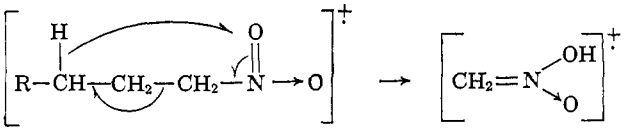
recting further fragmentation. This is also exemplified by the occurrence to only a very minor extent[11b] of any products resulting from a "McLafferty rearrangement."



The molecular ions are only discernible with the lowest member of the series. Loss of the oxygen atoms in the form of O and H_2O gives in some cases the equivalent of a nitrile molecular ion which decomposes further. This seems to be the most interesting feature of the compounds studied. The ubiquitous hydrocarbon ions are formed as well. Oxygen rearrangement with the production of fragments containing only C, H, and O does not seem to occur. The fragmentation pattern of the various nitroalkanes is sufficiently characteristic for "fingerprinting" purposes although position isomers may produce very similar spectra. Especially noteworthy is the great difference between the spectra of nitroalkanes and their isomeric nitrite esters which are usually formed together during the common procedures of preparation. Mass spectrometry would thus appear to be one of the simplest tools for the qualitative and quantitative analysis of such mixtures.

Experimental Section

The mass spectra (both high and low resolution) were measured with an AEI MS-9 instrument using the heated inlet system, ionization energy 70 e.v., inlet temperature about 60°, source temperature about 130°. The apparent resolution for high-resolution measurements was 17,000.

Purification of the samples used was achieved by preparative gas chromatography using a Wilkens Aerograph instrument with a polybutylene glycol column, 30 p.s.i. helium pressure. The retention times were as shown in Table II.

Table II

Compd.	Temp., °C.	Retention time, min.
1-Nitropropane	100	3.8
2-Nitropropane	70	9.5
1-Nitrobutane	110	7.2
1-Nitroheptane	150	16.0
Nitrocyclopentane	150	7.2
Nitrocyclohexane	150	10.3

Mass Spectrometry in Structural and Stereochemical Problems. LXXXI.[1] Stereospecificity in a Hydrogen-Transfer Reaction Characteristic of 6-Keto Steroids[2]

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Contribution from the Department of Chemistry, Stanford University, Stanford, California. *Received June 21, 1965*

The mass-spectral fragmentation behavior of 6-keto steroids has been studied in detail by examining the mass spectra of various deuterated and C-3 substituted cholestan-6-ones. Special emphasis has been placed on elucidating the course of the reaction associated with the loss of carbon atoms 1, 2, 3, and 4 of ring A less one hydrogen atom. This hydrogen transfer was found to be remarkably stereospecific, approximately 40% arising from the 3α- and the remainder from the 2α-position. A mechanism is proposed to account for this stereospecificity and for the observation that the intensity of the peak under consideration varies greatly depending upon the experimental conditions. In connection with a description of the syntheses of the various deuterated analogs of cholestan-6-one attention is called to the unexpectedly strong inhibitory influence of a 6β-substituent upon the extent of base-catalyzed deuterium exchange in the corresponding 3-ketone.

Introduction

Following our initial survey[4] of the mass spectra of steroid ketones, in which the carbonyl group occupied all of the possible nuclear positions, a detailed study of the fragmentation behavior of each positional type was undertaken in our laboratory by means of deuterium labeling. A summary of the results with 1-, 2-, 3-, 7-, 11-, and 16-keto steroids has already appeared[5] as has a subsequent detailed coverage of 15-keto steroids.[6] The main conclusion reached from this body of work is that while a single carbonyl group (in contrast to a dimethylamino or ethylenedioxy function[7]) is not very effective in localizing the charge predominantly on oxygen and thus controlling fragmentation, much information of mechanistic utility can be derived from these studies. Especially pertinent is

(1) Paper LXXX: C. Djerassi and S. D. Sample, *Nature*, in press.

(2) Financial support by the National Institutes of Health (Grant No. CA-07195) of the U. S. Public Health Service) is gratefully acknowledged. The purchase of the Atlas CH-4 mass spectrometer was made possible through NASA Grant No. NsG 81-60.

(3) (a) National Science Foundation Predoctoral Fellow, 1963–1964. (b) Recipient of a NATO fellowship from the Scientific Commission of the Belgian Ministry of Foreign Affairs while on leave from the University of Ghent, Belgium.

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

(5) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, Inc., San Francisco, Calif., 1964, Chapter 20.

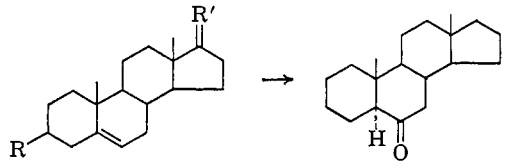
(6) C. Djerassi, G. von Mutzenbecher, J. Fajkos, D. H. Williams, and H. Budzikiewicz, *J. Am. Chem. Soc.*, 87, 817 (1965).

(7) See Chapter 18 in ref. 5.

the insight that has been gained into the factors affecting hydrogen-transfer reactions.[8,9] This has been the principal motive which has prompted us to extend this deuterium-labeling approach to typical members of the remaining classes (positions 4, 6, 12, and 17) of nuclear-substituted monoketo steroids and in the present paper we report our results with 6-keto steroids, notably with cholestan-6-one (XXI). Judging from the previously published[4] mass spectrum of this substance, the one fragmentation process which appeared to be associated specifically with a carbonyl function located at position 6 is the loss of ring A (carbon atoms 1, 2, 3, and 4) less one hydrogen atom. Consequently, the preparative work connected with deuterium labeling necessitated the introduction of deuterium in only a few nuclear positions, but in view of the remarkable stereospecificity (*vide infra*) of this hydrogen transfer, it was necessary in several instances to effect stereospecific monodeuteration. The details of these synthetic studies will be described first before considering the mass spectra and their implications.

Synthetic Studies

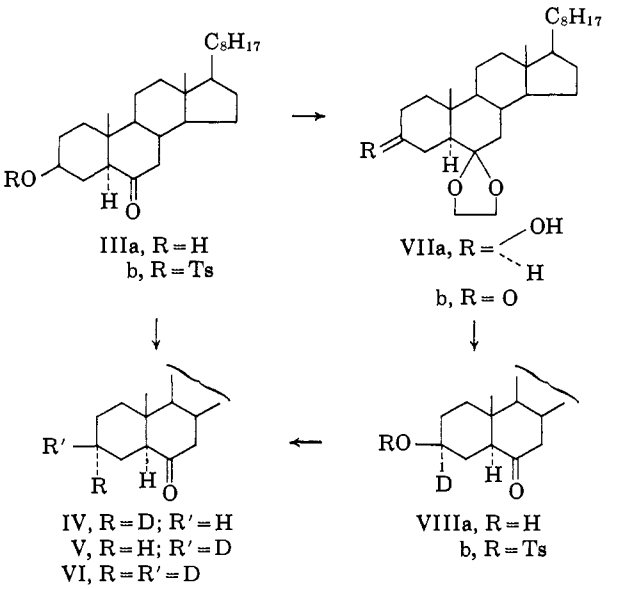
The simplest 6-keto steroid is the hitherto undescribed 5α-androstan-6-one (II), which was required as a reference compound for comparison of its mass spectrum (Figures 5 and 6) with that of more highly substituted analogs, notably of the cholestane series. Its synthesis commenced with the readily available dehydroisoandrosterone (Ia),[10] which was reduced by the Wolff–Kishner procedure to Δ⁵-androsten-3β-ol (Ib).[11] The subsequent steps proceeded by well-trodden paths from the cholesterol series[12] *via* 3β-chloro-Δ⁵-androstene (Ic) to Δ⁵-androstene (Id), followed by hydroboration and direct chromic acid oxidation[13] to the required ketone II.



Ia, R = OH; R′ = O
b, R = OH; R′ = H_2
c, R = Cl; R′ = H_2
d, R = H; R′ = H_2

II

The deuterium labeling was effected in the cholestane series starting with cholestan-3β-ol-6-one (IIIa).[14] Conversion to the tosylate IIIb and reduction with lithium aluminum deuteride followed by reoxidation by the Jones procedure[15] of the simultaneously formed 6-hydroxyl group provided 3α-d_1-cholestan-6-one (IV). For the preparation of the other two C-3 labeled ketones (V and VI), the starting hydroxyketone IIIa was first transformed into the ethylene ketal VIIa and then oxidized with the chromium trioxide–pyridine reagent[16] to the 3-keto-6-ethylene ketal VIIb. Reduction with lithium aluminum deuteride and removal of the protective grouping from C-6 provided 3α-d_1-cholestan-3β-ol-6-one (VIIIa), which was then subjected to the same reaction sequence as the unlabeled precursor IIIa. Thus tosylation at C-3, reduction of VIIIb with lithium aluminum hydride, and finally reoxidation at C-6 afforded 3β-d_1-cholestan-6-one (V), while substitution of lithium aluminum deuteride for the hydride yielded 3,3-d_2-cholestan-6-one (VI). As was to be anticipated from the reactions employed, the isotopic purity of the products (IV, V, and VI) was high (see Table I).



IIIa, R = H
b, R = Ts

VIIa, R = OH, H
b, R = O

IV, R = D; R′ = H
V, R = H; R′ = D
VI, R = R′ = D

VIIIa, R = H
b, R = Ts

The starting material for the C-2 labeled cholestan-6-ones was Δ²-cholesten-6-one (IXa),[14] which was transformed into the ethylene ketal IXb. Oxidation with *m*-chloroperbenzoic acid led to the crystalline 2α,3α-oxido-6-ethylene ketal X, which was reduced with lithium aluminum deuteride and the ketal grouping removed with acid. The resulting product (XIa) gave no melting point depression on admixture with authentic[17] cholestan-3α-ol-6-one (XIa without deuterium), thus demonstrating that the precursor epoxide X had indeed the assigned 2α,3α-configuration. In view of the well-documented[18] course of diaxial opening of such epoxides upon reduction with lithium aluminum hydride, the orientation of the deuterium atom in XIa must be β. The now superfluous 3α-hydroxy function of XIa was removed by lithium aluminum hydride reduction of its derived tosylate XIb followed by reoxidation at C-6 to give the desired 2β-d_1-cholestan-6-one (XII).

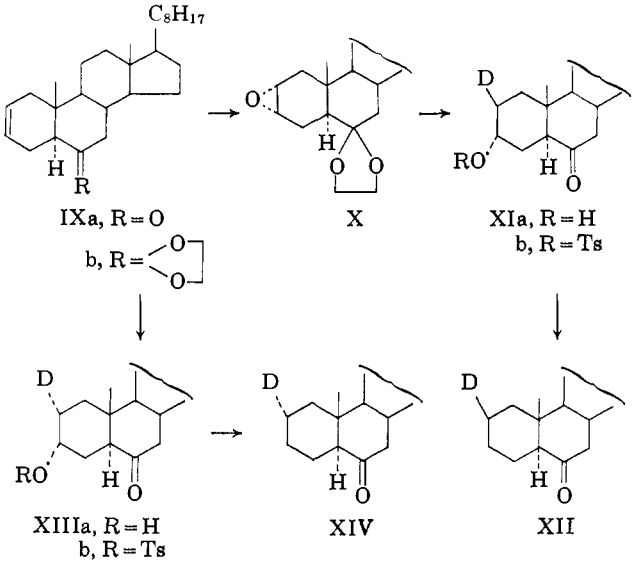
As will become apparent from a perusal of the subsequent discussion of the mass spectrometric results, 2α-d_1-cholestan-6-one (XIV) represents a key compound in the present investigation. The first attempt to prepare this substance, though completely abortive, merits brief mention. The starting material was the

(8) C. Djerassi, *Pure Appl. Chem.*, **9**, 159 (1964).
(9) H. Fritz, H. Budzikiewicz, and C. Djerassi, *Ber.*, in press.
(10) We are indebted to Syntex, S. A., Mexico City, for a generous gift of this substance.
(11) See R. H. Shapiro and C. Djerassi, *J. Am. Chem. Soc.*, **86**, 2825 (1964).
(12) R. E. Ireland, T. I. Wrigley, and W. G. Young, *ibid.*, **80**, 4604 (1958).
(13) H. C. Brown and C. P. Garg, *ibid.*, **83**, 2951 (1961).
(14) R. M. Dodson and B. Riegel, *J. Org. Chem.*, **13**, 424 (1948).
(15) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).
(16) J. R. Holum, *J. Org. Chem.*, **26**, 4814 (1961); G. I. Poos, G. E. Arth, R. E. Beyler, and L. H. Sarett, *J. Am. Chem. Soc.*, **75**, 422 (1953).
(17) C. W. Shoppee and G. H. R. Summers, *J. Chem. Soc.*, 1790 (1952).
(18) For extensive literature citations see J. G. Phillips and V. D. Parker in "Steroid Reactions," C. Djerassi, Ed., Holden-Day, Inc., San Francisco, Calif., 1963, Chapter 14.

Table I. Principal Mass Spectral Peaks of Cholestan-6-one (XXI) and Deuterated Analogs[a]

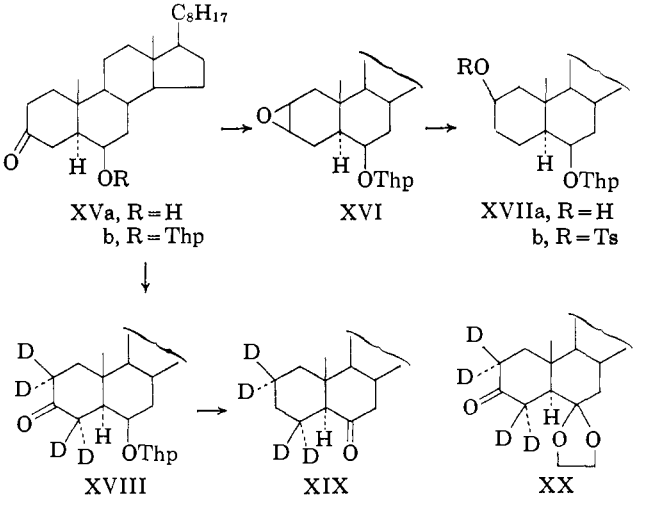
Compd.	Isotopic purity	M+	M − 15	M − 55	M − 113	M − 124	M − 140	M − 141	M − 155	M − 263
Cholestan-6-one (XXI)		386	371	331	273	262	246	245	231	123
2α-d_1-Cholestan-6-one (XIV)[b]	d_1 81% d_0 19%	387	372 (q)	331 (54%) 332 (46%)	274 (q)	262 (q)	247 (q)	245 (q)	232 (q)	124 (q)
2β-d_1-Cholestan-6-one (XII)	100%	387	372 (q)	331 (q)	274 (q)	262 (q)	247 (q)	246 (q)	232 (q)	124 (q)
2,2,4,4-d_4-Cholestan-6-one (XIX)[b]	d_4 50% d_3 46% d_2 3% d_1 1%	390	375 (q)	331 (40%) 332 (60%)	277 (q)	262 (q)	250 (q)	249 (q)	235 (q)	127 (q)
3α-d_1-Cholestan-6-one (IV)	d_1 96% d_0 4%	387	372 (q)	331 (65%) 332 (35%)	274 (q)	262 (q)	247 (q)	246 (q)	232 (q)	124 (q)
3β-d_1-Cholestan-6-one (V)	d_1 98% d_0 2%	387	372 (q)	331 (q)	274 (q)	262 (q)	247 (q)	246 (q)	232 (q)	124 (q)
3,3-d_2-Cholestan-6-one (VI)	d_2 92% d_1 4% d_0 4%	388	373 (q)	331 (62%) 332 (38%)	275 (q)	262 (q)	248 (q)	247 (q)	233 (q)	125 (q)
5α,7,7-d_3-Cholestan-6-one[b]	d_3 18% d_2 34% d_1 34% d_0 14%	389	374 (q)	334 (q)	276 (q)	264 (q)	249 (q)	248 (q)	234 (q)	123 (q)

[a] Mass spectra determined in CEC Model 21-103C mass spectrometer. The symbol q signifies a quantitative shift (>95%). [b] All peak shifts are corrected for isotopic contaminants. [c] The following problem is encountered in calculating the peak shift m/e 331 → 332. If one assumes that in the d_3- and d_4-species the deuterium is equally distributed between the four possible positions, then one arrives at a value of 86% of the 2α-position being substituted by deuterium (50% from d_4, 34.5% (3/4 of 46% d_3) from d_3, and 1.5% (1/2 of 3% d_2) from d_2). On that basis, a 70% shift to m/e 332 would be calculated. However, it is much more likely (see discussion) that the d_3-species has the 2,2,4 distribution, in which case a 60% transfer would result, as shown in the table.



known[19] cholestan·6β·ol-3·one (XVa), which was transformed by conventional steps (bromination at C·2, tetrahydropyranyl ether formation at C-6, lithium tri·*t*·butoxyaluminum hydride reduction, and base·catalyzed cyclization of the bromohydrin) to 2β,3β·oxidocholestan·6β·ol tetrahydropyranyl ether (XVI). The usual[18] *trans*·diaxial opening of the oxide ring with lithium aluminum hydride gave the 2β·ol XVIIa, which was converted into the tosylate XVIIb. It was then hoped that lithium aluminum deuteride reduction would provide the 2α·d_1·cholestan·6β·ol tetrahydro·pyranyl ether, but instead exclusively elimination occurred, since acid cleavage of the product and oxidation afforded Δ²·cholesten·6·one (IXa).

Another and successful route to 2α·d_1·cholestan·6·one (XIV) was based on the observation[20] that hydroboration of Δ²·cholestene (IX without C·6 substituent) proceeded predominantly from the α·side and that the principal product (50%) was cholestan·3α·ol. Since the presence of an ethylene ketal function at C·6 (see IXb) would not be expected[21] to increase the proportion of the undesired β·face attack, deuteriobora·tion of the unsaturated ketal IXb followed by hydrogen peroxide oxidation was undertaken. The expected[20] mixture of isomers was separated by preparative thin layer chromatography, first at the ketal stage and then again after acid cleavage. The desired 2α·d_1·cholestan·3α·ol·6·one (XIIIa) could be located by comparison with authentic,[17] unlabeled material and after trans·formation to the tosylate XIIIb, it was converted by



(19) C. W. Shoppee and G. H. R. Summers, *J. Chem. Soc.*, 3361 (1952).

(20) F. Sondheimer and M. Nussim, *J. Org. Chem.*, 26, 630 (1961); A. Hassner and C. Pillar, *ibid.*, 27, 2914 (1962).

(21) M. Nussim, Y. Mazur, and F. Sondheimer, *ibid.*, 29, 1120 (1964), have shown that it is the *axial* branch of an ethylene ketal which affects the stereochemical course of the hydroboration in the steroid series.

the usual procedure (see XIb → XII) into 2α-d_1-cholestan-6-one (XIV).

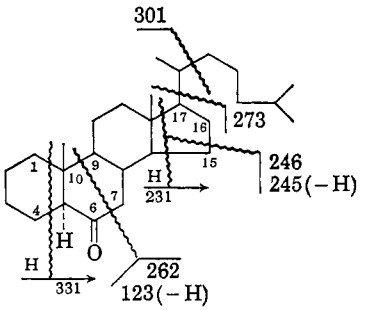
In order to label C-4 in cholestan-6-one with deuterium, it seemed simplest to prepare 2,2,4,4-d_4-cholestan-6-one (XIX), since any hydrogen migration from C-2 would already have been established from the mass spectra of XII and XIV. For this purpose, cholestan-6β-ol-3-one (XVa)[19] was converted into its tetrahydropyranyl ether XVb and then heated two times each for 5 hr. and once for 17 hr. with a mixture of sodium in deuterium oxide and O-d_1-methanol. Removal of the 3-keto group was effected by alternate lithium aluminum hydride reduction and tosylate formation (see preparation of VI) and after acid hydrolysis of the tetrahydropyranyl ether protecting group and reoxidation, the labeled ketone XIX was obtained. As shown in Table I, in spite of the repeated treatment, deuterium exchange was incomplete and only 50% of d_4-species was produced. Since cholestan-3-one and other ring B unsubstituted 3-keto steroids undergo ready deuterium exchange[22] (at least 80% d_4-species after one exchange), it seemed of interest to examine the effect of another C-6 substituent upon this reaction. When the same deuterium exchange conditions (three reflux periods with fresh reagents) were applied to cholestane-3,6-dione 6-ethylene ketal (VIIb) in order to prepare the d_4-analog XX, followed by the usual steps (see VIIb → VIII → VI, R = R′ = H), there was obtained a deuterated cholestan-6-one mixture of even poorer isotopic incorporation (12% d_0, 20% d_1, 23% d_2, 33% d_3, 12% d_4).

With the limited evidence at hand, one can only speculate about the origin of this effect. The common denominator in XVb and VIIb is the presence of a 6β-substituent and since it has been suggested[23] that one of the reasons for the preferred enolization toward C-2 in 5α-3-keto steroids is the increased buttressing between the 6β-hydrogen atom and the angular methyl group in Δ³- as compared to Δ²-enols, one might suggest that any 6β-substituent bulkier than hydrogen would tend to make enolization toward C-4 even less favorable. An alternate possibility may be that, in order to relieve the 6β–10β interaction in these substituted ketones, ring B is deformed toward a boat form in which the 6β-substituent is now completely eclipsed with the C-4 hydrogen atom in a Δ³-enol, while this is not the case in a Δ²-enol where C-4 is tetrahedral. Either explanation implies that the low d_4-content in XVIII and XX is due to incomplete deuterium introduction at C-4 rather than at C-2.[24] The relatively high proportion of d_0- and d_1-species (total of 32%) in the ketal VIIb suggests that enolization even toward C-2 is inhibited to a certain extent.

Discussion of Mass Spectra

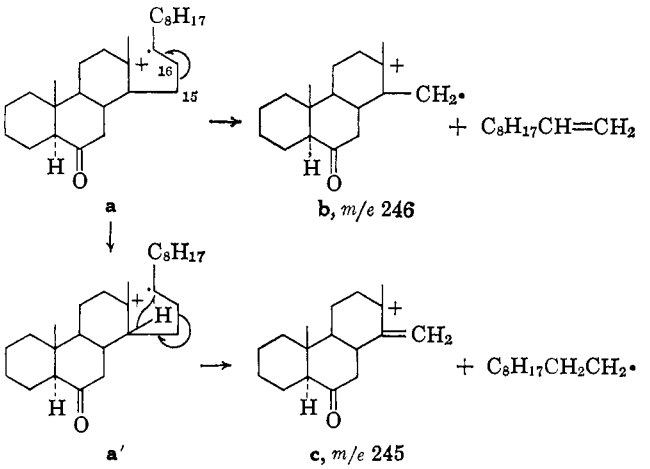
The mass spectrum of cholestan-6-one (XXI)[19] as determined in an all-glass, heated (200°) inlet system with a CEC Model 21-103C mass spectrometer is reproduced in Figure 1. The striking intensity variations observed upon alterations in the mass spectrometric measurement techniques (see Figures 2, 3, and 4) will be commented upon further on. By comparing the cholestan-6-one spectrum (Figure 1) with that (Figure 5) of 5α-androstan-6-one (II), it will be noted that only one of the important peaks above *m/e* 100, namely the one at *m/e* 123, coincides in both spectra. Since the two substances (XXI *vs.* II) differ only in the nature of the C-17 substituent, it follows that most of the significant higher mass range ions retain the "right hand" portion of the molecule. The correctness of this statement is supported by Table I, which summarizes the shifts of the most important peaks in the various deuterium-labeled cholestan-6-ones.

The origin of the peaks between *m/e* 273 and the molecular ion (*m/e* 386) in the cholestan-6-one spectrum (Figure 1) can be determined quite easily from an inspection of Table I. Thus *m/e* 371 corresponds to the loss of a methyl radical, *m/e* 368 to the loss of water,[25] *m/e* 353 to the elimination of both methyl and water, *m/e* 331 to the expulsion of carbon atoms 1, 2, 3, and 4 from ring A less one hydrogen atom (mechanism to be discussed below), *m/e* 301 to the fission of the 20–22 bond, and *m/e* 273 to rupture of the 17–20 linkage.



XXI

The peak at *m/e* 262 in Figure 1 can be ascribed quite unambiguously (see Table I) to fission of ring B (6–7 and 9–10 bonds) with charge retention on the hydrocarbon moiety. As shown schematically in structure XXI, the *m/e* 246 and 245 peaks are involved in ring D cleavage processes, which can be visualized most readily as originating from a molecular ion such as **a**. Scission of the 15–16 bond then leads to **b**, the



(22) See for instance R. H. Shapiro, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, 86, 2837 (1964).

(23) For summary see E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, "Conformational Analysis," Interscience Publishers, Inc., New York, N. Y., 1965, p. 348.

(24) See also footnote *c* in Table I.

(25) The loss of water in cyclic ketones is a fairly common occurrence in mass spectrometry: see for instance H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, Inc., San Francisco, Calif., 1964, pp. 20, 143.

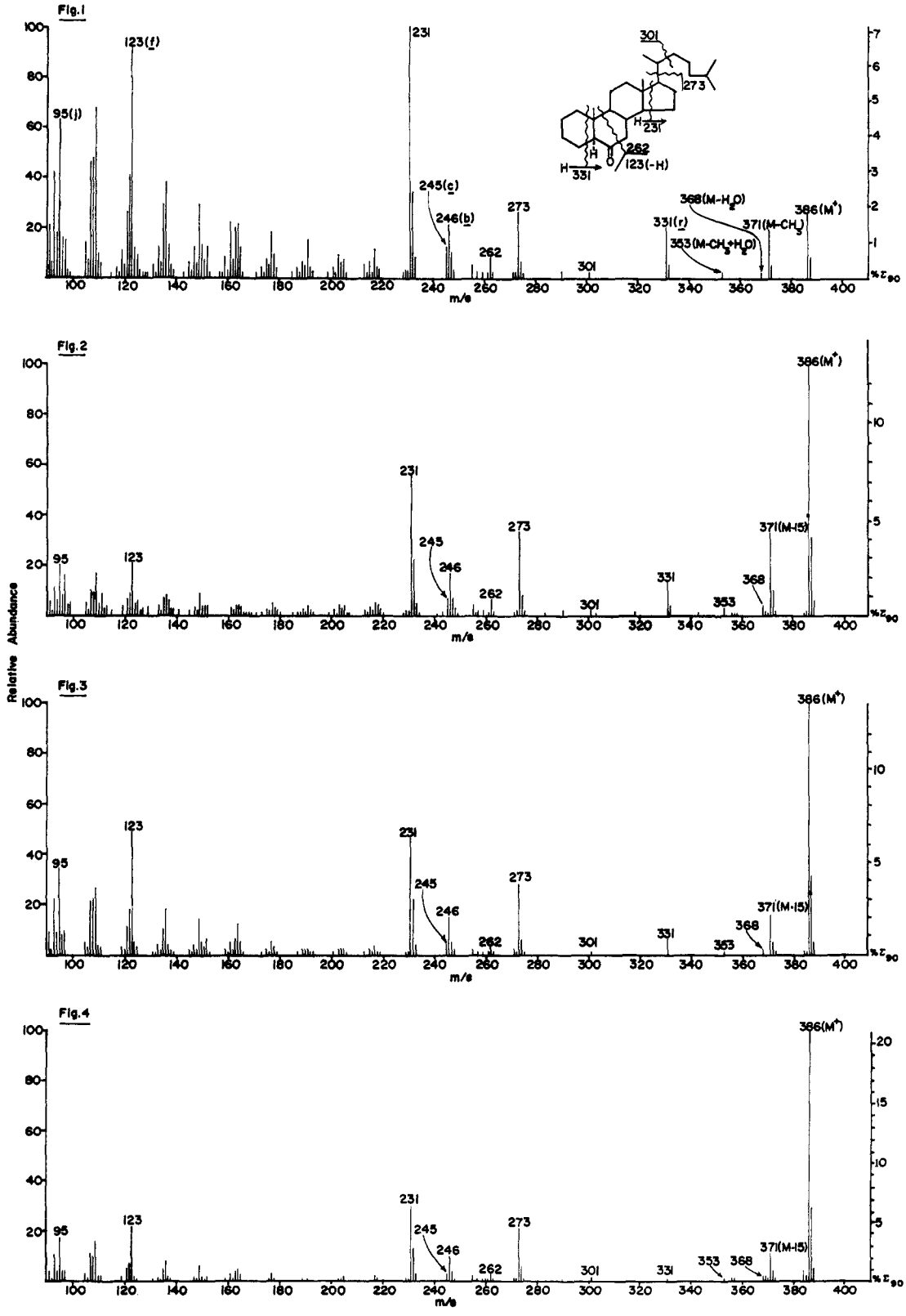
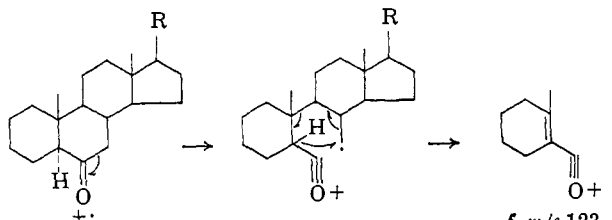


Figure 1. Mass spectrum of cholestan-6-one (XXI) determined in CEC Model 21-103C mass spectrometer with heated (200°), all-glass inlet system (ion source temperature, 250°).
Figure 2. Mass spectrum of cholestan-6-one (XXI) determined in CEC Model 21-103C mass spectrometer by direct insertion into ion source (250°).
Figure 3. Mass spectrum of cholestan-6-one (XXI) determined in AEI Model MS-9 mass spectrometer by direct insertion into ion source (150°).
Figure 4. Mass spectrum of cholestan-6-one (XXI) determined in Atlas Model CH-4 mass spectrometer by direct insertion into ion source (TO4 ion source, temperature, 70°).

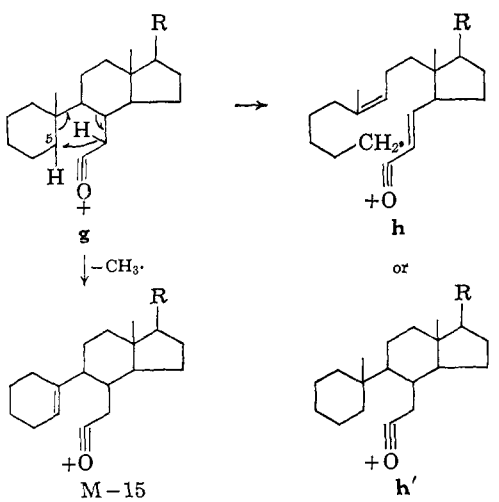
equivalent of an ionized cyclopropane, and a stable neutral olefin. A hydrogen transfer must accompany the generation of the *m/e* 245 peak and, while no deuterium labeling was performed in that region of the

molecule, a likely candidate is the C·14 hydrogen atom (see **a**′) giving rise to the allylic carbonium ion **c**.

The intense *m/e* 231 peak is associated with the well-known[8] cleavage of ring D together with one hydrogen atom, a process which occurs in nearly all C·17·substituted steroids and which need not be discussed further in the context of the present paper, other than to mention that its direct formation (at least in part) from the molecular ion is supported by the recognition of a metastable peak at *m/e* 138.4 ($231^2/386 = 138.24$). The origin of the important *m/e* 123 peak, which is also found in the 5α-androstan·6·one spectrum (Figure 5), is clearly defined by the shifts in the deuterated analogs (see Table I) since migration of the 5α·hydrogen atom is involved. Evidently, the molecular ion **d** undergoes first the typical α·cleavage of ketones to **e** followed by hydrogen transfer through a six·membered intermediate to yield the stable conjugated species **f** (*m/e* 123).



The various fragmentation reactions exhibited by a simple 6·keto steroid such as cholestan·6·one are summarized schematically in structure XXI and it will be noted that only three peaks, *m/e* 123, 262, and 331, can be attributed directly to the presence of the 6·keto function. The peaks at *m/e* 123 and 262 are associated with α·cleavage on one side of the carbonyl group and it may be asked why fragments corresponding to α·fission between carbon atoms 5 and 6 are not also evident, especially since fission of a more highly substituted bond should be favored. As far as the formation of an oxygen·containing fragment analogous to **f** is concerned, the explanation for its absence probably lies in the fact that hydrogen transfer in the resulting species **g** would furnish **h** or **h**′, which cannot lead to any highly stabilized species upon further bond fission. The molecular ion **g** may well be the progenitor of the M − 15 species (especially intense in Figures 5 and 6), but isotopic labeling would have to be performed to settle this point.



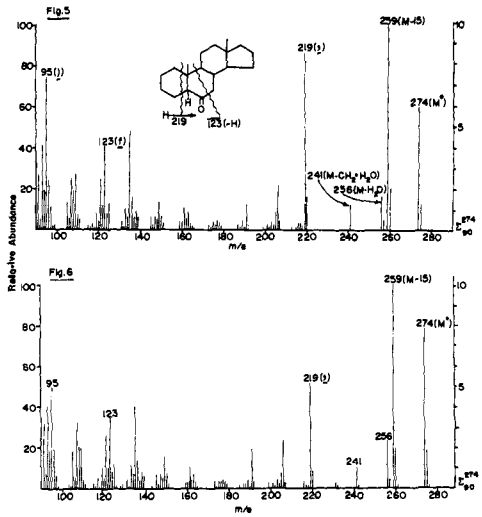
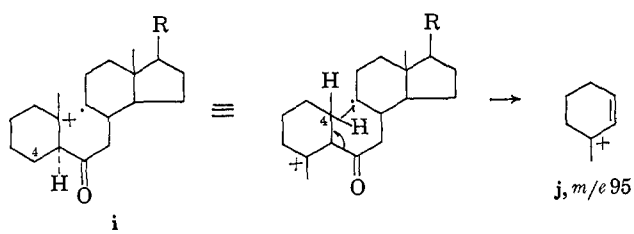


Figure 5. Mass spectrum of 5α·androstan-6-one (II) determined in CEC Model 21-103C mass spectrometer with heated (200°) all·glass inlet system (ion source temperature, 250°).
Figure 6. Mass spectrum of 5α-androstan-6-one (II) determined in Atlas Model CH·4 mass spectrometer (ion source temperature, 70°).

The hydrocarbon counterpart of *m/e* 262 due to alternate α·cleavage (fission of 5–6 and 9–10 bonds) should occur at *m/e* 96. Such a peak is comparatively small in Figures 1 and 5, but there is a very substantial peak at *m/e* 95, which could be visualized to arise from a molecular ion such as **i**, followed by transfer of a C·4 hydrogen atom to the C·9 radical site and fission of the 5–6 bond to afford the allylic carbonium ion **j**. Unfortunately, the *m/e* 90–100 region is sufficiently populated (see Figures 1 and 5) that no quantitative estimates of peak shifts in the deuterated analogs are possible. Qualitatively, it can be stated that at least a portion of the fragment of mass 95 is correctly depicted by structure **j**.

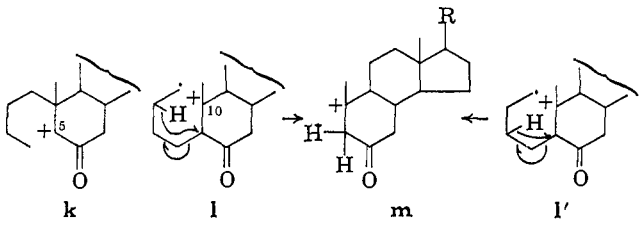


Mechanistically, there remains only a clarification of the genesis of the *m/e* 331 peak in the cholestan·6·one spectrum (Figure 1) which corresponds to the *m/e* 219 peak in the spectrum (Figure 5) of 5α-androstan·6·one (II). Formally, its genesis involves a β· rather than α·cleavage with respect to the carbonyl group as well as the transfer of one hydrogen atom from ring A to the charge·retaining fragment. It is precisely to uncover the origin of the itinerant hydrogen atom that the various labeled derivatives of cholestan·6·one were synthesized.

An inspection of the peak shifts summarized in Table I leads to the remarkable conclusion that the hydrogen transfer is almost completely stereospecific. A comparison of the data for the three analogs IV, V, and VI labeled at C·3 demonstrates that 35–38% of the migrating hydrogen arises from the 3α· and

essentially none from the 3β-position. Virtually the entire remainder (46–60%) originates from the 2α-position (see XII, XIV, and XIX in Table I), the somewhat wider range being due to uncertainties in calculations because of the lower isotopic purity of XIV (see also Experimental Section and footnote 36) and XIX. In any event, since only one hydrogen migrates to the charged fragment, at least two separate processes must be involved in the generation of this ion and any explanation must take into account the noteworthy stereospecificity of this hydrogen migration.

In principle, the required fissions of the 1–10 and 4–5 bonds could proceed through species in which the charge is either localized on oxygen or on carbon. In the latter event, localization at C-5 (**k**) is extremely unlikely because of the undesirability of placing a positive charge next to a carbonyl group. The alternate possibility is a molecular ion such a **l** with the charge situated at C-10; the indicated hydrogen migrations from C-2 (**l**) or C-3 (**l′**) would then lead to *m* (*m/e* 331 in Figure 1 and *m/e* 219 in Figure 5) and a butenyl radical.

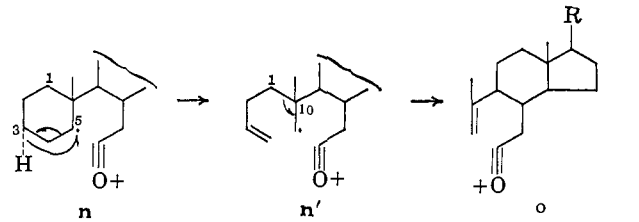


k **l** **m** **l′**

We consider such explanations very implausible because once the 1–10 bond is broken (**l** or **l′**) there exists no rationale for the observed rigorous stereospecificity of hydrogen transfer from the (original) α-side only. Indeed, the only way in which such stereospecificity can be accounted for is to assume that the hydrogen migration occurs prior to or concurrently with fission of a bond in ring A. Since such an assumption requires that the charge be localized on oxygen, there being no other energetically obvious site for it, this in turn necessitates cleavage of a bond terminating at C-5, since there exists no stabilizing factor for first cleaving a bond terminating at C-10.

If we pursue this reasoning, we could postulate initial α-fission next to the positively charged carbonyl group to provide the species **n** and hydrogen transfer in the intact chair form of the cyclohexane ring. If this were to occur, only two hydrogen atoms are appropriately situated for such a transfer, namely those with the 3α- or 1α-orientation. Transfer of the latter will be of no help in the subsequent step (fission of the 1–10 bond) since a carbene would then be generated at C-1. Migration of the 3α-hydrogen atom, on the other hand, with synchronous fission of the 4–5 linkage (see **n**), would lead to **n′**, where the radical site at C-5 would provide the driving force for the subsequent rupture of the 1–10 linkage in order to generate a 5–10 double bond. The final species would then possess the energetically attractive formulation **o**. It is quite likely that the steps outlined separately in **n** and **n′** would proceed in a synchronous fashion.

The only difficulty with such a proposal is to account for the stereospecificity in the hydrogen transfer from C-2. In the chair form of intermediate **n**,

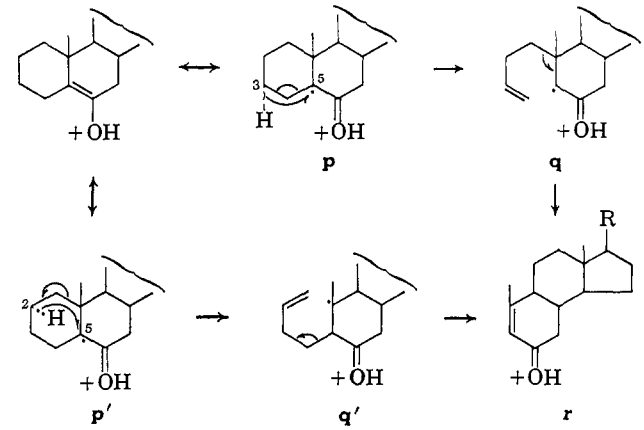


n **n′** **o**

(*m/e* 331, R = C_8H_{17})
(*m/e* 219; R = H)

the 2α-hydrogen atom cannot be transferred. Once the cyclohexane ring flips into a flexible form, hydrogen migration is easily possible from either the 2α- or the 2β-position (depending upon which of the flexible forms is considered), which is contrary to the experimental observation (Table I).

This difficulty is obviated by generating a radical site at C-5 without, however, breaking the 5–6 bond (see **n**) and thus limiting greatly the number of flexible forms in ring A. This can be achieved readily by assuming[26] that this particular fragmentation process commences with the molecular ion of the enol (**p**) rather than of the ketone. For reasons discussed above under **n**, hydrogen transfer from C-3 in the intact chair form can only originate from the 3α-locus (**p** → **q**), fission of the 1–10 bond then yielding the conjugated oxonium ion **r**. To explain the stereospecificity from the C-2 position, we need only to invoke conformational "flipping" of the chair form of ring A which will then permit transfer of the 2α- (see **p′**) but most certainly not the 2β-hydrogen atom, the remaining steps (**p′** → **q′** → **r**) being unexceptional. This picture also offers an explanation for the preponderance (see Table I) of the 2α- (46–60%) transfer over that originating from 3α (35–38%) since in the flexible form the 2α-hydrogen atom can approach the C-5 radical site considerably closer than can the 3α-hydrogen atom in the chair form (or the 3β-hydrogen in the flexible form).



p **q** **p′** **q′** **r**

The above explanation is based on the premise that a portion of the 6-keto steroid is ionized as the enol rather than keto form. That enolization in the inlet system prior to ionization can occur has been demonstrated by the encounter[27] of nearly 10% of a d_1-species in the mass spectrum of α-decalone in a mass spectrometer first equilibrated with deuterium oxide,

(26) We are indebted to our colleague, Dr. J. I. Braumann, for this suggestion.

(27) E. Lund, H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 1528 (1963).

Table II. Per Cent Total Ionization (Σ_{90}) of Selected Peaks of Cholestan-6-one (XXI) and 5α-Androsten-6-one (II)

Instrument	Figure	Approximate ion source temp., °C.	M^+ (*m/e* 386), %	$M^+ - 55$ (*m/e* 331), %	*m/e* 231, %
		Cholestan-6-one (XXI)			
CEC 21-103C 200° glass inlet system	1	250	1.85	1.42	7.12
CEC 21-103C direct inlet system	2	250	12.95	1.68	7.25
AEI MS-9 direct inlet system	3	150	13.55	0.81	6.23
Atlas CH4 TO4 direct inlet system	4	70	21.18	0.21	6.35
		5α-Androstan-6-one			
			M^+ (*m/e* 274), %	$M^+ - 55$ (*m/e* 219), %	
CEC 21-103C 200° glass inlet system	5	250	5.91	8.48	
Atlas CH4 TO4 direct inlet system	6	70	7.95	5.20	

as well as by the partial loss of deuterium in the spectrum of d_4-cyclododecanone[28] in an inlet system which has not been equilibrated with deuterium oxide.

Further support for this assumption is also provided by the mass spectra of cholestan-6-one (XXI) measured under different conditions (see Table II). The spectrum reproduced in Figure 1 was obtained by means of an all-glass inlet system heated to 200° in a CEC Model 21-103C where the ion source temperature is *ca.* 250°. By expressing the abundance of the various ions in terms of per cent total ionization over the range *m/e* 90 → M^+, it will be noted that the intensity of the molecular ion amounts to only 1.85, while at the other extreme a value of 21.18 is encountered for the molecular ion when measured with a "cold" (70°) ion source by the direct inlet system of the Atlas CH-4 mass spectrometer (Figure 4). Intermediate values are obtained (Table II) utilizing a direct inlet system[29] attached to a CEC instrument (ion source temperature 250°) or to an AEI MS-9 mass spectrometer with only a moderately hot (150°) ion source. Equally striking are the variations (Table II) in the per cent total ionization of two fragment ions. The above discussed *m/e* 331 species, which supposedly originates from an enol molecular ion (**p**), ranges from Σ_{90} 1.68 to 0.21, while the *m/e* 231 fragment, which is derived from a molecular ion such as **a**, covers a much narrower range (Σ_{90} 6.23 to 7.25) and thus seems to be much less dependent on experimental conditions.

The Spitellers[30] have already discussed fully the effect of the ion-source temperature upon the intensity of the molecular and certain fragment ions, and the present results substantiate their conclusions (*e.g.*, Figure 1 *vs.* Figure 4 and Table II). The distance between the sample probe (well over 1 m. in the instrument used to obtain Figure 1; a few millimeters in the instruments used for Figures 2–4) probably also plays a role as can be seen from a comparison of Figures 1 and 2. Thermal wall reactions prior to ionization undoubtedly affect the enolization process,[31] and this is probably the other factor (aside from the ion-source temperature) which accounts for the variations in per cent total ionization of the ions listed in Table II. This is particularly well demonstrated by considering the ratio in Σ_{90} of *m/e* 386 and *m/e* 331 in the first two horizontal columns of Table II, since they range from 1.3:1 to 7.7:1 simply by changing the inlet system.

A closer evaluation of the mass spectra (Figures 1–4) shows that, even under the most favorable conditions for fragmentation (Figure 1), the occurrence of the mechanistically most interesting process (leading to **r**, *m/e* 331) is relatively small and is virtually absent in the Atlas spectrum (Figure 4) of cholestan-6-one. In large part, this is due to competing fragmentations associated with the side chain, since in the 5α-androstan-6-one spectrum (Figure 5) the corresponding peak (*m/e* 219) is the most intense one (Σ_{90} 8.48%) in the entire spectrum and still amounts to 50% of the base peak (Σ_{90} 5.20%) when measured (Figure 6) in an Atlas instrument (same experimental conditions as in Figure 4).

Jones and Kime[32] have recently prepared a substantial number of C-3 substituted cholestan-6-ones and it was of interest to examine their mass spectral behavior in order to contrast it with that of the unsubstituted parent XXI and thus determine the effect of additional ring A substituents. As examples, there are reproduced the Atlas spectra of 3β-methoxy-cholestan-6-one (XXII, Figure 7) and of 3β-chloro-cholestan-6-one (XXIII, Figure 8). Except for the α-cleavage between C-6 and C-7 (see **f** = *m/e* 123 in Figures 1 and 5), which in the present cases leads to peaks at *m/e* 153 (XXII, Figure 7) and *m/e* 157 (XXIII, Figure 8), the fragmentation processes triggered by the C-6 carbonyl group are largely repressed, the most significant feature being again cleavages associated with the side chain or the loss of the C-3 substituent (*m/e* 384) in the form of methanol (Figure 7) or hydrogen

(28) K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, pp. 218, 219.

(29) J. F. Lynch, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *Experientia*, **19**, 211 (1963).

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

(31) This has recently been demonstrated in a very striking fashion in our laboratory (H. Budzikiewicz and C. Djerassi, unpublished observation) by the virtually complete exchange of all enolizable hydrogens of a ketone upon the simultaneous admission of the ketone and deuterium oxide through a heated glass inlet system.

(32) D. N. Jones and E. E. Kime, *Proc. Chem. Soc.*, 334 (1964). We are grateful to Dr. Jones of the University of Sheffield for providing us with several 3-substituted cholestan-6-ones.

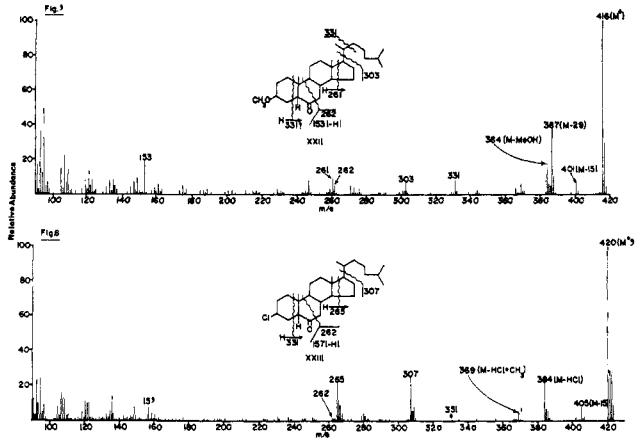


Figure 7. Mass spectrum of 3β-methoxycholestan-6-one (XXII) determined in Atlas Model CH-4 mass spectrometer.
Figure 8. Mass spectrum of 3β-chlorocholestan-6-one (XXIII) determined in Atlas Model CH-4 mass spectrometer.

chloride (Figure 8). Indeed in spectra obtained with a heated glass inlet system in a CEC Model 21-103C mass spectrometer no molecular ion could be observed with 3β-chloro- or 3β-acetoxycholestan-6-ones, the highest peak corresponding to M − HCl or M − HOAc. The latter process is so pronounced that even under the most favorable conditions (direct inlet procedure in Atlas CH-4 mass spectrometer) only a minute molecular ion peak could be observed. Such eliminations of the C-3 substituent of course complicate the mass spectral analysis since further fragment ions derived from the olefin are superimposed upon those originating from the parent molecular ion.

In summary, the present study with 6-keto steroids like the earlier ones[5,6] with other nuclear substituted keto steroids demonstrates that a single carbonyl group in a large hydrocarbon environment like the steroid system has only a weak directing effect upon the over-all fragmentation. This behavior should be contrasted with that of the corresponding 6-ethylene ketals[33] or dimethylamino derivatives[34] which exert a much more dominant role upon the electron-impact-induced fragmentation. On the other hand the presently discovered stereospecific hydrogen transfer reaction illustrates again the mechanistic benefits that may be reaped from a detailed study of the mass spectra of deuterium-labeled steroids.

Experimental Section[35]

5α-Androstan-6-one (II). To a mixture of 6.0 g. of Δ⁵-androsten-3β-ol (Ib)[11] and 5 ml. of dimethylaniline in 80 ml. of 1:1 chloroform–ether was added 6 ml. of thionyl chloride at 0°. After stirring for 1 hr., the mixture was poured into water, the 3β-chloro derivative Ic was isolated with ether, and the crude material, dissolved in 100 ml. of ether, was added to a solution of 2.5 g. of sodium in 100 ml. of liquid ammonia.[12] After refluxing the mixture for 2 hr., the ammonia was allowed to evaporate and the product was isolated in the usual manner with ether. Filtration in pentane solution through an alumina column and recrystallization from acetone provided 3.9 g. of Δ⁵-*androstene* (Id), m.p. 70–72°. The analytical specimen exhibited m.p. 77–78°, $[\alpha]D$ −101° after further recrystallization from acetone.

Anal. Calcd. for $C_{19}H_{30}$: C, 88.30; H, 11.70. Found: C, 88.50; H, 11.60.

To an ice-cold solution of 2.33 g. of Δ⁵-androstene (I) and 2.0 g. of boron trifluoride etherate in 25 ml. of dry ether was added dropwise in an atmosphere of nitrogen a solution of 410 mg. of sodium borohydride in 25 ml. of diglyme. After 1 hr. at room temperature, water was added carefully to destroy the excess reagent followed by a solution of 4.4 g. of sodium dichromate and 3 ml. of sulfuric acid in 17 ml. of water. The mixture was stirred for 90 min. at room temperature and diluted with water, the product was extracted with ether, and the residue was purified by chromatography on alumina (125 g.) by elution with benzene. After recrystallization from methanol–water the ketone (1.5 g.) exhibited m.p. 87–89°, which was raised to m.p. 90–90.5° upon further recrystallization, $[\alpha]D$ −28° (*c*, 0.5).

Anal. Calcd. for $C_{19}H_{30}O$: C, 83.15; H, 11.02. Found: C, 82.97; H, 11.07.

3α-d₁-Cholestan-6-one (IV). To a suspension of lithium aluminum deuteride (40 mg.) in 5 ml. of dry ether was added 120 mg. of cholestan-3β-ol-6-one tosylate (IIIb).[14] After heating under reflux for 1 hr., the mixture was cooled to 0°, the excess deuteride was decomposed with saturated sodium sulfate solution, and the residual moisture was removed by drying with anhydrous magnesium sulfate. The inorganic residue was filtered and washed several times with ether. The combined filtrate was then evaporated to dryness and the colorless oil was dissolved in 4 ml. of pure acetone, cooled to 0° in an atmosphere of nitrogen, and treated dropwise with 8 *N* chromic acid solution[15] until a yellow color persisted. Dilution with water, extraction with ether, and recrystallization of the crude product (m.p. 97–100°) from acetone gave 70 mg. of the pure labeled ketone IV, m.p. 100–101°.

Cholestane-3,6-dione 6-Ethylene Ketal (VIIb). A mixture of 3.0 g. of cholestan-3β-ol-6-one (IIIa), benzene (250 ml.), ethylene glycol (20 ml.), and *p*-toluenesulfonic acid monohydrate (0.5 g.) was heated under reflux for 13 hr. using a water separator. After cooling, pyridine (0.25 ml.) was added, the mixture was washed several times with water, and the benzene layer was dried over sodium sulfate and evaporated to dryness. The residual hydroxy ketal VIIa could not be crystallized readily and was, therefore, oxidized directly by dissolving it in pyridine (10 ml.) and adding the solution at 0° to a slurry of 3.0 g. of chromium trioxide in 20 ml. of pyridine.[16] The mixture was stirred vigorously for 1 hr. at 0° and then allowed to stand undisturbed for an additional 20 hr. The dark suspension was diluted with ether and the solid material was removed by filtration. The filter cake was washed thoroughly with ether and the combined ether solutions were washed with water 15 times until

(33) H. Audier, J. Bottin, A. Diara, M. Fetizon, P. Foy, M. Golfier, and W. Vetter, *Bull. soc. chim. France*, 2292 (1964).
(34) Z. Pelah, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, 87, 574 (1965).
(35) All melting points were determined on the Kofler block. Unless noted otherwise, rotations were determined in chloroform solution and the neutral alumina for chromatography was Merck activity I. The mass spectra were determined under the conditions outlined in Table II using an ionization energy of 70 e.v. We are indebted to Dr. Alan M. Duffield, Dr. Dieter Becher, and Mr. John Smith for these measurements.

the odor of pyridine was barely detectable. Evaporation of the ether and recrystallization from methanol containing a trace of pyridine yielded 2.5 g. of fine needles, m.p. 114–116°. The analytical sample exhibited m.p. 118–119°, $[\alpha]_D$ +27° (*c*, 1.06), ν_{max}^{KBr} 1707 cm.$^{-1}$.

Anal. Calcd. for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 78.34; H, 11.03.

3β-d₁-Cholestan-6-one (V). To a suspension of lithium aluminum deuteride (100 mg.) in 20 ml. of dry ether was added 730 mg. of the ethylene ketal VIIb and the mixture was heated under reflux for 1 hr. The excess reagent was destroyed with saturated sodium sulfate solution and the inorganic salts were dissolved with 10% hydrochloric acid. The layers were separated and the ether phase was washed thoroughly with 10% hydrochloric acid (which sufficed for the cleavage of the ketal) and then water. Drying and evaporation of the solvent furnished *3α-d₁-cholestan-3β-ol-6-one* (VIIIa), m.p. 138–139°, which was transformed directly without further purification into the tosylate VIIIb by means of *p*-toluenesulfonyl chloride in pyridine solution. Recrystallization from acetone provided 425 mg. of the tosylate VIIIb, m.p. 166.5–169° (lit.[14] m.p. 169°).

A portion (93 mg.) of the tosylate was reduced with lithium aluminum hydride followed by chromic acid oxidation in the manner described above in the preparation of IV. Recrystallization from acetone led to 55 mg. of the required labeled ketone V, m.p. 99–100°.

3,3-d₂-Cholestan-6-one (VI). 3α-d₁-Cholestan-3β-ol-6-one tosylate (VIIIb) was reduced with lithium aluminum deuteride and the product oxidized with 8 *N* chromic acid as described above. Recrystallization from acetone gave in over 80% yield the doubly labeled ketone VI, m.p. 99–100°.

2α,3α-Oxidocholestan-6-one Ethylene Ketal (X). A mixture of 420 mg. of Δ²-cholesten-6-one (IXa),[14] benzene (80 ml.), ethylene glycol (5 ml.), and *p*-toluenesulfonic acid monohydrate (20 mg.) was heated for 20 hr. while attached to a water separator. The reaction mixture was then processed as described above for the ketal VIIa and the crude oily product IXb was dissolved in 15 ml. of dry ether and allowed to stand at room temperature in the dark for 3 days with *m*-chloroperbenzoic acid (300 mg.) (kindly supplied by FMC Corp., Carteret, N. J.). After washing with bicarbonate solution and water, the dried solution was evaporated and the residue was crystallized from methanol containing a few drops of pyridine, yield 470 mg., m.p. 122–125°. The analytical sample of the oxido ketal X exhibited m.p. 123–125°, $[\alpha]_D$ +41° (*c*, 0.96).

Anal. Calcd. for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 77.98; H, 10.93.

2β-d₁-Cholestan-6-one (XII). The reduction of 270 mg. of 2α,3α-oxidocholestan-6-one ethylene ketal (X) was effected in ether solution (1 hr. of reflux) with 90 mg. of lithium aluminum deuteride and the crude product was heated for 20 min. in 5 ml. of ethanol containing 3 drops of concentrated acid to achieve complete cleavage of the ketal grouping. Chromatography on 30 g. of alumina and elution with 3:1 benzene–acetone followed by recrystallization from dilute methanol afforded 151 mg. of *2β-d₁-cholestan-3α-ol-6-one* (XIa), whose melting point (159–161°) was not depressed when mixed with a specimen of the nondeuterated analog (lit.[17] m.p. 159–160.5°).

Conversion of the alcohol XIa (96 mg.) into the tosylate XIb (83 mg., m.p. 148–149°) (lit.[17] m.p. 145.5–147° for unlabeled tosylate) was accomplished in the usual manner with *p*-toluenesulfonyl chloride in pyridine (17 hr., 20°) and an 80-mg. portion of XIb was reduced with lithium aluminum hydride and reoxidized with chromium trioxide exactly as described for the conversion of IIIb to IV; yield (after recrystallization from acetone) 48 mg., m.p. 101–102°.

2α-d₁-Cholestan-6-one (XIV). Δ²-Cholesten-6-one ethylene ketal (225 mg., 0.53 mmole, IXb) in 5 ml. of dry tetrahydrofuran was added to an ice-cold solution of sodium borodeuteride (126 mg.) in dry diglyme (14 ml.) containing 105 mg. of boron trifluoride etherate and the mixture was kept at room temperature overnight. The intermediate alkylborane was oxidized by stirring the reaction mixture for 45 min. with 1 ml. each of 10% sodium hydroxide and 30% hydrogen peroxide, water was added, and the product was isolated with ether. The residual oil (238 mg.) was separated into four fractions by preparative thin layer chromatography on silica gel with benzene–ether (1:1) and each fraction was separately warmed with ethanolic hydrochloric acid to cleave the ketal grouping. The fraction containing the desired *2α-d₁-cholestan-3α-ol-6-one* (XIIIa) was identified by utilizing the unlabeled substance[17] as reference standard and was purified further by thin layer chromatography (1:1 benzene–ether on silica gel). After recrystallization from methanol, there was obtained 41 mg. of XIIIa, m.p. 151–158° (lit.[17] m.p. 159–160.5° for unlabeled compound), which judging by the melting point must still have been contaminated by another hydroxycholestan-6-one produced during the hydroboration.[36] The subsequent steps (tosylation (XIIIb), lithium aluminum hydride reduction, and reoxidation) were performed in the previously described manner to give, after recrystallization from acetone 2α-d₁-cholestan-6-one (XIV) of m.p. 95–97°.

2,2,4,4-d₄-Cholestan-6-one (XIX). A mixture of 250 mg. of cholestan-6β-ol-3-one (XVa), freshly distilled dihydropyran (266 mg.), benzene (60 ml.), and one crystal of *p*-toluenesulfonic acid was stirred overnight at room temperature, then poured into a saturated bicarbonate solution and extracted with ether. The oily tetrahydropyranyl ether XVb was heated under reflux for 5 hr. with 20 mg. of sodium, 20 ml. of O-*d* methanol, and 0.5 ml. of deuterium oxide. The solvent was removed and the exchange process was repeated twice more, the last time by extending the reflux time to 17 hr. The exchanged ketone XVIII was reduced with lithium aluminum hydride and then

(36) This probably explains, at least in part, the somewhat low value (46%) in the hydrogen transfer (see Table I) to the *m/e* 331 ion as compared to 60% (see also footnote *c* in Table I) calculated from the 2,2,4,4-*d₄*-cholestan-6-one (XIX). The most likely contaminant is 2β-*d₁*-cholestan-3β-ol-6-one, thus giving rise to 2β-*d₁*-cholestan-6-one (XII) which is known (Table I) not to contribute any deuterium in the transfer step. In a thin layer chromatogram its spot overlapped partially with that corresponding to the 3α-isomer XIIIa and it is quite conceivable that a complete separation had not been effected. The 3β-*d₁*-cholestan-2β-ol-6-one is excluded because during the lithium aluminum hydride reduction of its tosylate the product would have been an olefin (see Discussion).

transformed by the usual sequence (tosylation, lithium aluminum hydride reduction, acid cleavage of tetrahydropyranyl ether, and reoxidation with chromium trioxide) into 2,2,4,4-d_4-cholestan-6-one (XIX) of 50% isotopic purity (see Table I).

When cholestane-3,6-dione 6-ethylene ketal (VIIb) was exchanged three times in the same manner as the tetrahydropyranyl ether XVc, the isotopic composition of the resulting cholestan-6-one was 12% d_0, 20% d_1, 23% d_2, 33% d_3, and 12% d_4.

Mass Spectrometry in Structural and Stereochemical Problems. LXXXII.[1] A Study of the Fragmentation of Some Amaryllidaceae Alkaloids[2]

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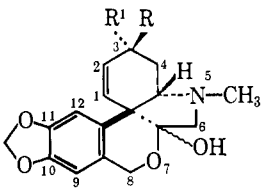
Contribution from the Departments of Chemistry, Stanford University, Stanford, California, and Iowa State University, Ames, Iowa. *Received July 6, 1965*

The mass spectra of 16 Amaryllidaceae alkaloids are reported. High-resolution mass spectrometry and deuterium labeling were used in the interpretation of many of the spectra. Of particular significance is the observation that minor changes in stereochemistry are frequently sufficient to cause appreciable differences in the mass spectra of many of the stereoisomers.

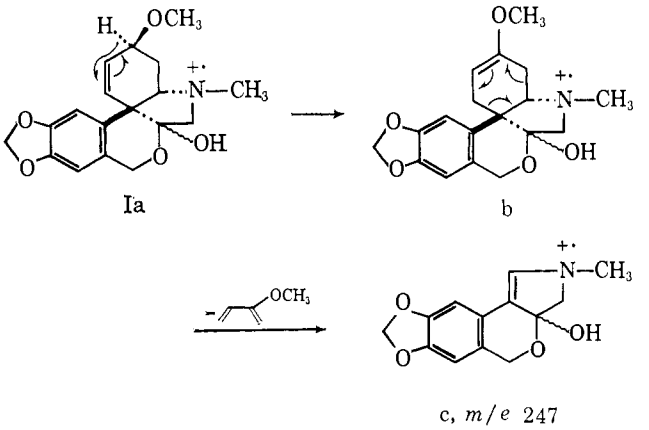
The Amaryllidaceae alkaloids constitute a large and chemically extensively investigated group of naturally occurring bases.[4] Although many classes of alkaloids have been subjected to mass spectral scrutiny[5] no detailed investigation of the Amaryllidaceae group has as yet been reported. It was felt that such a mass spectral survey was especially appropriate since extensive isolation and structure studies on novel Amaryllidaceae alkaloids are under way in various laboratories and mass spectrometry would be expected to play an important role in such investigations.[6]

Tazettine and Criwelline. Tazettine (I) and criwelline (II) differ only in the configuration of the methoxyl group at C-3, but this is sufficient to cause marked



I, R = OCH_3; R′ = H
II, R = H; R′ = OCH_3

variations in relative abundance of the ions in the spectra (Figures 1 and 2) of these alkaloids. The dominant ion in the spectrum (Figure 1) of tazettine occurs at mass 247 (M − 84), while a shift to *m/e* 250 is reflected in the spectrum of tazettine-N-d_3. High-resolution mass spectral measurements[7] demonstrated the homogeneity and composition of this ion as $C_{13}H_{13}NO_4^+$. The appearance of a metastable ion at mass 184.6 ($247^2/331$ = 184.6) established that at least a portion of this ion yield originated from a one-step decomposition of the molecular ion of tazettine (I). Such a scheme is depicted in Ia → b → c (*m/e* 247) in which, following an initial hydrogen transfer, ring C is fragmented by a retro-Diels–Alder process[8] with the formation of c.



Ia b c, *m/e* 247

(1) For paper LXXXI, see C. Djerassi, R. H. Shapiro and M. Vandewalle, *J. Am. Chem. Soc.*, **87**, 4892 (1965). The present paper also represents part XXVI of a series on the chemistry of Amaryllidaceae alkaloids.

(2) We are indebted to the U. S. Public Health Service (Grants No. GM-11309 and AM-04257 to Stanford University) and the National Science Foundation (Grant No. GP-253 to Iowa State University) for financial support. The purchase of the Atlas CH-4 mass spectrometer was made possible through NASA grant No. NsG 81-60.

(3) NASA Fellow 1964–1965 at Iowa State University.

(4) (a) W. C. Wildman in "The Alkaloids," Vol. VI, R. H. F. Manske, Ed., Academic Press Inc., New York, N. Y., 1960, p. 372, and references therein; (b) H. M. Fales and W. C. Wildman, *J. Am. Chem. Soc.*, 82, 3368 (1960); (c) H. -G. Boit, "Ergebnisse der Alkaloidchemie bis 1960," Akademie Verlag, Berlin, 1961, p. 410.

(5) See H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. I, Holden-Day, Inc., San Francisco, Calif., 1964.

(6) (a) Mass spectrometry has been employed recently in the structure elucidation of the Amaryllidaceae alkaloid amaryllisine by A. L. Burlingame, H. M. Fales, and R. J. Highet, *J. Am. Chem. Soc.*, **86**, 4976 (1964). (b) During the preparation of the present manuscript, we learned from Professor A. L. Burlingame of the University of California that he had performed high-resolution mass measurements on buphanisine (IV) and related bases ("Advances in Mass Spectrometry," Pergamon Press, London; Vol. III in preparation) with conclusions very similar to ours; see also K. L. Pering, M. S. Thesis, University of California, 1965.

(7) Determined by Dr. M. Barber using an A.E.I. double focussing MS-9 mass spectrometer.

(8) For full discussion and pertinent references, see H. Budzikiewicz, J. I. Brauman, and C. Djerassi, *Tetrahedron*, 21, 1855 (1965).