Mass Spectrometry in Structural and Stereochemical Problems. 249.[1] Elucidation of the Course of the Characteristic Ring D Fragmentation of Unsaturated Steroids[2]

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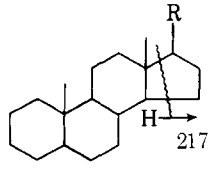
Abstract: A series of deuterium-labeled Δ^7- and $\Delta^{8(14)}$-steroidal olefins has been synthesized in order to study the electron impact induced ring D fragmentation characteristic of these compounds. Such information is important in the structure elucidation of unknown steroids and is especially relevant in Δ^7-sterols which are fairly common among marine organisms. The characteristic ring D cleavage has been found to result from loss of carbons 15, 16, and 17 together with their substituents and one additional hydrogen atom. Evidence is presented for the electron impact induced isomerization of Δ^7 to $\Delta^{8(14)}$ steroids; in the latter, the allylically activated C-7 is the origin of 74% of the hydrogen transferred with minor transfer from carbons 9, 12, and 18. The mechanisms proposed for the steroidal olefins formally require the intermediacy of an ionized carbene fragment. The C-19 (C-18 in Δ^7-pregnene) angular methyl group in the steroidal olefins was labeled with deuterium allowing analysis of the origin of the methyl group lost from the molecular ion. The extensive use of remote functionalization techniques provided access to deuterium labeling at carbons 9, 11, 12, 18, and 19.

A characteristic feature of the mass spectra of C-17 substituted steroids arises from loss of carbons 15, 16, and 17 in a fragment which includes the side chain and one additional hydrogen atom; in cholestane this is the base peak of the mass spectrum (*m/e* 217).[4]



This fragmentation is of considerable importance as a diagnostic tool in determining the nature of the C-17 substituent in saturated steroids which do not undergo facile loss of the C-17 side chain. In addition, the numerous studies which have been conducted on this cleavage in androstane,[5] pregnane,[6] cholestane,[6] lanostane,[7] and *D*-homo- and *D*-norsteroids[8] have yielded a wealth of mechanistic information on the complex processes which are involved.[9]

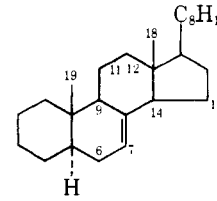
It has been reported[10] that the mass spectra of Δ^5- and Δ^7-unsaturated steroids display an analogous peak (*m/e* 215 in the cholestenes) presumably due to a similar fragmentation, and recent work with labeled cholesterols[11] shows that loss of the ring D fragment is responsible for this peak in the Δ^5 steroids. However, neither Δ^5 nor Δ^7 steroids show a peak corresponding to expulsion of the ring D fragment without hydrogen transfer (*m/e* 216 in the cholestenes) which is a major feature of the mass spectra of the saturated steroids.

It has been shown[6] that in saturated steroids the major source of the hydrogen transferred and lost with the side chain comes from position 14 (about 70%), an a priori unexpected locus since it involves the supposedly unlikely fission of two bonds connected to the same carbon atom. In addition, it was shown that loss of the ring D fragment without hydrogen transfer is the result of a reciprocal hydrogen transfer between carbons 16 and 18. Both fragmentations as well as a number of others can be rationalized through a common intermediate (a) as shown in Scheme I.[6] Ionization of the 13–17 bond relieves the strain inherent in ring D and produces the most stable possible structure, a tertiary carbonium and secondary radical a. The geometry of a is such that the radical site can approach the 14α proton, transfer of which gives the ionized olefin (b). Loss of the ring D fragment at this point would formally give an ionized carbene (c); however, by postulating a 1–2 hydride shift (Scheme II, b → d) or skeletal rearrangement (b → e) tertiary allylic carbonium ions can be generated.

Other relevant fragmentations which can be rationalized as proceeding through a include the loss of the side chain together with carbon atoms 16 and 17 followed by expulsion of the C-19 methyl substituent to give g as well as a reciprocal proton transfer between carbons 16 and 18 (a → h → i) followed by loss of the ring D fragment without any additional hydrogen to give j.

In the Δ^7 steroids there is no proton available at C-8 eliminating the possibility of a 1,2-hydrogen shift (d), and these compounds are extremely unlikely to undergo ring expansion as in e as the 8–9 bond is vinylic. If in the Δ^7 steroids as in the saturated steroids it is the 14α proton which is transferred and lost with the ring D fragment, the possibility of an ionized carbene intermediate (e.g., c) or a 1,3-hydrogen shift from carbon atoms 9 or 12 would be the most attractive routes for such ring D fragmentation. Furthermore, the mass spectra of $\Delta^{8(14)}$-unsaturated steroids also show a peak corresponding to loss of ring D with proton transfer, and they too lack a proton at C-8 and seem unlikely to undergo ring expansion. Indeed the $\Delta^{8(14)}$ steroids have no C-14 proton and appear to suffer an unfavorable vinylic cleavage. These facts raised questions as to whether the mechanism was identical with that operating with saturated steroids and, if so, which protons were being transferred and lost with the ring D fragment. Certainly, if it could be established that the 14α hydrogen was transferred and lost with ring D in the Δ^7 steroids, more serious consideration would have to be given to an ionized carbene structure c.

Synthesis. The unlabeled cholest-7-ene (**1**) and its $\Delta^{8(14)}$ isomer **2** were accessible by standard methods.[12]



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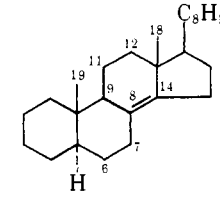
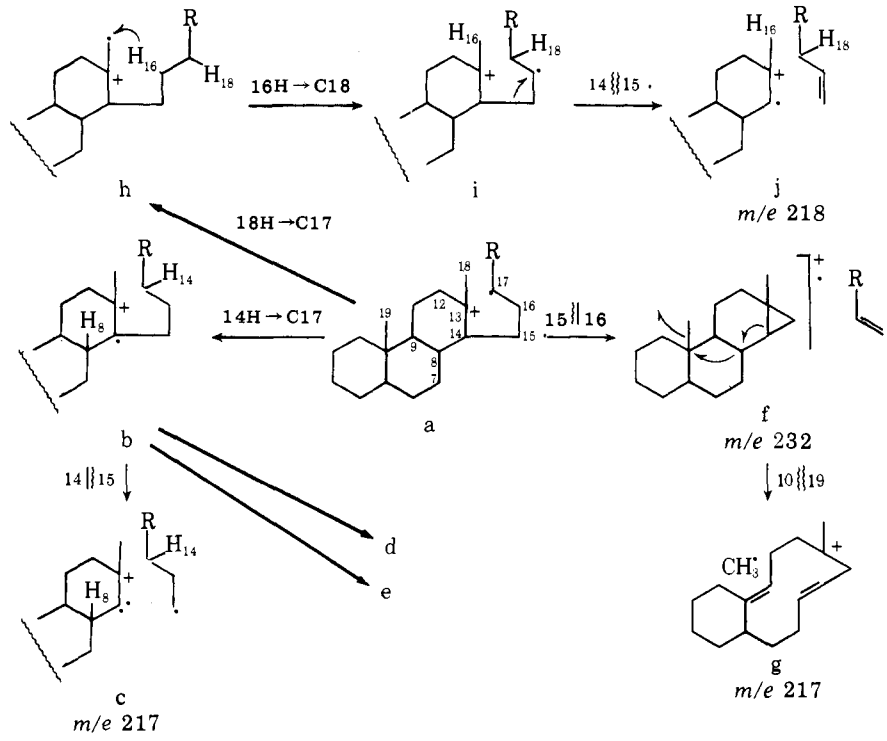


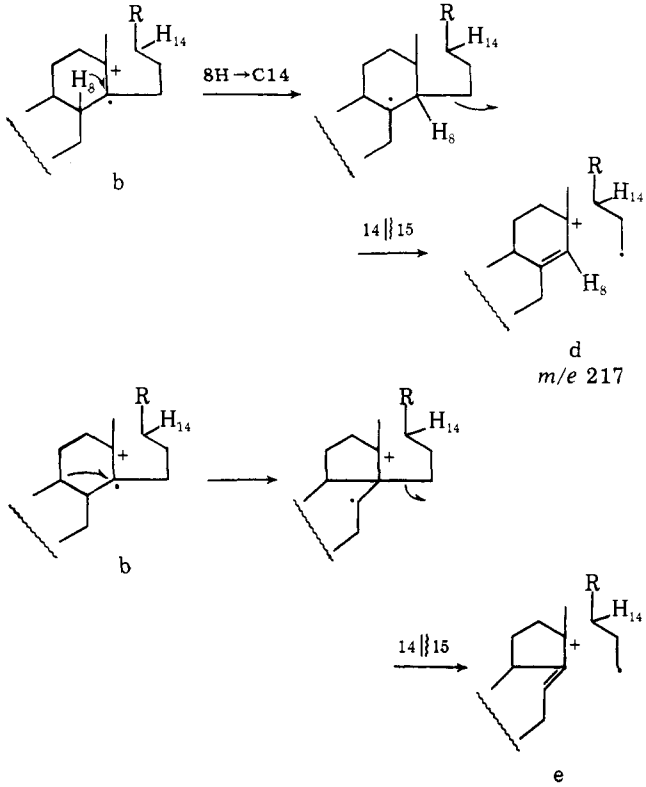
2

The C-19 labeled cholestenes were prepared using a route previously[13] worked out for labeling analogous compounds in

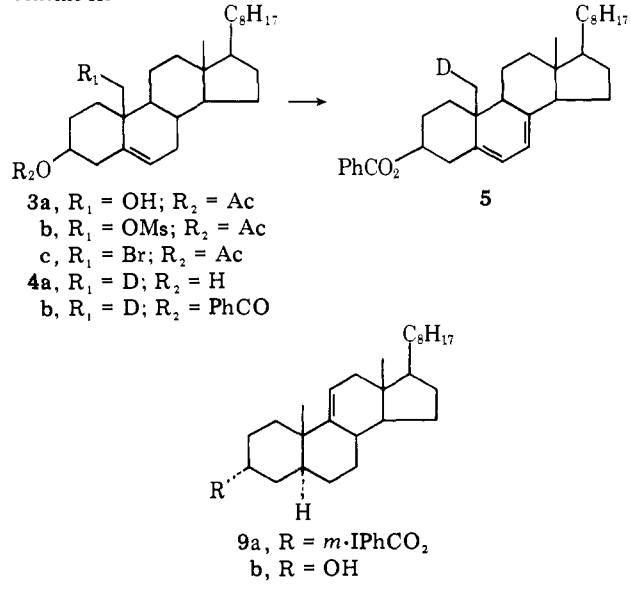
Scheme I



Scheme II



Scheme III

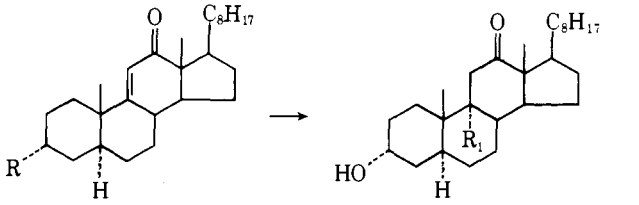


the androstene series as shown in Scheme III. Cholest-5-ene-3β,19-diol 3-acetate (**3a**)[14,15] was mesylated, the mesylate[16] **3b** exchanged with sodium bromide, and the bromide **3c** reduced with lithium aluminum deuteride to cholest-4-en-3β-ol-*19-d*$_1$ (**4a**). The deuterated cholesterol benzoate **4b** was converted to cholesta-5,7-dien-3β-ol-*19-d*$_1$ (**5**) as described by Hunziker and Mullner.[17] The diene **5** was converted to cholest-7-ene-*19-d*$_1$ (**6**) and cholest-8(14)-ene-*19-d*$_1$ (**7**) as described for the unlabeled compounds.[12]

tert-Butyl chromate oxidation[18] of cholest-9(11)-en-3α-ol *m*-iodobenzoate (**9a**)[19] followed by hydrolysis provided cholest-9(11)-en-3α-ol-12-one (**10b**), a common intermediate in the synthesis of the 9- and 12-labeled cholestenes (Scheme IV). Catalytic hydrogenation of **10b** provided cholestan-3α-ol-12-one (**11**). After reduction of the tosylhydrazone of **11** with $NaBD_3CN$ in the presence of DCl/AcOD[20] the resulting cholestan-3α-ol-*12,12-d*$_2$ (**12**) was oxidized to cholestan-3-one-*12,12-d*$_2$ (**13**) which was converted to cholest-4-en-3-one-*12,12-d*$_2$ (**14**) (Scheme V) by the method of Rosenkranz et al.[21] and reduced to cholest-5-en-3β-ol-*12,12-d*$_2$ (**15**) by the method of Dauben and Eastham.[22] Cholest-7-ene-*12,12-d*$_2$ (**16**) and cholest-8(14)-ene-*12,12-d*$_2$ (**17**) were accessible from **15** in the same manner as the 19-labeled compounds **6** and **7**.

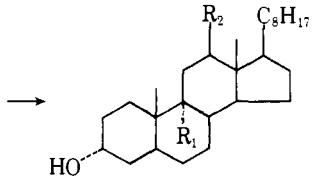
Catalytic deuteration of the α,β-unsaturated ketone **9b**

Scheme IV



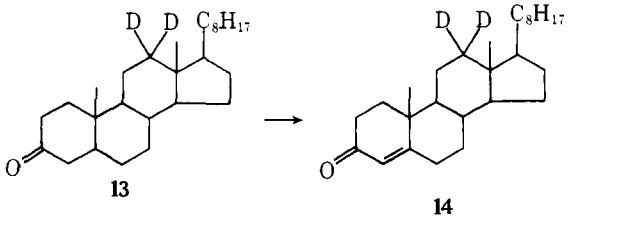
10a, R = *m*-IPhCO$_2$
b, R = OH

11, R = H
18, R = D



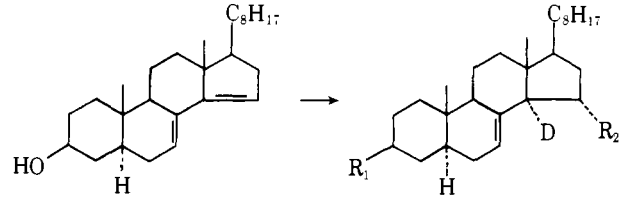
12, R_1 = H; R_2 = D_2
19, R_1 = D; R_2 = H_2

Scheme V



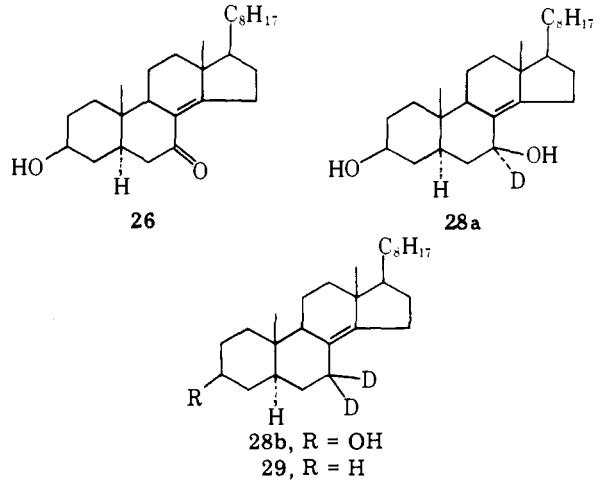
13 14 15

Scheme VI



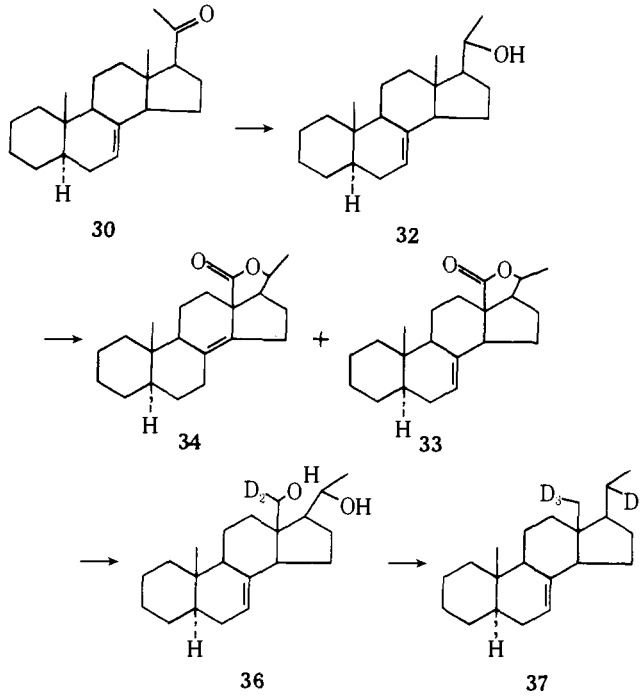
23 24b, R_1 = R_2 = OH
25, R_1 = R_2 = H

Scheme VII



26 28a
28b, R = OH
29, R = H

Scheme VIII



30 32 34 33 36 37

followed by treatment with basic methanol provided cholestan-3α-ol-12-one-9α-d_1 (**18**). Wolff-Kishner reduction of **18** to cholestan-3α-ol-9α-d_1 (**19**) provided access to cholest-7-ene-9α-d_1 (**21**) and cholest-8(14)-ene-9α-d_1 (**22**) in the same manner as the 12-labeled steroids through the Δ^5-steroidal intermediate cholest-5-en-3β-ol-9α-d_1 (**20**).

Cholesta-7,14-dien-3α-ol (**23**) was readily deuterioborated and oxidized[24] to cholest-7-ene-3β,15α-diol-*14*α-d_1 (**24b**) (Scheme VI) which could be reduced to the desired cholest-7-ene-*14*α-d_1 (**25**) by the Ireland phosphorodiamidate reduction.[25]

Advantage was taken of the recently described observation of Cunningham and Overton[26] to prepare the important C-7 labeled $\Delta^{8(14)}$ olefins (Scheme VII). Cholest-8(14)-en-3β-ol-7-one (**26**)[27] was reduced with lithium aluminum hydride to cholest-8(14)-ene-3β,7β-diol-7α-d_1 (**28a**) which was subjected to deuterolysis in the presence of aluminum chloride. The resulting cholest-8(14)-en-3β-ol-7,7-d_2 (**28b**) was converted to the tosylate and reduced to give cholest-8(14)-ene-7,7-d_2 (**29**).[12]

Labeling of the C-18 angular methyl group was effected in the pregnene series taking advantage of a 20α-alcohol substituent for remote functionalization purposes (Scheme VIII). Thus reduction of 5α-pregn-7-en-20-one (**30**)[1] with sodium in ethanol gave the desired 20β and 20α diols **31** and **32**, respectively, and while the more easily accessible 20β alcohol failed to serve as a route to the desired lactone **33**, the 20α alcohol could be oxidized using iodine and lead tetraacetate[28] to give the lactone **33** in fair yields. Although the oxidation conditions caused isomerization of some of the Δ^7 lactone **33** to the $\Delta^{8(14)}$ compound **34**, these were separable by fractional recrystallization. The former was reduced to the diol **36** using lithium aluminum deuteride which was reduced to the hydrocarbon **37** by the Ireland phosphorodiamidate reduction using deuterated solvents.[7,25]

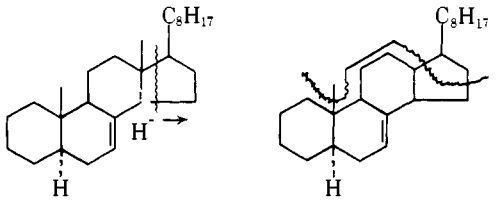
Results and Discussion

In the unsaturated as well as in the saturated steroids the observed ring D fragmentation peak is actually the result of two separate fragmentations as shown schematically for cho-

Table I. Shifts[a] of Mass Spectral Peaks of Deuterated $\Delta^{8(14)}$ Steroids

Compd	Isotopic purity, %	M^+	$M - CH_3$	$M - C_8H_{17}$	$M - C_{10}H_{20}$	$M - C_{11}H_{23}$
Cholest-8(14)-ene (**2**)		370	355	257	230	215
Cholest-8(14)-ene-*7,7-d_2* (**29**)	1 d_0 5 d_1 94 d_2	372	357	259	232	216 (67%) 217 (33%)
Cholest-8(14)-ene-*9α-d_1* (**22**)	38 d_0 62 d_1	371	356	258	231	215 (6%) 216 (94%)
Cholest-8(14)-ene-*12,12-d_2* (**17**)	3 d_0 27 d_1 70 d_2	372	357	259	232	216 (5%) 217 (95%)
Cholest-8(14)-ene-*19-d_1* (**7**)	6 d_0 92 d_1 2 d_2	371	355 (22%) 356 (78%)	258	231	215 (9%) 216 (91%)

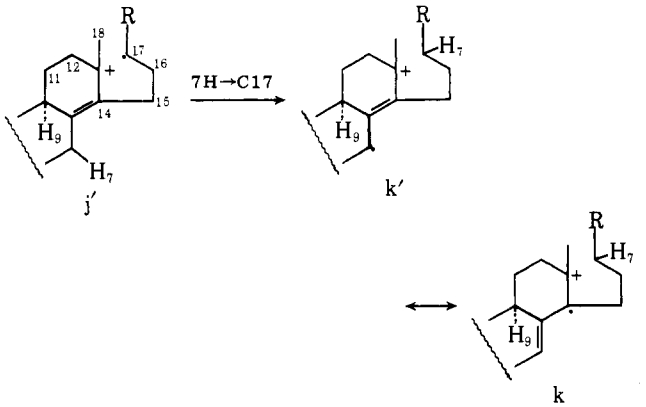
[a] The shift values have been corrected for ^{13}C contributions and effects due to isotopic composition. The spectra were measured at 70 eV.



h′, *m/e* 215 i′, *m/e* 215

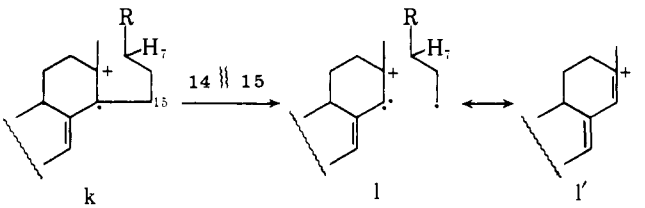
lest-7-ene in terms of structures h′ and i′. Since the contributions of each of these fragments vary with the position of the double bond and other substituents in the steroid, the data given in the Discussion section and in Table III have been corrected for this possible ambiguity and are given in terms of percent of the peak due to ring D fragmentation rather than just percent shift of the observed peak, thus allowing comparison between the different labeled steroids.[29]

The results for the labeled cholest-8(14)-enes listed in Table I are exactly as expected by analogy to the saturated series. Initial cleavage of the allylic 13–17 bond would relieve the strain inherent in the *trans*-hydrindan system and create the most stable possible intermediate, a tertiary allylic cation and a secondary radical (j′). In the case of cholestane, it has been



j′ k′ k

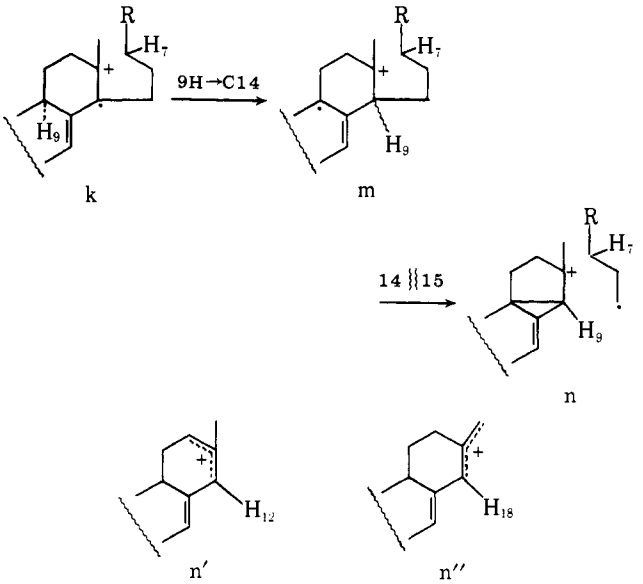
shown that the positions which are sterically accessible to the C-17 radical site are 7, 14, and 18; in fact major transfer from positions 14 and 18 does occur[6] in cholestane. In the absence of a proton at C-14, it is not surprising that the major transfer site in the cholest-8(14)-enes is from the allylically activated C-7 position accounting for 74% of the hydrogen transfer and resulting in the resonance stabilized ion radical k′, which is equivalent to an ionized butadiene (cf. k). In the pregnane and cholestane series cleavage of two bonds on one carbon has been considered a priori as evidence for a hydrogen shift or skeletal rearrangement in order to avoid postulation of an ionized carbene intermediate. Cleavage of the 14–15 bond in k would give an ionized allylic carbene l which may intuitively seem improbable. However such an ionized carbene is isoelectronic with a vinyl carbonium ion (e.g., l′) which from solution



k l l′

chemistry is known to be considerably more stable than was formerly believed.[30] Furthermore, in an environment such as the mass spectrometer which provides relatively large amounts of energy and short ion lifetimes, it is conceivable that ions such as l might exist even though charge dissipation via solvation (as in solution chemistry) is impossible. The intermediacy of the carbene l could be avoided by invoking a 1,3-allylic hydrogen shift from C-9 to C-14 to give m which could then eliminate the ring D fragment to give n. If C-12 or C-18 are involved in such hypothetical 1,3-hydrogen shifts, then butadienyl carbonium ions of type n′ and n″ could be invoked.

Positions 9 and 12 are the origin of a small amount of transferred hydrogen (7 and 6%, respectively). This is not unexpected since the C-17 radical in j′ cannot approach these positions with ring C in its original conformation. However,



k m n n′ n″

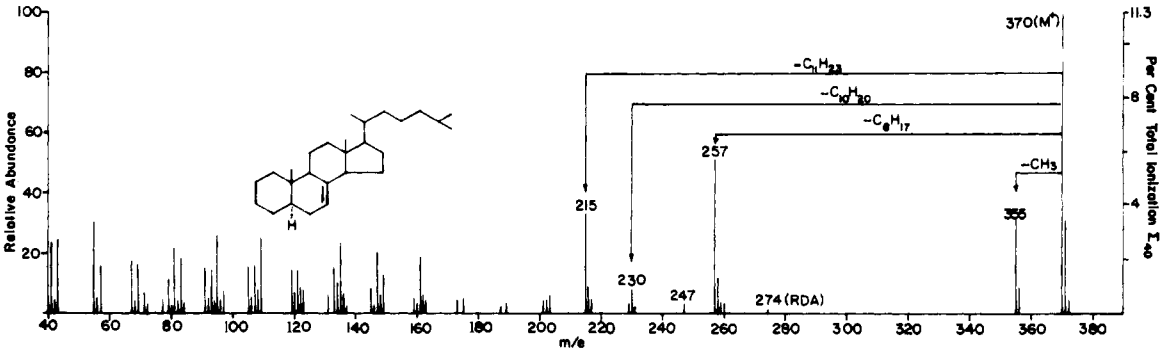


Figure 1. Mass spectra of 5α-cholest-7-ene (**1**) (70 eV).

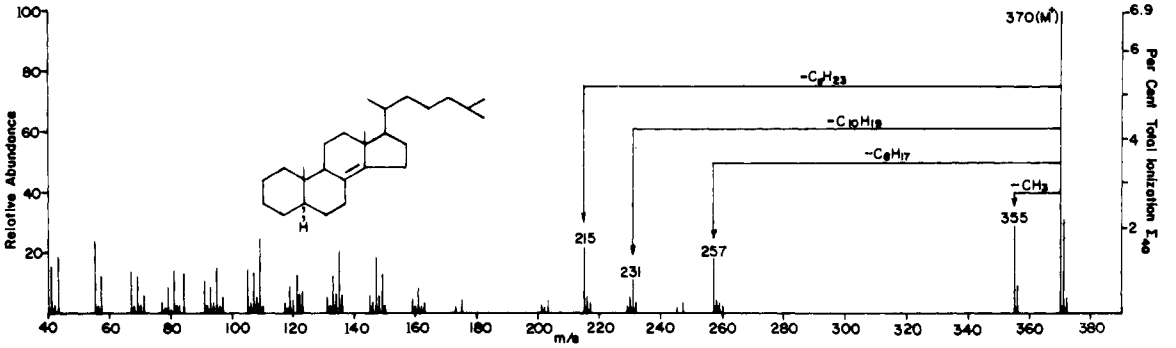
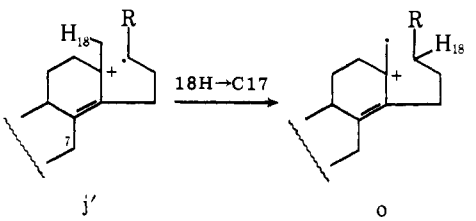


Figure 2. Mass spectra of 5α-cholest-8(14)-ene (**2**) (70 eV).

ring C free of the C/D trans ring junction would be free to flip into conformations other than its original chair form which allow the radical better access to positions 9 and 12. Position 11 is virtually inaccessible and no hydrogen transfer from this position was observed.[3]

Deuterium labeling of the angular C-19 methyl group showed (Table I) that in $\Delta^{8(14)}$-cholestene 9% of the ring D fragmentation peak is the result of an entirely different cleavage pattern involving loss of the side chain and two carbons from ring D along with the 19-methyl group (i') similar[29] to that depicted for the saturated steroids in Scheme I (a → g). This leaves only 13% of the hydrogen transfer unaccounted for, and aside from any isotope effects it is assumed that this originates for the most part from C-18. In the saturated steroids, the radical at carbon 17 is known[6] to abstract hydrogen from C-18 which is activated by the charge centered on C-13. The C-18 radical can then abstract a proton from C-16 causing cleavage of the 14–15 bond and loss of the ring D fragment as in a → j (Scheme I). This elimination of the ring D fragment without an additional hydrogen is a major feature in the mass spectrum of cholestane but is virtually absent in the $\Delta^{8(14)}$ and Δ^{7} steroids. In the $\Delta^{8(14)}$ isomers the major hydrogen transfer site is the allylically activated C-7. Evidently abstraction from the primary C-18 as depicted in structure o does not compete well with the energetically more favorable abstraction from C-7.



Loss of an angular methyl group from the molecular ion, also a major feature of the mass spectra of $\Delta^{8(14)}$ steroids, is the result of rupture of the allylic 13–18 bond. The presence of the 8(14) unsaturation appears to have a marked effect on the site from which the methyl group is lost rather than on the ion abundance. In pregnane and therefore presumably in cholestane, 20% of the $M^+ - Me$ signal is due to loss of C-18, whereas in the $\Delta^{8(14)}$ steroids loss of C-18 accounts for 78% (cf. Table IV).

Isomerization of the Δ^7 double bond to the $\Delta^{8(14)}$ position after electron impact is suggested by the similarity of the spectra (see Figures 1 and 2) of the isomeric cholestenes. The major differences in the spectra of the Δ^7 and $\Delta^{8(14)}$ steroids are a consistently larger loss of side chain in the Δ^7 isomers and the presence of a small peak due to the thermally forbidden retro-Diels–Alder fragmentation[1] in the Δ^7 steroids which is virtually absent from the spectra of the $\Delta^{8(14)}$ isomers. Further evidence for double bond isomerization comes from examination of the data (Table IV) for the $M^+ - CH_3$ peak in the various 19-labeled steroids. In pregnane and other C-17 substituted hydrocarbons, interaction between the C-17 side chain and the 18-methyl group increases the strain inherent in the C/D trans ring junction. This factor and the generation of a secondary vs. primary radical at C-17 are probably responsible for the increased ring D fission in pregnane as compared to androstane.[5] Rupture of the 13–17 linkage effectively blocks the cleavage of a second bond to C-13, and in pregnane only 20% of the $M^+ - CH_3$ peak is due to loss of C-18. The Δ^7 double bond is in a "neutral" position in the steroid skeleton, and would not be expected to encourage loss of either of the angular methyl groups, whereas the $\Delta^{8(14)}$ double bond allylically activates the 13–18 bond, thus favoring loss of the 18-methyl group. As seen from Table IV, the expulsion of the C-18 angular methyl group from the molecular ion accounts for the bulk of the $M^+ - CH_3$ peak in both Δ^7- and $\Delta^{8(14)}$-cholestenes (76 and 78%, respectively) supporting the idea that isomerization of the Δ^7- to the $\Delta^{8(14)}$-cholestenes is occurring in the spectrometer after electron impact.

The most unexpected result from the mass spectra of the labeled Δ^7 steroids is the major transfer (Table III) of hydrogen from C-7 (29%). Transfer of a vinylic C-7 proton is a priori

Table II. Shifts[a] of Mass Spectral Peaks of Deuterated Δ^7 Steroids

Compd	Isotopic purity, %	M^+	$M - CH_3$	$M - C_8H_{17}$[b]	$M - C_{10}H_{20}$[c]	$M - C_{11}H_{23}$[d]
Cholest-7-ene (**1**)		370	355	257	230	215
Cholest-7-ene-*7*-d_1[20a] (**39**)	11 d_0 77 d_1 12 d_2 0 d_3	371	356	258	231	215 (24%) 216 (76%)
Cholest-7-ene-*9α*-d_1 (**21**)	14 d_0 86 d_1	371	356	258	231	215 (12%) 216 (88%)
Cholest-7-ene-*12,12*-d_2 (**16**)	3 d_0 27 d_1 70 d_2	372	357	259	232	216 (6%) 217 (94%)
Cholest-7-ene-*14α*-d_1 (**25**)	7 d_0 93 d_1	371	356	258	231	215 (23%) 216 (77%)
Cholest-7-ene-*19*-d_1 (**6**)	10 d_0 90 d_1	371	355 (24%) 356 (76%)	258	231	215 (16%) 216 (84%)
Pregn-7-ene (**38**)[1]		286	271	257	230	215
Pregn-7-ene-*18,18,18,20*-d_4 (**37**)	1 d_2 12 d_3 85 d_4 2 d_5	290	272 (68%) 275 (32%)	260	233	217 (12%) 218 (88%)

[a] See footnote *a*, Table I. [b] This heading should read $M^+ - C_2H_5$ for pregnenes. [c] $M^+ - C_4H_8$ for pregnenes. [d] $M^+ - C_5H_{11}$ for pregnenes.

Table III. Deuterium Transfers (%)[a] in Ring D Cleavage of Labeled Steroidal Olefins

Compd	Position labeled 18	14	12	11	9	7	Total, %
5α-Cholest-7-ene	14[b]	27	7		14	29	91
5α-Cholest-8(14)-ene			6		7	74	87
5α-Cholest-7-en-3α-ol methyl ether[3]		30[c]		0	19		49
5α-Cholest-8(14)-en-3α-ol methyl ether[3]				0		68	68

[a] These values are corrected for ^{13}C contributions, isotopic composition, and the presence of fragments originating from other cleavage patterns as described in the text. [b] Datum from Δ^7-pregnene (corrected, see ref 29). [c] Spectrum from Varian-MAT CH-7 GC/MS.

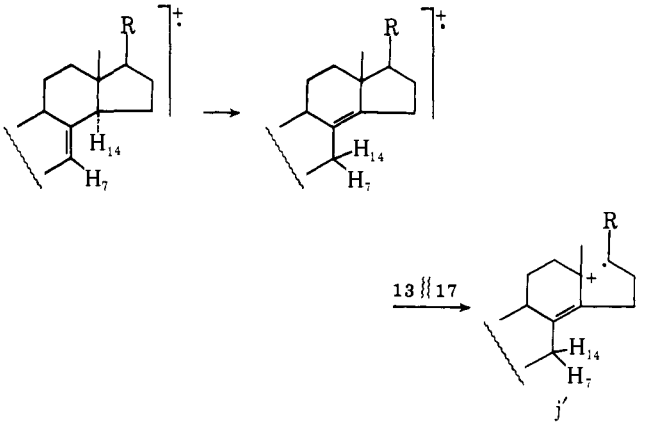
Table IV. Relative Percent Loss of C-18 and C-19 in the $M^+ - CH_3$ Fragment of Various Steroids[a]

	C-19 loss	C-18 loss
70 eV		
Pregnane[6]	80–83	17–20
Androstane[5]	37	63
Δ^7-Androstene[1]	37	63
Δ^7-Pregnene	32	68
Δ^7-Cholestene	24	76
$\Delta^{8(14)}$-Cholestene	22	78
$\Delta^{7,9(11)}$-Cholestadiene[3]	80	20
12 eV		
Δ^7-Pregnene	27	83
Δ^7-Cholestene	18	82
$\Delta^{8(14)}$-Cholestene	14	86
$\Delta^{7,9(11)}$-Cholestadiene[3]	87	13

[a] Values are corrected for ^{13}C contributions and isotopic composition. Δ^7-Pregnene was labeled at C-18. In androstane and pregnane both angular methyl groups were labeled, and the remaining steroids were labeled at C-19.

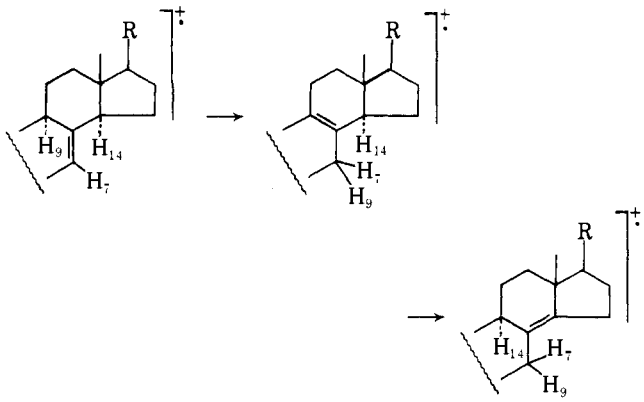
very unlikely unless one assumes prior isomerization of the Δ^7 double bond to the $\Delta^{8(14)}$ position. Isomerization to the equally substituted $\Delta^{8(9)}$ olefin must also be considered, but the low deuterium transfer (Table III) from C-9, and the results from the C-12 and C-19 labeled compounds, indicate that this does not occur to any great extent—similar to the situation existing in solution chemistry.[12] Once double bond isomerization has occurred, the two hydrogens originally attached to C-7 and C-14 are both available for abstraction from C-7 (see j′ in

Scheme IX



Scheme IX) by the C-17 radical site. Indeed, the labeling data (Table III) reveal that the amount of hydrogen transfer originating from C-14 (27%) is equal within the error of the measurement to the transfer (29%) from C-7. Thus 56 ± 2% of the hydrogen transfer must actually originate from C-7 in the isomerized steroid. Since the labeling results (Table III) show that in $\Delta^{8(14)}$-cholestene 74% of the hydrogen transferred to the expelled ring D fragment originates from C-7, it follows that at least 76% of the Δ^7-cholestene isomerizes to $\Delta^{8(14)}$-cholestene in the mass spectrometer in order to achieve 56 ± 2% transfer from C-7 (29% transfer of the label from C-7 in the original Δ^7 steroid). The deuterium transfer data (Table III) from other positions in the Δ^7 steroids are also consistent with the assumption of prior isomerization of the π bond to the 8(14) position.

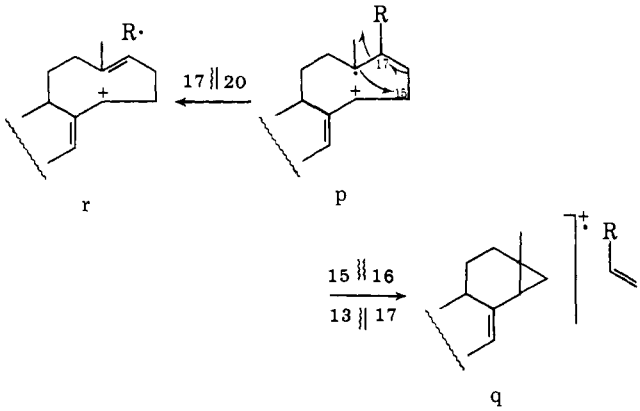
Scheme X



Isomerization of cholest-7-ene-9α-d_1 (**21**) to cholest-8(14)-ene-9α-d_1 (**22**) in solution as described in the Experimental Section resulted in some loss of deuterium from C-9 as shown in Tables and I and II. This was not unexpected as any isomerization proceeding through the tetrasubstituted $\Delta^{8(9)}$ olefin would cause loss of the label. A similar isomerization in the mass spectrometer would result in scrambling of the 7, 9, and 14 labels and increased transfer from C-9 (see Scheme X). Since transfer from C-9 in the Δ^7 compounds (14%) is only slightly higher than in the $\Delta^{8(14)}$ and saturated steroids (7 and 9%, respectively) this pathway may intuitively appear to be of minor importance. However, by analogy to the discussion above, each deuterium transferred from C-9 to C-7 by isomerization has only a 50% chance of being lost in any hydrogen abstraction originating from C-7 since there are two hydrogens there. In addition, transfer from C-7 in the isomerized $\Delta^{8(14)}$ steroid occurs only to the extent of 74% (Table III). Because of this, the 7% greater transfer (14% vs. 7%; cf. Table III) from C-9 in the Δ^7 steroids would represent a much larger (i.e., 19%) extent of randomization of the labels at carbons 7, 9, and 14. Since this randomization would result in a lower apparent transfer of deuterium from the C-7 and C-14 labeled compounds, the 76% isomerization of the Δ^7 to $\Delta^{8(14)}$ steroids described above should be considered a minimum number.

The only available 18-labeled Δ^7 steroid (Δ^7-pregnene) showed 14%[29] transfer from C-18. This is in good agreement with the amount of (13%) of hydrogen which has not been accounted for in Table III in $\Delta^{8(14)}$-cholestene. Again, in the Δ^7 steroids as in $\Delta^{8(14)}$ steroids—but in marked contrast to saturated steroids[6]—loss of the ring D fragment without proton transfer (j in Scheme I) is virtually absent.

The shifts (see Table II) on 19-labeled Δ^7-cholestene spectra showed, as expected, that some of the ring D fragmentation peak is the result of a completely different cleavage involving loss of the angular methyl group as described above for the $\Delta^{8(14)}$ steroids and earlier[6] for saturated steroids. This alternative fragmentation is responsible for a larger portion of the observed peak in the Δ^7- (16%) over the $\Delta^{8(14)}$- (9%) cholestenes. The increased incident of this fragmentation in Δ^7 as compared to $\Delta^{8(14)}$ steroids can best be rationalized through an initial rupture of the allylic 13–14 bond in the unisomerized Δ^7 steroid to give species p which would then lead to the required intermediate q (counterpart of species f in Scheme I).[29]



The intermediacy of p has already been cited[10a] to explain the facile loss of side chain r and accounts also for the greater intensity of this peak (Σ_{40} 5.7 in cholest-7-ene (**4**) compared to Σ_{40} 1.9 in cholest-8(14)-ene (**5**), Figures 1 and 2) seen in the Δ^7 compared to the $\Delta^{8(14)}$ steroids since a similar rationalization in the latter would require cleavage of the allylic 15–16 bond providing an energetically less favorable primary radical, primary allylic cation species.

Experimental Section

General. Low-resolution mass spectra were obtained by Mr. R. G. Ross on an AEI MS-9 spectrometer using a direct inlet system. ^1H and ^{13}C NMR spectra were obtained on Varian T-60 or XL-100 spectrometers using carbon tetrachloride or deuteriochloroform, respectively, as solvents and tetramethylsilane as an internal standard. Elemental analyses were determined by the Stanford Microanalytical Laboratory, Stanford University. Unless noted otherwise, optical rotations were measured in carbon tetrachloride with a Perkin-Elmer Model 141 spectropolarimeter. UV spectra were measured on a Cary 14 spectrometer. Melting points were measured on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Gas chromatography was performed on a Hewlett-Packard 402 high-efficiency gas chromatograph using a 6-ft glass column with the indicated support material and liquid phase. Chemical shifts for the C-18 and C-19 angular methyl resonances were calculated by the method of Zürcher.[31] Further experimental details are available in the thesis of L. Partridge.[3]

Cholest-5-en-3β-ol-*19-d_1* Benzoate (4b). Cholest-5-en-3β,19-diol 3-acetate 19-mesylate[15,16] (**3b,** 3.8 g) was dissolved in 250 mL of 2-propanol (dried with magnesium and iodine), 7 g of LiBr was added, and the solution was refluxed for 1.5 h.[13] Extraction yielded 3.6 g of an oil; TLC showed no trace of the mesylate (R_f of the bromide **3c** 0.5 on silica developing with 10 % ether/hexane vs. R_f of the mesylate 0.2).

The crude 19-bromide **3c** (0.89 g) was refluxed under N_2 with lithium aluminum deuteride (0.9 g) in dry diglyme. After 2 h the solution was cooled and worked up using aqueous Rochelle salt and extracted into ether. The resulting crude cholesterol-*19-d_1* (**4a**) was converted to the benzoate, chromatographed on silica, and eluted with 5% ether/hexane to give 0.55 g of pure product (**4b**): mp 146–148 °C (from ethanol); NMR δ 7.70 (m, 5 H, aromatic), 5.38 (m, 1 H, vinyl), 4.80 (m, 1 H, C-3 axial), 0.68 (s, 3 H, C-18); isotopic composition 10% d_0, 90% d_1.

Cholesta-5,7-dien-3β-ol-*19-d_1* Benzoate (5). Cholest-5-en-3β-ol-d_1 benzoate (**4b,** 200 mg) was brominated using dibromantin and dehydrobrominated with trimethyl phosphite in refluxing mixed xylenes by the procedure described by Hunziker and Müllner[17] for the unlabeled compound. Recrystallization from acetone/methanol gave **5** (130 mg) as yellowish plates which could be recrystallized in the same way to give pure product: mp 137–139 °C (lit.[17] 140 °C); UV 271, 281, 294 nm identical with that of unlabeled material;[17] NMR δ 7.70 (m, 5 H, aromatic), 5.50 (m, 2 H, vinyl), 4.90 (m, 1 H, C-3 axial (no singlet at 0.97, C-19)), 0.62 (s, 3 H, C-18).

Cholest-7-ene-*19-d_1* (6). The hydrocarbon **6** was made from cholesta-5,7-dien-3β-ol-*19-d_1* benzoate (**5**) in the same manner as the unlabeled compound **1**.[12] The final product **6** was chromatographed on 12% $AgNO_3$/silica: mp 87–89 °C; M^+ *m/e* 371;[32] NMR δ 5.12 (m, 1 H, vinyl), no singlet for C-19 (calcd 0.76), 0.53 (s, 3 H, C-18 calcd 0.53).

Cholest-8(14)-ene-*19-d_1* (7). This compound was obtained from cholest-7-ene-*19-d_1* (**6**) in exactly the same way as the unlabeled compound **2**:[12] mp 53–54 °C; M^+ *m/e* 371;[32] NMR no vinyl protons, no singlet for C-19 (calcd 0.66), δ 0.82 (s, 3 H, C-18 calcd 0.82).

Cholest-9(11)-en-3α-ol-12-one (9b). Following the procedure of Breslow[19] a solution of cholestan-3β-ol *m*-iodobenzoate (**8a,** 20 g) and phenyl iodine dichloride (9.5 g) in 1 L of CH_2Cl_2 was irradiated with a 250-W floodlamp for 1.5 h under N_2 while being cooled by a blower.

The 9α-chloro steroid **8b** thus produced was dehydrochlorinated by addition of 80 g of silica (activity 203, 70–230 mesh column grade) and evaporation of the solvent at 80 °C using a rotary evaporator. The mixture was then kept at 80 °C for 1 h followed by elution through a 250-g column of silica using 5% ether/hexane. The crude sterol ester **9a** (70% **9a**, 30% **8a** by NMR) isolated in this way was hydrolyzed and the NMR spectrum compared to that of the crude cholest-9(11)-en-3α-ol (**9b**) prepared by the method of Breslow et al.[19] and found to be identical. NMR (after subtraction of the resonance due to cholestan-3α-ol) δ 5.23 (m, 1 H, vinyl), 3.95 (m, 1 H, C-3 equatorial), 0.92 (s, 3 H, C-19 calcd 0.92), 0.58 (s, 3 H, C-18 calcd. 0.58).

It was most convenient to continue using the unhydrolyzed *m*-iodobenzoate mixture **9a**. Thus the crude cholest-9(11)-en-3α-ol *m*-iodobenzoate (**9a**, 19 g) was taken up in a solution of 20 mL of CCl_4, 19 mL of acetic anhydride, and 30 mL of acetic acid which was heated with stirring just to reflux, at which point the heating mantle was removed and a *tert*-butyl chromate reagent made from 20 g of CrO_3[18] was added dropwise to the now homogeneous solution over a period of 15 min. The thick, green solution was refluxed for 4 h with stirring and cooled before addition of enough saturated aqueous oxalic acid to neutralize the excess oxidizing agent and 200 mL of ether. The organic layer was decanted and the precipitated chromium salts were rinsed with two more 200-mL portions of ether. The combined ether extracts were washed two times with 5% concentrated HCl and three times with water, 5% Na_2CO_3, and brine and dried over $MgSO_4$. The combined organic extracts were evaporated to give 20 g of a greenish oil which was chromatographed on 700 g of silica.

Ether/hexane (5/95) eluted 10.3 g of oily cholest-9(11)-en-3α-ol-12-one *m*-iodobenzoate (**10a**) which was hydrolyzed to give 6.2 g (45% overall from cholestanol after correcting for recovered starting material **8a**) of cholest-9(11)-en-3α-ol-12-one (**10b**) as an amorphous, white solid: mp 139–143 °C; IR 3400, 1685 cm^{-1}; UV 238 nm (ε 9160); $[\alpha]^{20}_D$ +72.3°; NMR δ 5.48 (s, 1 H, vinyl), 3.93 (m, 1 H, C-3 equatorial), 1.02 (s, 3 H, C-19 calcd 1.02), 0.92 (s. 3 H, C-18 calcd 0.92); M^+ *m/e* 400. Further characterization was achieved through the acetate **10c**: mp 119–121 °C; $[\alpha]^{20}_D$ +84°; NMR δ 5.57 (s, 1 H, vinyl), 4.98 (m, 1 H, C-3 equatorial), 2.98 (s, 3 H, acetate), 1.07 (s, 3 H, C-19 calcd 1.07), 0.93 (s, 3 H, C-18 calcd 0.93); M^+ *m/e* 442.

Anal. Calcd for $C_{29}H_{46}O_3$: C, 78.68; H, 10.47. Found: C, 78.64; H, 10.32.

Cholestan-3α-ol-12-one (11). The α,β-unsaturated ketone **10** (6.2 g) was hydrogenated catalytically in 300 mL of ethyl acetate at atmospheric pressure at 30 °C using 2 g of 10% Pd/C. Temperatures below 20 °C or impurities in the substrate inhibit the reduction. Filtration and evaporation of the solvent gave 6.2 g of a white solid which was chromatographed on 300 g of silica and eluted with 10% ether/benzene to give a mixture of cholestan-3α-ol and an unidentified cholesten-3α-ol. Ether/benzene (20%) eluted 4.7 g of the desired product (**11**): mp 147–149 °C; IR 3400, 1690 cm^{-1}; $[\alpha]^{20}_D$ +85.3°; NMR δ 4.02 (m, 1 H, C-3 equatorial), 1.03 (s, 3 H, C-18 calcd 1.03), 0.88 (s, 3 H, C-19 calcd 0.88).

Anal. Calcd for $C_{27}H_{46}O_2$ (M^+ of acetate 444. Found 444): C, 80.54; H, 11.51. Found: C, 80.34; H, 11.50.

Cholestan-3α-ol-*12,12-d*$_2$ (12). Cholestan-3α-ol-12-one (**11**, 6.0 g) was refluxed overnight with tosylhydrazine (6.0 g) and 200 mg of *p*-toluenesulfonic acid in 250 mL of dry methanol. The volume was reduced to 60 mL, the solution was cooled, and 7.0 g of tosylhydrazone was collected. A second crop of 0.9 g was crystallized from the mother liquor after further evaporation of the solvent.

The combined tosylhydrazones were refluxed for 0.5 h in 50 mL of methanol-d_1; the solvent was evaporated and the procedure repeated twice to exchange the active protons.[20] The vacuum dried tosylhydrazone was reduced by the general method of Borsch et al.[20] using DCl in AcOD and $NaBD_3CN$ (7 g): mp 186–187 °C (lit.[12] 185 °C); M^+ *m/e* 390; NMR δ 3.9 (m, 1 H, C-3 equatorial), 0.78 (s, 3 H, C-19 calcd 0.78), 0.65 (s, 3 H, C-18 calcd 0.65); isotopic composition 3% d_0, 27% d_1, 70% d_2.

Cholest-7-ene-*12,12-d*$_2$ (16). Cholestan-3α-ol-*12,12-d*$_2$ (**12**) was oxidized using Jones reagent to cholestanone-*12,12-d*$_2$ (**13**) which was converted to cholest-4-en-3-one-*12,12-d*$_2$ (**14**) by the method of Rosenkranz et al.[21] and reduced to cholest-5-en-3β-ol-*12,12-d*$_2$ (**15**) as described by Dauben and Eastham.[22] The cholestene **16** was prepared in the manner described for the 19-labeled compound **6**: mp 87–88 °C (lit.[12] 87 °C); M^+ *m/e* 372;[32] NMR identical with that of unlabeled material **1**.

Cholest-8(14)-ene-*12,12-d*$_2$ (17). The $\Delta^{8(14)}$ isomer **17** was obtained from **16** in the same way as the unlabeled compound **2**: mp 54–55 °C (lit.[12] 53–54 °C); M^+ *m/e* 372;[44] NMR identical with that of **2**.

Cholestan-3α-ol-12-one-*9α-d*$_1$ (18). Cholest-9(11)-en-3α-ol-12-one (**10b**, 2 g) in 5 mL of methanol-d_1 was added at 31 °C to 0.4 g of 10% Pd/C in 200 mL of ethyl acetate which had been equilibrated with two portions of D_2 gas for a total of 4 h. The reduction was completed in 1 h under D_2 at 30 °C. Using less catalyst or lower temperatures tended to favor scrambling of the deuterium while scaling up the reduction gave poor isotopic purity even with greatly extended equilibration times. After filtration and evaporation of the solvent, the C-11 deuterium was exchanged using basic methanol. Extraction gave the crude product contaminated with cholestan-3α-ol and an unidentified cholesten-3α-ol which were removed by chromatography on silica with 10% ether/hexane. Ether (20%) afforded hexane **18**: mp 147–149 °C; M^+ 403; NMR, IR identical with those of unlabeled material **11**; isotopic composition 14% d_0, 86% d_1.

Cholestan-3α-ol-*9α-d*$_1$ (19). Cholestan-3α-ol-12-one-*9α-d*$_1$ (**18**, 2.2 g) was dissolved in 100 mL of dry distilled diethylene glycol with 30 mL of *tert*-butyl alcohol and 30 mL of 97% hydrazine under N_2 and heated to 90 °C. Potassium hydroxide (6 g) was added and the solution was refluxed (132 °C) for 1 h. The mixture was distilled until the pot temperature reached 208 °C and refluxed for 5 h at which point it was cooled, diluted with H_2O, and extracted into ether. The combined ether extracts were evaporated to give 2.0 g of **46** as a white solid with mp 186–187 °C after crystallization from methanol (lit.[26] mp 183 °C): NMR δ 3.9 (m, 1 H, C-3 equatorial), 0.78 (s, 3 H, C-19 calcd 0.78), 0.65 (s, 3 H, C-18 calcd 0.65).

Cholest-7-ene-*9α-d*$_1$ (21). Cholestan-3α-ol-*9α-d*$_1$ (**19**) was converted to cholest-5-en-3α-ol-*9α-d*$_1$ benzoate (**20**) and then to the product **21** in the same manner as the 12-labeled compound **16**: mp 86–88 °C (from acetone); M^+ *m/e* 371;[32] NMR identical with that of the unlabeled material **1**.

Cholest-8(14)-ene-*9α-d*$_1$ (22). The $\Delta^{8(14)}$ isomer **22** was obtained from **21** in exactly the same manner as the unlabeled compound **2**: mp 53–54 °C; M^+ *m/e* 371;[32] NMR identical with that of **2**.

Cholest-7-ene-3β,15α-diol (24a). Cholesta-7,14-dien-3β-ol[23] (**23**, 300 mg) was hydroborated and oxidized using the general method of Nussim et al.[24] to give 180 mg of **24a**: mp 184.5–186 °C crystallized from ether/hexane; $[\alpha]^{20}_D$ +45.6°; M^+ *m/e* 402; NMR δ 5.4 (m, 1 H, vinyl), 415 (m, 1 H, C-15), 3.50 (m, 1 H, C-3 axial), 0.81 (s, 3 H, C-19 calcd 0.81), 0.57 (s, 3 H, C-18 calcd 0.57).

Anal. Calcd for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 80.64; H, 11.56.

Cholest-7-ene-3β,15α-diol-*14α-d*$_1$ (24b). The sterol **23** (360 mg) was reduced with B_2D_6 generated as described by Nussim et al.[24] and recrystallized from ether/hexane to give 200 mg of **24b**: mp 181–183 °C; NMR identical with that of **24a**; $[\alpha]^{20}_D$ +46.9°; isotopic composition 7% d_0, 93% d_1.

Cholest-7-ene-*14α-d*$_1$ (25). Cholest-7-ene-3β,15α-diol-*14α-d*$_1$ (**24b**, 95 mg) was stirred under N_2 in 10 mL of dry THF with triphenylmethane as an indicator and *n*-butyllithium was added until the red color remained for at least 10 min.[7,25] As soon as the indicator began to fade, bis(dimethylamino)phosphorochloridate (distilled) (0.7 g) in 2 mL of *N,N,N′,N′*-tetramethylethylenediamine was added and the solution stirred for 2 h. Water was added, the product was extracted into ether, and the extracts were evaporated to give a clear oil which was chromatographed on 10 g of silica eluted with 10% methanol/ether.

The crude 3β,15α-phosphorodiamidate diester was reduced by treatment with lithium (50 mg) in *n*-propylamine (6 mL) under argon overnight with stirring.[7,25] The excess lithium was removed mechanically and the reaction quenched with H_2O. After ether extraction, chromatography on silica eluted with hexane provided cholest-7-ene-*14α-d*$_1$ (**25**) in 80% overall yield:[32] mp 86–87 °C (crystallized from acetone); M^+ *m/e* 371; NMR identical with that of the unlabeled material **1**.

Cholest-8(14)-ene-3β,7β-diol (27). Cholest-8(14)-en-3β-ol-7-one[27] (**26**, 250 mg) was reduced with lithium aluminum hydride (100 mg) in dry ether at room temperature for 0.5 h. The reduction was worked up using aqueous Rochelle salt and the product **27** recrystallized from acetone to give 200 mg: mp 163–164 °C; $[\alpha]^{20}_D$ +41.0° ($CHCl_3$); NMR δ 4.13 (m, 1 H, C-7), 3.55 (m, 1 H, C-3 axial), 0.86 (s, 3 H, C-18 calcd 0.86), 0.74 (s, 3 H, C-19 calcd 0.72).

Anal. Calcd for $C_{27}H_{46}O_2$: C, 80.54; H, 11.51. Found: C, 80.75; H, 11.50.

Cholest-8(14)-ene-3β,7β-diol-*7α-d*$_1$ (28a). The above described

reduction of **26** was repeated with lithium aluminum deuteride: mp 161–163 °C; NMR identical with that of **27** except for absence of δ 4.13 signal.

Cholest-8(14)-ene-7,7-d_2 (29). The crude diol **28a** was treated with lithium aluminum deuteride/aluminum chloride according to the general procedure of Cunningham and Overton.[26] The resulting crude cholest-8(14)-en-3β-ol-7,7-d_2 (**28b,** 90 mg) was treated with *p*-toluenesulfonyl chloride in pyridine and reduced.[12] The resulting oil was chromatographed on 5 g of 12% $AgNO_3$/silica and eluted with hexane to give **29:** mp 51 °C (lit.[12] 53 °C); NMR identical with that of **2;** M^+ *m/e* 371.[32]

5α-Pregn-7-en-20β-ol (32). 5α-Pregn-7-en-20-one[1] (**30,** 21.0 g) was reduced in 2 L of absolute ethanol using metallic sodium (90 g) which was added slowly over a 2-h period with stirring. Partial evaporation of the solvent and extraction gave 22 g of a yellow oil which was chromatographed on 1100 g of silica and eluted with 10% ether/hexane to give 6.5 g of recovered **30** contaminated with the 17α epimer followed by 4.3 g of 5α-pregn-7-en-20β-ol (**31**): mp 112–114 °C (crystallized from acetone); $[\alpha]^{20}_D$ −37°; IR 3400 cm^{-1}; NMR δ 5.10 (m, 1 H, vinyl), 3.62 (m, 1 H, C-20), 1.10 (d, 3 H, J = 6.6 Hz, C-21), 0.78 (s, 3 H, C-19 calcd 0.78), 0.61 (s, 3 H, C-18 calcd 0.62).

Anal. Calcd for $C_{21}H_{34}O$: C, 83.38; H, 11.33. Found: C, 83.26; H, 11.14.

Further elution led to the desired 20α epimer (8.5 g) **32** which was recrystallized from methanol to yield white plates which lost solvent at 60 °C to give a white powder melting at 142–144 °C; $[\alpha]^{20}_D$ 13.4°; IR 3400 cm^{-1}; NMR δ 5.10 (m, 1 H, vinyl), 3.62 (m, 1 H, C-20), 1.17 (d, 3 H, J = 6.6 Hz, C-21), 0.78 (s, 3 H, C-19 calcd 0.78), 0.53 (s 3 H, C-18 calcd 0.53).

Anal. Calcd for $C_{21}H_{34}O$: C, 83.38; H, 11.33. Found: C, 83.13; H, 11.44.

Assignments of the 20β and 20α configurations were made on the basis of NMR, rotations, and TLC retention as compared to the known pregnan-20-ols.[33]

5α-Pregn-7-ene-18,20α-lactone (33). 5α-Pregn-7-en-20α-ol (**32,** 800 mg) was added with iodine (680 mg) to a hot suspension of 5.0 g of lead tetraacetate (freshly recrystallized from glacial acetic acid and dried at 35 °C (5 mm)) and $CaCO_3$ (dried at 70 °C, 1 mm) (1.6 g) in 100 mL of methylcyclohexane which had been kept at reflux under N_2 for 2 h. Use of unrecrystallized $Pb(OAc)_4$, lower boiling solvent, or failure to reflux the $Pb(OAc)_4$ with $CaCO_3$ in advance led to drastically reduced yields, and oxidation of the 20β alcohol **31** was not successful. The mixture was refluxed with vigorous stirring under N_2 with light from a 250-W floodlamp until the iodine color had disappeared (about 40 min). The reaction mixture was cooled to 15 °C and filtered and the filter cake was rinsed with cyclohexane; the combined filtrates were rinsed twice with 5% sodium thiosulfate and once with water, 0.5 mL of pyridine was added, and the organic layer was dried over $MgSO_4$. All rinses were extracted with ether and the combined extracts were evaporated at 35 °C under vacuum.[28]

The resulting yellow oil was taken up in 70 mL of acetone, cooled to 0 °C, and oxidized by dropwise addition of 3 mL of a solution made of 1.3 g of CrO_3 and 1 mL of H_2SO diluted with 4 mL of H_2O and cooled to 10 °C. The mixture was kept between 0 and 5 °C for 30 min and then added to 200 mL of 5% Na_2CO_3 and extracted into ether. The ether was rinsed with H_2O and brine and dried over $MgSO_4$ and all rinses were extracted again with ether. The combined extracts were evaporated at 40 °C to give a yellow oil which was chromatographed on 120 g of silica and eluted with 20% ether/hexane to give 420 mg of a pale yellow oil. Since the product began to decompose on standing, giving off I_2, it was dissolved in 100 mL of acetone under a stream of CO_2 and reduced with 5 mL of a $CrCl_2$ solution.[21a] The reaction mixture was stirred for 0.5 h and then extracted into ether. The combined extracts were evaporated to give 370 mg of a colorless oil which represented a 1:1 mixture of the Δ^7 and $\Delta^{8(14)}$ lactones **33** and **34,** respectively. Since the Δ^7 lactone is stable to all subsequent reaction conditions, the isomerization ($\Delta^7 \rightarrow \Delta^{8(14)}$) is assumed to take place during the lead tetraacetate oxidation.

Recrystallization from methanol gave a 70/30 mixture of the $\Delta^{8(14)}/\Delta^7$ lactones and further recrystallization gave 120 mg of pure 5α-pregn-8(14)-ene-18,20α-lactone (**34**) as white plates: mp 154–158 °C; $[\alpha]^{20}_D$ + 183°; IR 1755 cm^{-1}; NMR δ 4.60 (m, 1 H,C20), 1.33 (d, 3 H, J = 6.6 Hz, C-21), 0.82 (s, 3 H, C-19).

Anal. Calcd for $C_{21}H_{30}O_2$: C, 80.21; H, 9.62. Found: C, 79.83; H, 9.46.

Fractional recrystallization of the mother liquors yielded 90 mg of 5α-pregn-7-ene-18,20α-lactone (**33**) nearly free of $\Delta^{8(14)}$ lactone and chromatography on 25 g of silica followed by elution with 10% ether/hexane gave 45 mg of pure **33** which was crystallized from hexane to give a gummy solid: $[\alpha]^{20}_D$ +1.9°; IR 1755 cm^{-1}; NMR δ 5.20 (m, 1 H, vinyl), 4.52 (m, 1 H, C-20), 1.33 (d, 3 H, J = 6.6 Hz, C-21), 0.87 (s, 3 H, C-19). An analytical sample was prepared by preparative gas chromatography (10% OV-17 240 °C): mp 149–152 °C after crystallization from hexane; M^+ *m/e* 314.

Anal. Calcd for $C_{21}H_{30}O_2$: C, 80.21; H, 9.62. Found: C, 79.93; H, 9.72.

5α-Pregn-7-ene-18,20α-diol (35). The lactone **33** (120 mg) was reduced in dry ether at room temperature with $LiAlH_4$ (200 mg). The mixture was stirred for 30 min and worked up using aqueous Rochelle salt and extracting with ether to give 110 mg of a white solid which was recrystallized from acetone: mp 140–152 °C; $[\alpha]^{20}_D$ + 5.1°; IR 3400 cm^{-1}; NMR δ 5.10 (m, 1 H, vinyl), 4.10 (m, 1 H, C-20), 4.00 (m, 2 H, hydroxyl protons), 3.25 (m, 2 H, C-18), 1.14 (d, 3 H, J = 6.6 Hz, C-21), 0.81 (s, 3 H, C-19); M^+ *m/e* 318.

Anal. Calcd for $C_{21}H_{34}O_2$: C, 79.19; H, 10.76. Found: C, 78.77; H, 10.70.

5α-Pregn-7-ene-18,20α-diol-*18,18*-d_2 (36). The diol 36 was prepared from **33** in the same way as the unlabeled compound except that lithium aluminum deuteride was used as the reducing agent: NMR δ 5.10 (m, 1 H, vinyl), 4.55 (m, 2 H, hydroxyl protons, 4.10 (m, 1 H, C-20), 1.14 (d, 3 H, J = 6.6 Hz, C-21), 0.81 (s, 3 H, C-19); M^+ *m/e* 320.

5α-Pregn-7-ene-*18,18,18,20*-d_4 (37). The labeled diol **36** (110 mg) was converted to the 18,20-phosphorodiamidate diester as in **29**[25] and chromatographed on 10 g of silica. Elution with 5% methanol/ether gave some less polar products and then 50 mg of a mixture of the two phosphorodiamidate monoesters while 10% methanol/ether eluted the desired 18,20-diester (55 mg, 28%).

The above diester (55 mg) was reduced in 6 mL of $D_2NC_3H_7$ (prepared by the method of Muccino[7]) using 20 drops of *tert*-butyl alcohol-d_1 (the product of a reduction attempted without *tert*-butyl alcohol was an unidentifiable tar) and ca. 50 mg of lithium metal while stirring under argon for 3 h.

The excess lithium was removed mechanically and the reaction quenched with H_2O. After ether extraction the combined extracts were evaporated, chromatographed on silica, and eluted with hexane to give 15 mg of an oil which refused to crystallize. Gas chromatography showed the presence of several minor products (<30% total). 5α-Pregn-7-ene-*18,18,18,20*-d_4 (**37**) was isolated pure by preparative VPC using a 10% OV-17 column (1/4 in. × 6 ft, 230 °C) and recrystallized from acetone/methanol: mp 69–70 °C (lit.[1] 68–69 °C); M^+ *m/e* 290; NMR identical with that of unlabeled authentic material, δ 5.10 (m, 1 H, vinyl), 0.78 (s, 3 H, C-19 calcd 0.78). No resonance was seen for the 18-methyl group as expected, but an unlabeled sample prepared in exactly the same way gave the required resonance, δ 0.43 (s, 3 H, C-18 calcd 0.43).

References and Notes

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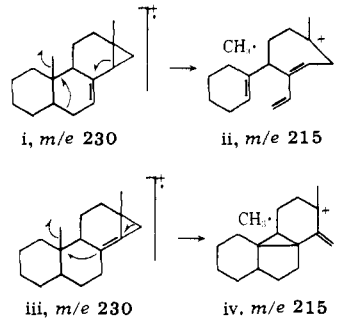

i, *m/e* 230 ii, *m/e* 215

iii, *m/e* 230 iv, *m/e* 215

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Cooperativity in Bimolecular Micelle-Catalyzed Reactions. Inhibition of Catalysis by High Concentrations of Detergent

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Abstract: The rate constant of micelle-catalyzed reactions as a function of detergent concentration has previously been treated as being analogous to positive cooperativity in enzymatic reactions. A kinetic model analogous to the Hill model accommodated data on the rate constants of many micellar reactions as a function of detergent concentration. Most bimolecular micelle-catalyzed reactions reach a rate maximum, which decreases with increasing detergent concentration. In an extension of the previous treatment this behavior is described in analogy with substrate inhibition of enzymatic reactions. Two terms are derived which quantitatively describe this inhibition: n' is an index of cooperativity in formation of the inactive detergent substrate complex, and $K_{n'}$ is the association constant for this complex formation. In some cases, the term n' asymptotically approaches unity at very high detergent concentration, indicating a lack of cooperativity in association under these conditions.

Reactions catalyzed by micelles have often been viewed as models of enzyme-catalyzed reactions.[1,2] This analogy is based on the gross structural similarities and properties of micelles and enzymes, and the fact that both micelles and enzymes bind substrate in a noncovalent manner prior to the catalytic step.[2] Recently, I drew an analogy between micellar reactions and reactions catalyzed by many regulatory enzymes.[3] The rate constants of micelle-catalyzed reactions when plotted vs. detergent concentration give sigmoid shaped curves. This behavior is analogous to positive cooperativity in enzymatic reactions, a sigmoid shaped dependence of velocity on substrate concentration. A kinetic model analogous to the Hill model,[4] which describes enzymatic reactions, accommodated published data on the rate constants of many micellar reactions as a function of detergent concentration.[3] This treatment assumed that a rate plateau is achieved at high detergent concentrations. While this assumption is generally valid for unimolecular spontaneous reactions, most bimolecular reactions which are catalyzed by micelles have rate maxima. At very high detergent concentration, the rates of reaction decrease with increasing detergent concentration. In an extension of the previous treatment, this behavior is now described in analogy with substrate inhibition of enzymatic reactions.

Theory and Methods

The simple mathematical model used previously[3] to describe the sigmoid shaped dependence of rate constant on detergent concentration begins with the assumption that a substrate, S, and a number, n, of detergent molecules, D, aggregate to form catalytic micelles, D_nS, which may then react to yield product:

$$\begin{array}{l} n\mathrm{D} + \mathrm{S} \underset{K_\mathrm{D}}{\rightleftharpoons} \mathrm{D}_n\mathrm{S} \xrightarrow{k_\mathrm{m}} \text{product} \\ \mathrm{S} \xrightarrow{k_0} \text{product} \end{array} \qquad (1)$$