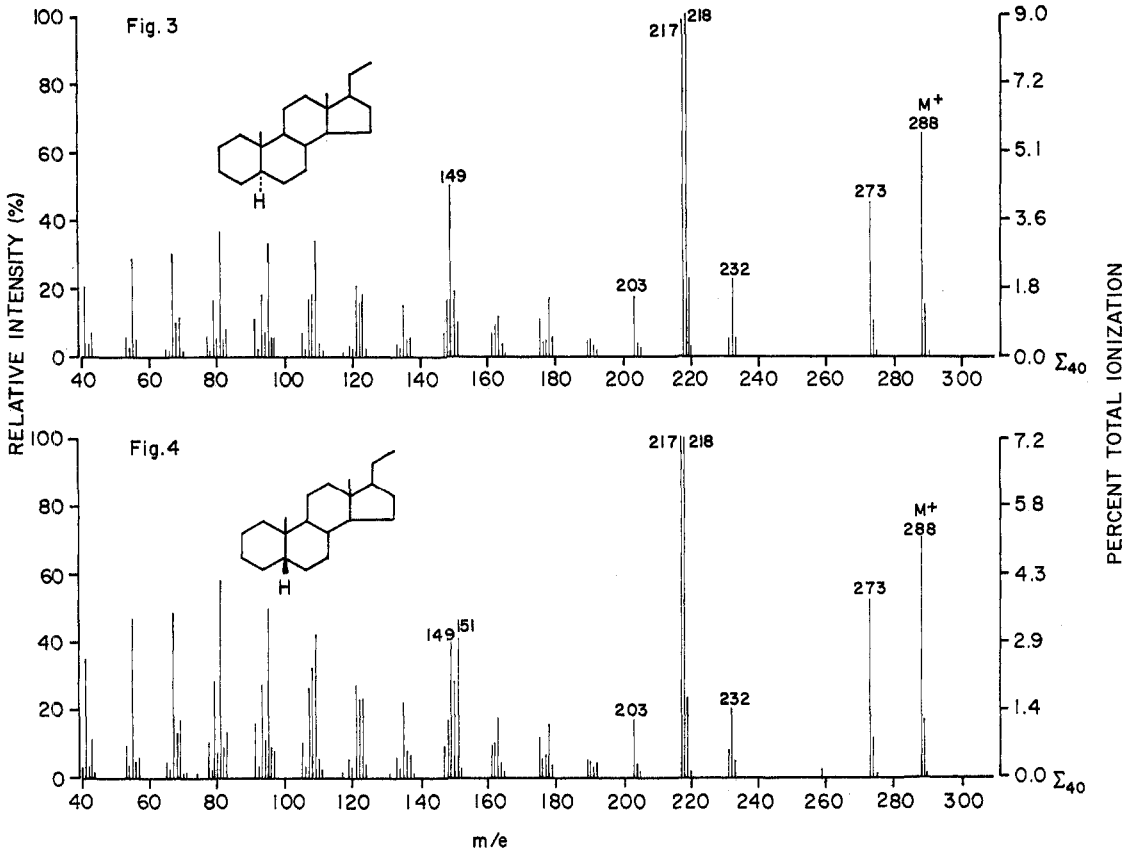
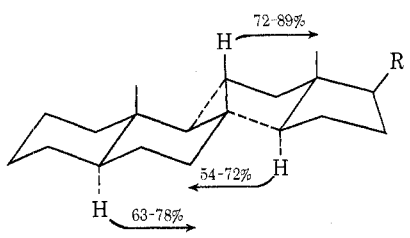


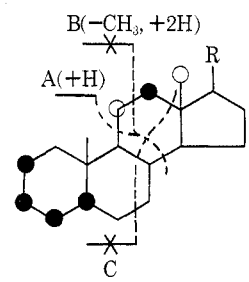
Figure 3.—Mass spectrum of 5α-pregnane (III), measured at 70 eV.
Figure 4.—Mass spectrum of 5β-pregnane (IV), measured at 70 eV.



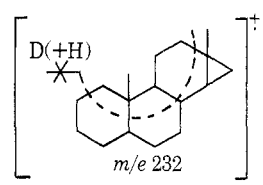
VII (*m/e* 149)

participation of different fragmentation reactions which can lead to similar *m/e* 149 fragment ions consisting of rings A and B, but are derived from parent ions other than the molecular ion. Refocused scanning for metastable peaks indicated the participation of six precursor ions in forming the *m/e* 149 ion,[10] but the extent of their participation is unknown.

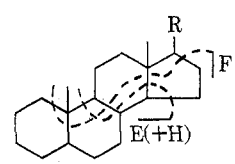
Examination of the mass spectra of some ring A deuterated 5β-cholestanes, which we had available from other studies, indicated that the lower intensity *m/e* 149 peak is due to more than one major fragment ions which originate from different parts of the molecule. The diagnostically important *m/e* 151 ion, on the other hand, appeared to be quite homogeneous, retaining carbon atoms 2, 3, and 4. Since this information and the loss of the side chain are compatible with several cracking patterns (see A–F in VIII–X) we decided to prepare additional deuterium and substituent labeled pregnanes and cholestanes to shed light on the genesis of this ion. The syntheses of these labeled compounds, the labeling results, and their interpretations are discussed below.



VIII (*m/e* 151)

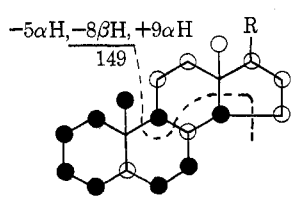


IX (*m/e* 151)



X (*m/e* 151)

this alternative is energetically much less likely. Rigorous differentiation between these two alternative would require carbon isotope labeling.



(10) C. C. Fenselau and F. P. Abramson, *Org. Mass Spectrom.*, **2**, 915 (1969).

Syntheses of Labeled Compounds[11]

The preparation of *2,2,3ξ,4,4-d*$_5$ and *2,2,3,3,4,4-d*$_6$ labeled 5β-cholestanes (XIV and XV) were carried out

(11) For a recent survey of the various deuterium-labeling techniques in the steroid field, see L. Tökés and L. Throop in "Organic Reactions in Steroid Chemistry," Vol. 1, J. Fried and J. A. Edwards, Ed., Van Nostrand-Reinhold, Princeton, N. J., 1972, Chapter 4.

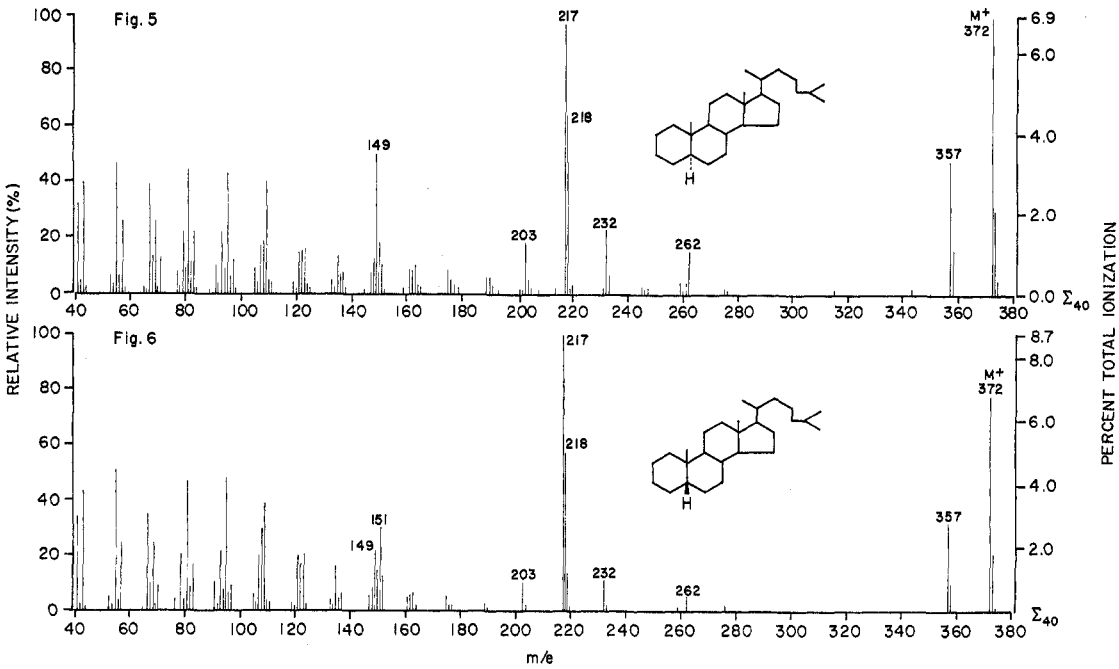
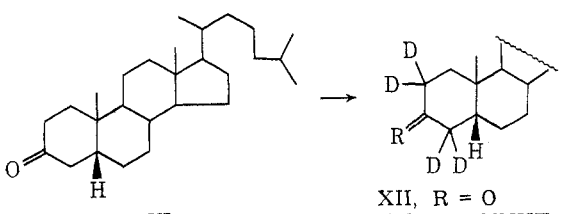
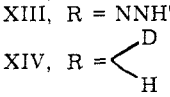


Figure 5.—Mass spectrum of 5α-cholestane (V), measured at 70 eV.
Figure 6.—Mass spectrum of 5β-cholestane (VI), measured at 70 eV.

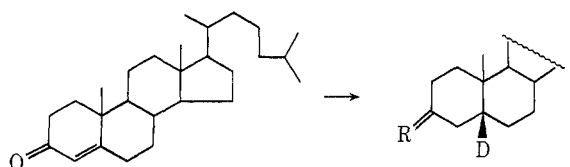
in connection with other studies, using 5β-cholestan-3-one-*2,2,4,4*-d_4 (XII) as a common intermediate in both syntheses. The pentadeuterio 5β-cholestane (XIV) was prepared by lithium aluminum deuteride treatment of the tosylhydrazone derivative (XIII) of XII followed by quenching of the reaction mixture with ethyl acetate and water. This reaction gave the expected[12] 5β-cholestane-*2,2,3ξ,4,4*-d_5 (XIV) in 73% isotopic purity. In the 5α series this reaction is known to give mainly the 3α-deuterio derivative (70%).[12] We made no attempt, however, to establish the configuration of the deuterium at C-3. Electrochemical reduction[13] of the tetradeuterio ketone XII in dioxane and deuteriosulfuric acid gave 5β-cholestane-*2,2,3,3,4,4*-d_6 (XV) in 85% isotopic purity.



XI

XII, R = O
XIII, R = NNHTs
XIV, R = 
XV, R = D_2

Deuteration of the 5β position was carried out to check if this site is involved in hydrogen transfer similar to that observed in the formation of the *m/e* 149 ion in the 5α series.[4] Catalytic deuteration of cholest-4-en-3-one followed by alkaline back-exchange of the deuterium from C-4, gave a mixture of 5α-d_1 and 5β-d_1 cholestan-3-ones which were separated by thin layer chromatography. Modified Huang-Minlon reduction of the pure 5β-d_1-cholestan-3-one (XVII) gave 5β-d_1-cholestane (XVIII) in 81% isotopic purity.



XVI

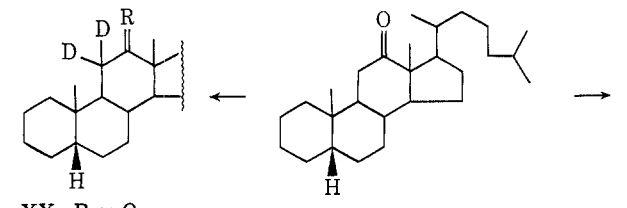
XVII, R = O
XVIII, R = H_2

The preparations of both *11,11*-d_2 and *12,12*-d_2 labeled 5β-cholestanes (XXII and XXIV) were carried out starting with 5β-cholestan-12-one[14] (XIX). Base-catalyzed deuterium exchange of XIX gave the *11,11*-d_2 ketone (XX) which was then converted into its tosylhydrazone derivative (XXI). Treatment of the tosylhydrazone with sodium borohydride, first in boiling methanol, then in boiling dioxane to speed up the reaction, consumed all the starting material and gave a mixture of less polar products. Thin layer chromatographies on silica gel, then on silver nitrate impregnated silica gel, gave pure 5β-cholestane-*11,11*-d_2 (XXII) in 21% yield, which was free of any olefin contaminants and exhibited 89% isotopic purity.

Deuteration of the C-12 position was carried out by lithium aluminum deuteride treatment of the tosylhydrazone derivative (XXIII) of XIX, followed by quenching of the reaction mixture with deuterium oxide. Owing to the very limited supply of the starting ketone (3 mg) and the low yield obtained from the reduction of this sterically hindered tosylhydrazone, the resulting 5β-cholestane-*12,12*-d_2 (XXIV) could be characterized only by thin layer chromatography and gc–mass spectrometric analysis.[15]

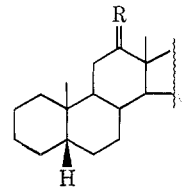
Availability of 18-methylpregn-4-ene-3,20-dione[16] (XXV) provided an attractive short route to a C-18 labeled derivative which is a key compound to test for

(12) M. Fischer, Z. Pelah, D. H. Williams, and C. Djerassi, *Chem. Ber.*, **98**, 3236 (1965).
(13) L. Throop and L. Tökés, *J. Amer. Chem. Soc.*, **89**, 4789 (1967).
(14) J. Gawronski and M. Kielczewski, *Rocz. Chem.*, **44**, 1175 (1970).
(15) It has been reported in ref 4 that under identical conditions lithium aluminum hydride treatment of *11,11*-d_2-5α-pregnane 12-tosylhydrazone yields *11,11*-d_2-5α-pregnane as the saturated hydrocarbon product.
(16) G. V. Baddeley, H. Carpio, and J. A. Edwards, *J. Org. Chem.*, **31**, 1026 (1966).



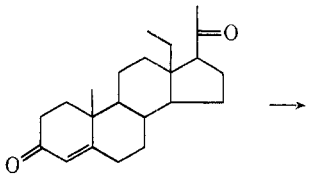
XX, R = O
XXI, R = NNHTs
XXII, R = H_2

XIX

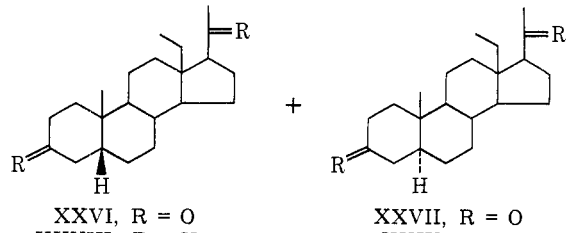


XXIII, R = NNHTs
XXIV, R = D_2

the possible participation of cleavage pattern D (see IX) in the formation of the *m/e* 151 ion. Catalytic hydrogenation of XXV gave a readily separable mixture of C-5 epimeric diones (XXVI and XXVII). As expected from the corresponding pregnane-3,20-diones, these epimers exhibited almost identical optical rotatory dispersion and circular dichroism curves as well as 19-H chemical shifts in the nmr in deuteriochloroform solution The very similar optical properties of the 3,20-diones, in spite of the opposite signs of the Cotton effects of C-5 epimeric 3 ketones, is due to the overwhelmingly strong positive Cotton effect of the 20-ketone function.[17] The stereochemical assignment of these epimers was based on the amplitude of the Cotton effects (the lower value being due to the negative contribution of the 3 ketone of the 5β skeleton) and on the 10-Hz chemical-shift difference of the 19-H resonances in hexadeuteriobenzene solution. The 5β configuration was assigned to the major product, which exhibited the less shielded 19-H resonance.[18]



XXV



XXVI, R = O
XXVIII, R = H_2

XXVII, R = O
XXIX, R = H_2

Modified Huang-Minlon reduction of these diones gave the desired 5β and 5α 18-methylpregnanes (XXVIII and XXIX) whose 19-H resonances were in good agreement with those of their pregnane analogs.[19]

(17) C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill, New York, N. Y., 1960, p 55.

(18) The chemical shifts of the 19-H resonances of 5α- and 5β-cholestan-3-ones in hexadeuteriobenzene solution are 0.66 and 0.75 ppm, respectively.

(19) For the 19-H resonances of 5α- and 5β-pregnanes, see N. S. Bhacca and D. H. Williams, "Application of NMR Spectroscopy in Organic Chemistry," Holden-Day, San Francisco, Calif., 1964, Chapter 2.

This confirmed the stereochemical assignments of the diones. The mass spectral characteristics in the *m/e* 147–152 region were also very similar in the spectra of the labeled and unlabeled pregnanes in both 5α and 5β series, which indicates that the methyl group was indeed a legitimate substituent label at C-18.

The 18-methyl-5β-pregnane (XXVIII) did not crystallize owing to the presence of a very small isomeric contaminant. This isomer could be separated only by gas chromatography and exhibited virtually identical nmr and mass spectral characteristics with those of the major product XXVIII. This contaminant is apparently the 17α isomer of 18-methyl-5β-pregnane arising from C-17 epimerization prior to the hydrazone formation of the 20 ketone.[20] The equal relative intensities of the *m/e* 149 and 151 fragment ions of both 17α and 17β 18-methyl-5β-pregnanes indicate that the genesis of the diagnostically important *m/e* 151 ion is independent of the stereochemistry at C-17.

Discussion of Mass Spectral Results

The 70-eV spectra of 5α and 5β androstanes, pregnanes, and cholestanes are reproduced in Figures 1–6. The shift values of the *m/e* 149–152 ions in the spectra of the labeled compounds are listed in Table I and summarized on structure VIII (full dots, label retained; shaded dot, one of the two deuteriums retained; circle, label lost). The most noticeable difference between the two androstane spectra (Figures 1 and 2) is the higher intensity of the $M^+ - CH_3$ (*m/e* 245) and *m/e* 135 ions in the spectrum of 5β-androstane as compared to the molecular ion. These relative intensity differences, however, are sensitive to variations of the ion source temperature and other experimental parameters, and can be used to identify the androstane epimers only in a comparative manner.

It has been established that in 5α-androstane (I) the expelled methyl radicals originate exclusively from the C-18 and C-19 angular methyl groups in 3:2 ratio, respectively.[5] The more intense $M^+ - CH_3$ fragmentation in 5β-androstane (II) may be due to enhanced loss of the C-19 methyl group to relieve the increased strain of the A/B cis ring junction. Confirmation of this hypothesis will await deuterium labeling of this position.

With C-17 side chain bearing hydrocarbons (Figures 3–6) the pronounced difference in the relative intensities of the *m/e* 149 and 151 peaks provides a much more general and reliable differentiation between the C-5 epimers. In the 5α series this mass range is dominated by an intense peak at *m/e* 149, whereas two major peaks, at *m/e* 149 (of somewhat reduced intensity) and 151, are characteristic for the 5β hydrocarbons. The same difference prevails also at low ionization potentials (20 and 15 eV).[21] The formation of the diagnostic *m/e* 151 fragment ion from 5β steroids is unaffected by the size of the side chain, by the configuration at C-17 (as indicated by the spectrum of 18-

(20) Gc–mass spectral analysis of the crude reduction mixture revealed the presence of some 18-methyl-5β-pregnan-20-one together with smaller quantities of its 17α epimer. The structural assignments of these ketones were based on the known [L. Tökés, R. T. LaLonde, and C. Djerassi, *J. Org. Chem.*, **32**, 1020 (1967)] fragmentation patterns of both 17β and 17α epimeric pregnan-20-ones.

(21) For the 20-eV spectra of C-5 epimeric cholestanes, ergostanes, and stigmastanes, see ref 6a.

TABLE I

SHIFTS[a] OF MASS SPECTRAL PEAKS OF THE LABELED ANALOGS OF 5β-STEROIDAL HYDROCARBONS

5β-Cholestanes	Isotopic purity, %	m/e			
		$C_{11}H_{17}^+$	$C_{11}H_{18}^+$	$C_{11}H_{19}^+$	$C_{11}H_{20}^+$
d_0 (VI)		149	150	151	152
2,2,3ξ,4,4-d_5 (XIV)	d_4, 24 d_5, 73 d_6, 3	149 (36%) 154 (64%)	150 (23%) 155 (77%)	151 (6%) 156 (94%)	152 (~90%)[b]
2,2,3,3,4,4-d_6 (XV)	d_4, 2 d_5, 13 d_6, 85	149 (32%) 155 (~60%)	150 (25%) 156 (75%)	151 (10%) 157 (90%)	152 (~90%)
5β-d_1 (XVIII)	d_0, 16 d_1, 81 d_2, 3	149 (52%) 150 (48%)	150 (36%) 151 (64%)	151 (7%) 152 (93%)	152 (92%) 153 (8%)
11,11-d_2 (XXII)	d_0, 2 d_1, 9 d_2, 89	149 (85%) 150 (15%)	150	151	152
12,12-d_2 (XXIV)	d_0, 6 d_1, 35 d_2, 57 d_3, 2	149	150	151 (52%) 152 (48%)	152
18-methyl[c] (XXVIII)		149[d]	150	151 165 (<5%)	

[a] The shift values are corrected for isotopic impurity as well as for ^{13}C contributions and are reliable to ±5%. [b] Owing to poor isotopic purity, the reliability of this shift is ±10%. [c] This sample is 18-methyl-5β-pregnane. [d] This ion is present in the expected relative intensity, but it cannot be determined with certainty how much of the m/e 135 and 149 ions is shifted by 14 mass units.

methyl-5β,17α-pregnane; see previous section), and by the presence of a methyl substituent at C-18 (see XXVIII in Table I). The ensuing discussion of the genesis of this fragment ion allows further predictions about the generality (or limitations) of its diagnostic value.

Examination of the spectra of the various model compounds and initially available ring A labeled 5β-cholestanes (XIV and XV) revealed the loss of the side chain and retention of carbon atoms 2, 3, and 4 in the m/e 151 ion and in about two-thirds of the m/e 149 ions. These ions, therefore, could be formed by a ring C cleavage which is analogous to the one leading to the majority of the m/e 149 ions in the 5α series[4] (see I–VI and cleavage A in VIII). There are other cleavage patterns, however, which can lead to the m/e 151 ions and are also compatible with these results (see patterns B–F on VIII–X), but most of them could be eliminated with the aid of subsequent labeling results (see Table I).

Complete loss of the two deuteriums from C-11 rules out the participation of the mechanistically quite reasonable B and C cleavages (see VIII). Pattern D, which would involve further fragmentation of the m/e 232 ion (see IX), is excluded by the loss of the C-18 methyl label. The remaining two fragmentation patterns E and F (see X) are compatible with all the currently available labeling results. They can be deemed unlikely, however, since both of them (especially E) would involve energetically very unfavorable fragmentation mechanisms. Deuterium labeling at positions 15, 16, or 19 could establish unequivocally the possible participation of path F, but carbon isotope labeling would be needed for the rigorous exclusion of pattern E (see footnote 9).

Retention of one of the two deuteriums from C-12 in the m/e 151 ion (see XXIV in Table I) provides further mechanistic support for cleavage pattern A. For a fragmentation mechanism to be consistent with our current knowledge of this ion, the requirement for the presence of a side chain at C-17 (independent of its configuration) and for the β configuration at C-5, as well as the labeling results shown in Table I, have to be taken into account. Such mechanisms are outlined in Scheme I.

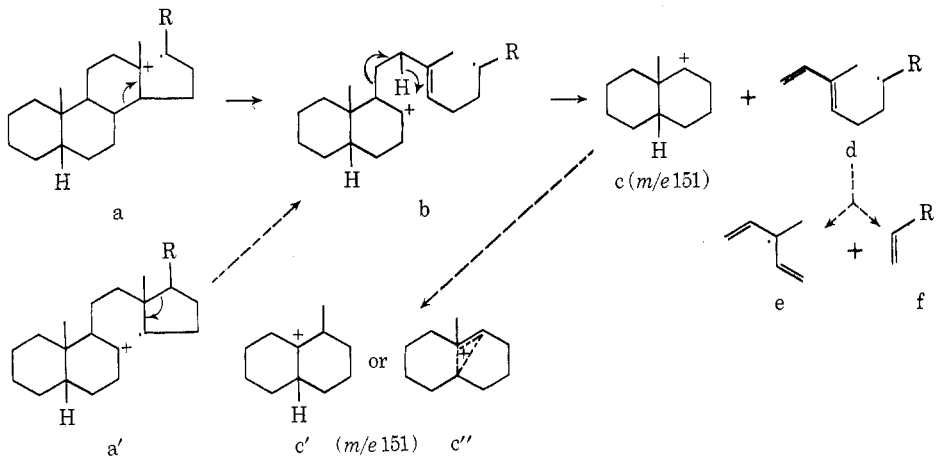
Rupture of the 13–17 bond in forming molecular ion a is the most important primary cleavage of C-17 side chain bearing steroidal hydrocarbons.[4,22] This molecular ion is known to undergo ring D cleavages and various hydrogen transfers, leading to most of the diagnostically important fragment ions in the high-mass range of the spectrum.[4] Alternatively, opening of ring C, *via* cleavage of the activated 8–14 bond, yields a homoallylically stabilized ion radical (b). The rupture of the 13–17 bond in the presence of a C-17 side chain in molecular ion a′ may be another possible fragmentation leading to ion b. Transfer of one of the allylic hydrogens from C-12 to C-8 in ion b triggers the fission of the 9–11 bond, forming the m/e 151 ion (c) and a diene radical (d). Both c and d may undergo further reactions to gain stabilization. Methyl migration[23] in the m/e 151 ion, for example, leads to a tertiary carbonium ion (c′) or cleavage of the activated 5–10 bond can yield a homoallylically stabilized ion (c″). Radical d can undergo fission to give a conjugated diene radical e and an olefin f.

These mechanisms are fully consistent with the labeling results shown in Table I. Molecular ions a or a′ account for the requirement for the presence of a side chain at C-17 (independent of its configuration), since the 13–17 bond cleavage is not very significant in 5α and 5β androstanes (I and II).[5,22] The C-5 stereochemistry may play an important role at the fragmentation of molecular ion b. Examination of the Dreiding models of the 5α and 5β epimers of ion b indicates that the C-17 radical site can reach easily to the 5α tertiary hydrogen, but it can not approach the 5β hydrogen to within rea-

(22) G. Eadon, S. Popov, and C. Djerassi, *J. Amer. Chem. Soc.*, **94**, 1282 (1972).

(23) For a recent review on electron impact induced rearrangements see R. G. Cooks, *Org. Mass Spectrom.*, **2**, 481 (1969).

SCHEME I

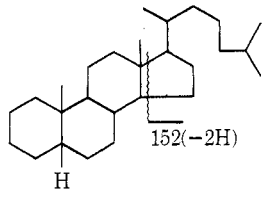


sonable bonding distance without encountering serious overlaps between various parts of the molecule. It is possible, therefore, that in the 5α series the transfer of the 5α hydrogen leads ultimately to the *m/e* 149 ion (70% deuterium loss has been found from this site[4]), while at this stage in the 5β series an alternate fragmentation path (b → c) is preferred which yields the *m/e* 151 ion. This is in good agreement with the reduced intensity of the *m/e* 149 ion in the 5β series, of which no more than 65–70% can originate from cleavage pattern A (see Table I).

Refocused measurement of metastable peaks[24] in the spectrum of 5β-cholestane (VI) confirmed that at least part of the *m/e* 149 and 151 ions is derived directly from the molecular ion. These measurements revealed a total of 14 possible parent ions for the *m/e* 149 ion and 10 for the *m/e* 151 ion, but these metastable peaks have no direct relevance to the relative significance of the participating cleavages. These results are in agreement with earlier findings[10] that the fragment ion which shows more complex deuterium-labeling results (*m/e* 149 in this case) is the one which exhibits the more numerous modes of formation.

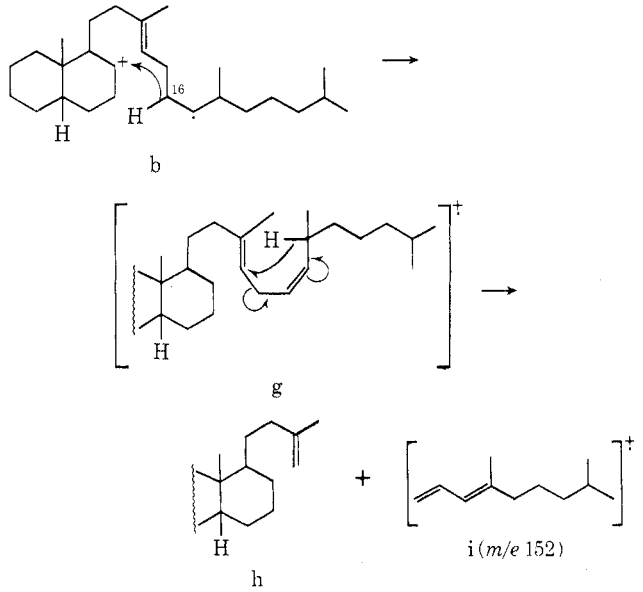
The *m/e* 152 ion in the spectrum of 5β-cholestane (Figure 6) is also a stereospecific fragmentation product. This ion is absent in Figures 1–5 and its presence only in the spectrum of 5β-cholestane can be explained by the retention of the side chain in it. This is further substantiated by the observed small peaks at *m/e* 166 and 180 in the spectra of methyl and ethyl homologs of 5β-cholestane, 5β-ergostane, and 5β-stigmastane, respectively, while these peaks are absent in the spectra of the corresponding 5α epimers.[25] The *m/e* 152 ion lost all ring A and ring C labels (see Table I). It is apparently due to a ring D fragmentation in which the charge is retained on the side chain bearing portion which loses two hydrogens to the neutral side (see XXX).

The stereochemical dependence of this fragmentation may be due to the same reason as proposed for the genesis of the *m/e* 151 ion (*vide supra*), namely,



XXX

hindered hydrogen transfer from the 5β position to the C-17 radical site in ion b. In discussing the various fragmentations of ion b it was shown above how transfer of the C-12 hydrogen leads to the *m/e* 151 fragment ion. Alternatively, hydrogen transfer in ion b may occur from C-16 just as well, since this position is also activated by the isolated radical site at C-17. This leads to an ionized diene g which can undergo a second hydrogen transfer from the tertiary and allylic C-20 position to C-13 in a six-membered transition state. This second hydrogen transfer then triggers the fission of the 14–15 bond to yield a neutral olefin and the ionized conjugated diene i (*m/e* 152). Confirmation of this fragmentation mechanism, however, will have to await further deuterium-labeling evidence.



Cracking pattern A, with or without a reciprocal hydrogen transfer, appears to be responsible for about 75% of the low-intensity *m/e* 150 ion. The remaining

(24) M. Barber and R. M. Elliot, ASTM E-14 Committee, 12th Annual Conference on Mass Spectrometry and Allied Topics, Montreal, Canada, 1964; J. H. Futrell, K. R. Ryan, and L. W. Sieck, *J. Chem. Phys.*, **43**, 1832 (1965); T. W. Shannon, T. E. Mead, C. G. Warner, and F. W. McLafferty, *Anal. Chem.*, **39**, 1748 (1967).

(25) Personal communications with Dr. E. J. Gallegos, Chevron Research Co., Richmond, Calif.

25% originates from other parts of the molecule. Owing to its low intensity this ion was not investigated in further detail.

In conclusion, C-17 side chain bearing steroidal hydrocarbons exhibit stereospecific fragmentation reactions which are diagnostic for the configuration at C-5 when the other asymmetric centers are in their "normal" (8β,9α,10β,13β) configuration. It has not been established as yet whether changing of one or more of the other asymmetric centers has any effect on these stereospecific fragmentations. It is known,[22] however, that in the spectrum of *D*-nor-5α-pregnane the diagnostic mass range is dominated by an intense peak at *m/e* 148 which may interfere with the interpretations. The mechanistic details uncovered in this study, including the site-specific hydrogen transfer from C-12 in the formation of the *m/e* 151 ion, are significant contributions to the understanding of the electron impact induced behavior of the steroidal hydrocarbon skeleton.

Diagnostically important stereospecific fragmentations, in the sense of the "presence or absence" of fragment ions rather than "relative intensity differences," usually involve bond forming as well as breaking steps. The stereospecificity of these fragmentations is primarily due to the specific requirements in the spatial relationship between the bond-forming species. Such a stereospecific fragmentation can be of practical diagnostic value only if it is the sole or at least the main contributor in the genesis of the fragment ion in consideration. This is the criterion which most hydrocarbon fragment ions fail to meet. It is not surprising, therefore, that polycyclic hydrocarbons, which are more likely to retain several asymmetric centers after the preferential cleavage of a strained bond, are more prone to exhibit stereospecific fragmentations than the bicyclic compounds used in earlier studies. The C-17 side chain on a tetracyclic steroidal skeleton behaves like a fragmentation-triggering "functional group" by promoting the rupture of the 13–17 bond. This dominant primary cleavage assures the formation of several reasonably homogeneous fragment ions which can facilitate the observation of stereospecific fragmentation reactions if the spatial significance of the isomeric center (C-5 in this case) is not destroyed by the primary cleavage.

Experimental Section[26]

5β-Cholestan-3-one-*2,2,4,4-d*$_4$ (XII).—A solution of 5β-cholestan-3-one (XI, 25 mg) in methanol-*O-d* (5 ml) was saturated with 10% sodium deuterioxide in deuterium oxide and then heated under reflux for 36 hr. After cooling, ether was added and the organic phase was quickly washed with ice-cold water. Drying (Na_2SO_4) and evaporation of the ether yielded 23 mg of 5β-cholestan-3-one-*2,2,4,4-d*$_4$ (XII, 91%) which exhibited 1% d_2, 12% d_3, and 87% d_4 isotope composition by mass spectrometric analysis.

5β-Cholestane-*2,2,3ξ,4,4-d*$_5$ (XIV).—The labeled ketone (XII, 10 mg) was dissolved in methanol-*O-d* (15 ml), *p*-toluenesulfonyl hydrazine (20 mg) and 1 drop of deuteriosulfuric acid were added, and the solution was heated under reflux for 4 hr. A few drops of water was added and the crystalline precipitate which formed upon refrigeration was collected, washed with aqueous methanol (1:1), then dried under vacuum at 50°. The resulting crystalline d_4-tosylhydrazone (XIII, 13 mg, 89%) was dissolved in dry monoglyme (20 ml), lithium aluminum deuteride (40 mg) was added, and the mixture was heated under reflux for 24 hr. The excess deuteride was decomposed by the addition of a few drops of ethyl acetate and water, then the heating was resumed for 10 min. Dilute hydrochloric acid was added and the resulting solution was diluted with water. Ether extraction, washing of the ether phase with dilute sodium bicarbonate solution and water, drying (Na_2SO_4), and evaporation of the solvent gave a glassy residue. Chromatography of the residue on silver nitrate impregnated silica gel plate in hexane and elution of the fraction which exhibited the same R_f value as authentic 5β-cholestane yielded 2.9 mg (34%) pure 5β-cholestane-*2,2,3ξ,4,4-d*$_5$ (XIV), mp 69–70° (MeOH). For isotope composition see Table I.

5β-Cholestane-*2,2,3,3,4,4-d*$_6$ (XV).—A solution of 5β-cholestan-3-one-*2,2,4,4-d*$_4$ (XII, 10 mg) in dry dioxane (5 ml) and 10% deuteriosulfuric acid in deuterium oxide (3 ml) was electrolyzed for 4 hr in the presence of a lead cathode at 200 mA current.[27] The reaction mixture was diluted with water and extracted with ether. Washing with dilute sodium bicarbonate solution and water, drying (Na_2SO_4), and evaporation of the ether gave a glassy residue. Chromatography on silver nitrate impregnated silica gel plate in hexane and elution of the hydrocarbon fraction gave 3 mg (31%) of pure 5β-cholestane-*2,2,3,3,4,4-d*$_6$ (XV), mp 68–69.5° (MeOH). For isotope composition see Table I.

5β-*d*$_1$-Cholestane (XVIII).—A solution of cholest-4-en-3-one (XVI, 100 mg) in 50 ml of methanol was deuterated at room temperature and atmospheric pressure in the presence of 10% palladium on charcoal catalyst. The deuterium uptake ceased in about 10 min. The catalyst was removed by filtration and the residue after the evaporation of the solvent was chromatographed on silica gel plates in 15% ethyl acetate in hexane. The more polar fraction, which had the same R_f value as authentic 5β-cholestan-3-one, gave 55 mg of the 4ξ,5β-d_2 product, which was dissolved in methanol, and the solution was saturated with 1 *N* sodium hydroxide solution. After heating under reflux for 5 hr, water was added and the mixture was extracted with ether. Washing with water, drying (Na_2SO_4), and evaporation of the ether gave 55 mg (54%) of 5β-d_1-cholestan-3-one (XVII), isotope composition 15% d_0, 79% d_1, and 6% d_2.

A solution of the 5β-d_1 ketone (XVII, 15 mg) in 2 ml of ethylene glycol, 1 ml of 1-butanol, and 0.5 ml of hydrazine hydrate was heated under reflux for 1 hr. After cooling to about 100°, potassium hydroxide (150 mg) was added and the reaction mixture was heated without a condenser until the temperature reached about 210°. The heating was then continued under an air-cooled condenser for 4 hr at 210–220°. After cooling, ether and water were added, the ether phase was washed and dried (Na_2SO_4), and the solvent was evaporated. The residue was filtered through a small silica gel column in hexane, yielding 12 mg (83%) of pure 5β-d_1-cholestane (XVIII), mp 69–70° (MeOH). For isotope composition see Table I.

5β-Cholestane-*11,11-d*$_2$ (XXII).—A solution of 5β-cholestan-12-one[14] (XIX, 7 mg) in methanol-*O-d* (5 ml) was saturated with 10% sodium deuterioxide in deuterium oxide and then heated under reflux for 2 days. After cooling ether was added and the organic phase was washed rapidly with ice-cold water. Drying (Na_2SO_4), evaporation of the ether, and thin layer chromatography of the residue gave crystalline 5β-cholestan-12-one-*11,11-d*$_2$ (XX, 5 mg, 72%) which exhibited 7% d_1 and 93% d_2 isotope composition by mass spectrometric analysis.

The labeled ketone (XX, 4.8 mg) was dissolved in methanol-*O-d* (4 ml), *p*-toluenesulfonylhydrazine (10 mg) and 1 drop of deuteriosulfuric acid were added, and the solution was heated under reflux for 4 hr. A few drops of water was added and the crystalline precipitate which formed upon refrigeration was collected, washed with aqueous methanol (1:1), then dried under vacuum at 50°. The resulting crystalline *11,11-d*$_2$-5β-cholestan-12-one tosylhydrazone (XXI, 5.8 mg), 84%, which contained only traces of starting material according to spot

(26) The mass spectra were measured on Atlas CH-4 (equipped with EFO-4B ion source) and CH-7 mass spectrometers at 70 eV ionizing potential unless otherwise stated. The refocused measurement of metastable peaks was carried out on an AEI-MS-9 mass spectrometer by Mr. R. Ross and Dr. D. M. Smith of Stanford University. The nmr spectra were measured on a Varian HA-100 spectrometer using tetramethylsilane as internal reference. The ir spectra were determined on a Perkin-Elmer Model 237 Infracord spectrometer, the ORD spectra on a JASCO-ORD/UV-5 spectrometer, and the CD curves were measured by Mrs. Ruth Records at Stanford University on a JASCO-ORD/CD spectrometer. The elemental analyses were determined by Miss L. Jaime on a Hewlett-Packard Model 185 CHN Analyzer.

(27) For detailed description of the electrolysis cell and reaction conditions see ref 11, pp 166–169.

chromatography, was heated with methanol (5 ml) for a few minutes and then 10 mg of sodium borohydride was added. After heating under reflux for 1 hr, an additional 10 mg of sodium borohydride was added and the heating was continued for 1 hr. Since a thin layer chromatographic spot test still showed the presence of some starting material, the methanol was distilled off under reduced pressure and the residue was treated again with fresh sodium borohydride (10 mg) in boiling dioxane (5 ml). After 5 hr of heating all starting material was consumed. Ether was added and the organic phase was washed with plenty of water. Drying (Na_2SO_4) and evaporation of the solvents gave an oily residue (5.2 mg) which was chromatographed on a silica gel plate in hexane. The fraction which showed identical R_f value with authentic 5β-cholestane, yielded 1.5 mg of semicrystalline product which according to gc–mass spectrometric analysis was contaminated with an olefin. Chromatography of this product on a silver nitrate impregnated silica gel plate in hexane gave pure 5β-cholestane-*11,11-d*$_2$ (XXII, 0.8 mg, 21%), mp 68–70° (MeOH), isotopic purity 89% (see Table I).

5β-Cholestane-*12,12-d*$_2$ (XXIV).—The tosylation of 5β-cholestan-12-one[14] (XIX, 3 mg) was carried out the same way as described above for the preparation of the *11,11-d*$_2$ tosylate (XXI) but using undeuterated methanol and sulfuric acid. The resulting crystalline tosylhydrazone (XXIII, 4.3 mg, 100%) was dissolved in dry dioxane (2.5 ml) and was heated under reflux with lithium aluminum deuteride (20 mg) for 20 hr. The excess deuteride was decomposed by the careful addition of a few drops of deuterium oxide and the heating was resumed for 10 min. Dilute hydrochloric acid and water were added. Ether extraction, washing of the ether phase with dilute sodium bicarbonate solution and water, drying (Na_2SO_4), and evaporation of the solvent gave a glassy residue (2.5 mg). Chromatography on silver nitrate impregnated silica gel plate in hexane and elution of the fraction which exhibited the same R_f value as authentic 5β-cholestane, gave pure 5β-cholestane-*12,12-d*$_2$ (XXIV, 0.5 mg, 17%); for isotope composition see Table I. The gc retention time and the mass spectrum of this sample were identical with those of authentic 5β-cholestane (VI) with the exception of the mass shifts of the deuterium-containing ions in its mass spectrum.

Hydrogenation of 18-Methylpregn-4-ene-3,20-dione (XXV).—18-Methylpregn-4-ene-3,20-dione[16] (XXV, 140 mg) in ethyl acetate (30 ml) was hydrogenated at room temperature and atmospheric pressure in the presence of 10% palladium-on-charcoal catalyst. When the hydrogen uptake ceased the catalyst was removed by filtration and the residue after evaporation of the solvent was chromatographed on silica gel plates in ether–hexane (3:7, the plates were developed twice). Elution of the less polar fraction gave 18-methyl-5α-pregnane-3,20-dione (XXVII, 43 mg, 31%): mp 183–183.5° (aqueous MeOH); ir (KBr) 1695 and 1710 cm^{-1}; $[\alpha]_D$ +123.3 ± 4.2° (*c* 1.1, $CHCl_3$); ORD (*c* 0.12, MeOH) $[\alpha]_{308}$ 3438 (pk), $[\alpha]_{267}$ −4144 (tr); CD (*c* 0.044, MeOH) $[\theta]_{288}$ +18.562 (max); nmr ($CDCl_3$) 0.99 (19-H), 2.17 ppm (21-H); nmr (C_6D_6) 0.53 (19-H), 1.885 ppm (21-H); mass spectrum *m/e* 330 (M^+). *Anal.* Calcd for $C_{22}H_{34}O_2$: C, 79.95; H, 10.37. Found: C, 80.01; H, 10.43.

The more polar fraction consisted of 18-methyl-5β-pregnane-3,20-dione (XXVI, 76 mg, 54%): mp 112.5–114° (aqueous MeOH); ir (KBr) 1705 cm^{-1}; $[\alpha]_D$ 123.2 ± 2.7° (*c* 1.37, $CHCl_3$); ORD (*c* 0.10, MeOH) $[\alpha]_{309}$ 2937 (pk), $[\alpha]_{266}$ − 2649 (tr); CD (*c* 0.10, MeOH) $[\theta]_{293.5}$ +15.80 (max); nmr ($CDCl_3$) 1.00 (19-H), 2.17 ppm (21-H); nmr (C_6D_6) 0.64 (19-H), 1.92 ppm (21-H); mass spectrum *m/e* 330 (M^+). *Anal.* Calcd for $C_{22}H_{34}O_2$: C, 79.95; H, 10.37. Found: C, 79.83; H, 10.58.

18-Methyl-5β-pregnane (XXVIII).—A solution of 18-methyl-5β-pregnane-3,20-dione (XXVI, 20 mg) in ethylene glycol (2.5 ml), 1-butanol (1 ml), and hydrazine hydrate (95%, 1 ml) was heated under reflux for 1.5 hr. After cooling to about 100° potassium hydroxide (150 mg) was added and the heating was continued without a condenser until the temperature of the reaction mixture reached about 210°. After heating for 8 hr at 210–220° under an air-cooled condenser, the reaction mixture was cooled, diluted with water, and extracted with ether. The ether extract was washed with water and dried (Na_2SO_4) and the solvent was evaporated. The oily residue was chromatographed on a small (1 g) silica gel column. Elution with hexane (10 ml) gave noncrystalline 18-methyl-5β-pregnane (XXVIII, 9.5 mg, 52%) which exhibited a single tlc spot in a variety of solvent systems: nmr ($CDCl_3$) 0.895 ppm (19-H); mass spectrum *m/e* 302 (M^+). According to nmr and gc–mass spectral analysis this compound was contaminated with about 5% of an isomeric product, tentatively identified as 18-methyl-5β,17α-pregnane. This contaminant exhibited a virtually identical mass spectrum with that of the main component and a 19-H signal in the nmr ($CDCl_3$) at 0.90 ppm as a shoulder on the 19-H resonance (0.895 ppm) of XXVIII.

18-Methyl-5α-pregnane (XXIX).—18-Methyl-5α-pregnane-3,-20-dione (XXVII, 25 mg) was reduced under the same conditions as described above for the 5β isomer, except that the reaction mixture was heated at 210–220° for 5 hr only, yielding pure 18-methyl-5α-pregnane (XXIX, 4 mg, 17%): mp 88–89° (MeOH); nmr ($CDCl_3$) 0.76 ppm (19-H); mass spectrum *m/e* 302 (M^+). *Anal.* Calcd for $C_{22}H_{38}$: C, 87.34; H, 12.66. Found: C, 87.14; H, 12.70. This product was homogeneous according to gc–mass spectral analysis.

Registry No.—I, 438-22-2; II, 438-23-3; III, 641-85-0; IV, 481-26-5; V, 481-21-0; VI, 481-20-9; XIV, 36783-17-2; XV, 36783-18-3; XVIII, 36783-19-4; XXII, 36783-20-7; XXIV, 36783-21-8; XXVI, 36783-22-9; XXVII, 36783-23-0; XXVIII, 36783-24-1; XXIX, 36783-25-2.

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