ISOTOPE LABELLING AND MASS SPECTROMETRY OF NATURAL PRODUCTS†

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INTRODUCTION

Mass spectrometry—as a tool in physical and chemical investigations—has been known for many years. Applications to structural problems in organic chemistry are of much more recent origin and this is especially true if we refer to natural products. Some examples of mass spectrometric studies in the steroid, terpenoid and alkaloid fields—with special reference to structural applications—were presented by me in a section lecture[1] at the second I.U.P.A.C. International Symposium on the Chemistry of Natural Products in Prague. The extent to which this area of mass spectrometry is now flourishing and the speed with which it is being accepted by natural product chemists is testified by the observation that in a recent book[2] dealing with the structure elucidation of alkaloids by mass spectrometry, only 8 per cent of all literature citations referred to papers which appeared prior to 1960, while nearly 50 per cent of the references came from the 1963 literature.

If mass spectrometry is to be exploited to its fullest extent, we should endeavour to reach that point of sophistication, which would permit a reasonable prediction about the principal fragmentation paths of a given molecule upon electron bombardment. This in turn implies that we have some knowledge about the breakdown behaviour triggered by the more common functional groups from studies with simple models, that this information is transferable to more complex situations—for instance substances where the functional group is inserted into a polycyclic framework—and that we can express the mechanistic conclusions in a fashion that is meaningful to the organic chemist. As pointed out in a recent book[3], we feel that at this early stage of development it is most profitable if we assume that the positive charge, produced when a single electron is removed from the molecule upon exposure to a stream of electrons, is localized at certain centres and that subsequent bond fissions are largely of the homolytic kind‡ and generally follow many of the standard concepts in physical organic chemistry. Thus tertiary carbonium ions or radicals will be energetically preferred over secondary and primary ones, allylic or benzylic activation will favour bond fissions, *etc.* Such an approach will usually be more effective when we are dealing with molecules possessing heteroatoms, where one of the non-bonding electrons is likely to be lost more readily,

† Paper (LVII) in the series "Mass Spectrometry in Structural and Stereochemical Problems." For preceding article see R. H. Shapiro and C. Djerassi. *Tetrahedron*, in press.

‡ We are following a convention outlined elsewhere (ref. 2, p. 2) that full arrows denote two-electron shifts, while "fishhooks" (⌒) express one-electron movements.

than in hydrocarbons, where the charge may be distributed over many different centres. The power of the heteroatom or other functionality (*e.g.* aromatic system) to "fix" the positive charge is often characterized by its ability to control the major fragmentation process of a molecule. As shown below, we find that an isolated carbonyl group is only moderately effective in governing the principal bond fissions, when placed in a hydrocarbon skeleton as found in the steroids, while an ethylene ketal grouping is much more powerful in that regard.

HIGH-RESOLUTION MASS SPECTROMETRY AND ISOTOPE LABELLING

We are thus faced with the problem of elucidating the mechanism of molecular fragmentation in the mass spectrometer or at least of being able to draw a consistent picture, which will aid us in the structure elucidation of unknown substances through an interpretation of their mass spectral fragmentation patterns. First and foremost, we must be in a position to determine which atoms of a molecule are retained in the charged fragment and which are lost in the neutral moiety. While there are many occasions where such a conclusion can be reached by simple consideration of the mass of the charged fragment, there are others—especially when heteroatoms are present—where this is not feasible because of the occurrence of isobaric fragments (*e.g.* CH_2 *v.* N). There are three ways of accomplishing this aim.

(a) Substituent labelling

Introduction of a substituent (*e.g.* methyl) will be effective, provided it does not affect the particular fragmentation under consideration. The presence or absence of the appropriate mass shift will then pinpoint the portion of the molecule retained in the fragment.

(b) Isotope labelling

As will be shown below, substitution of one of the constituent atoms by its isotope (usually D, ^{13}C, ^{15}N or ^{18}O) will offer the most meaningful information.

(c) High-resolution mass spectrometry

The resolving power[4] of commercially available high-resolution mass spectrometers is such that it is usually a simple matter to establish the empirical formula of a given ion. At times, such information is of considerable mechanistic utility and in that event, high-resolution mass spectrometry offers the simplest solution.

A very instructive example is the mass spectrum of pyrrolidone, which has been studied in detail in our laboratory[5] as a model for certain alkaloids. The spectrum displays a moderately intense peak at *m/e* 56, which could correspond to C_3H_4O (56·026213), C_3H_6N 56·050022) or C_2H_2NO (56·013637). High-resolution measurements with an MS-9 mass spectrometer† showed that all three ions were present in a ratio of 6 : 2 : 1, an

† These measurements were performed at the University of Liverpool through the co-operation of Professor G. W. Kenner.

observation which is of the utmost importance as it demonstrates that not one but three fragmentation routes are operative. These are outlined in *Figure 1*, using the conventions outlined previously[3] and assuming that the charge in the molecular ion is localized largely on oxygen (I) or nitrogen (II), followed by bond fission adjacent to the carbonyl carbon atom. It will be noted that of the three fragmentations, the preferred one (*A* in *Figure 1*) proceeds without hydrogen rearrangement, while transfer of one hydrogen atom is involved in processes *B* and *C*.

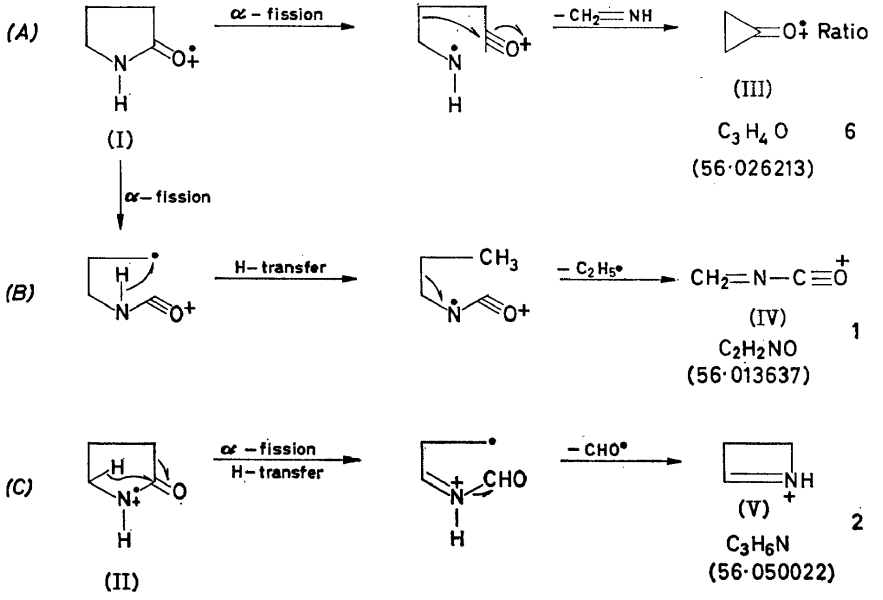


Figure 1. Multiple origin of *m/e* 56 peak in mass spectrum of pyrrolidone

The problem of the mechanism and the multiple origin of the *m/e* 56 ion in the pyrrolidone mass spectrum can also be attacked[5] by conventional-resolution mass spectrometry coupled with deuterium labelling. The hydrogen atom attached to nitrogen can be replaced by deuterium simply by equilibration with deuterium oxide in the mass spectrometer inlet system, while the C–3 hydrogen atoms adjacent to the carbonyl group can be equilibrated by heating pyrrolidone with deuterium oxide in the presence of potassium carbonate. Nearly 70 per cent of the peak remains unmoved after *N*-deuteration but is shifted by 2 mass units in the 3,3-d_2 derivative, thus demonstrating the predominance of path *A* (*Figure 1*). The minor contribution of path *C* can be demonstrated by the partial movement of the *m/e* 56 peak to *m/e* 57 in *N*-d_1 labelled pyrrolidone. The presence of small adjacent peaks around *m/e* 56 in the pyrrolidone mass spectrum does not permit an unequivocal demonstration of the presence or absence of small amounts of ion (IV), but this point could be settled with 5,5-d_2-pyrrolidone (no shift in (III), 2 mass unit shift in (IV), 1 mass unit shift in (V)).

We can conclude for this example, that while the most valuable mechanistic information would be derived from isotope labelling, since it also confirms the occurrence and origin of hydrogen transfers, the quickest approach is the single high-resolution measurement, which is required to demonstrate the multiple nature of the *m/e* 56 ion. The obvious recommendation in such a case, therefore, is to utilize both techniques for mechanistic purposes—high-resolution mass spectrometry to demonstrate the presence and composition of isobaric fragments, and deuterium labelling for a definition of the more intimate rearrangement processes.

A different conclusion about the relative merits of high-resolution data and deuterium labelling will be reached if one considers the mass spectrum (*Figure 2*) of 5α-androstan-1-one, a steroid which has been labelled[6] with deuterium in positions 2, 3, 4, 5, 6, 7, 16 and 19 (see asterisked positions in *Figure 2*). It was of some interest to determine the nature and origin of the important *m/e* 203 fragment in its mass spectrum (*Figure 2*). High-resolution measurements† demonstrated the apparent homogeneity (98 per cent $C_{15}H_{23}$) of this fragment. While the conclusion is correct in terms of empirical composition, it is misleading to assume that only one species is responsible for it.

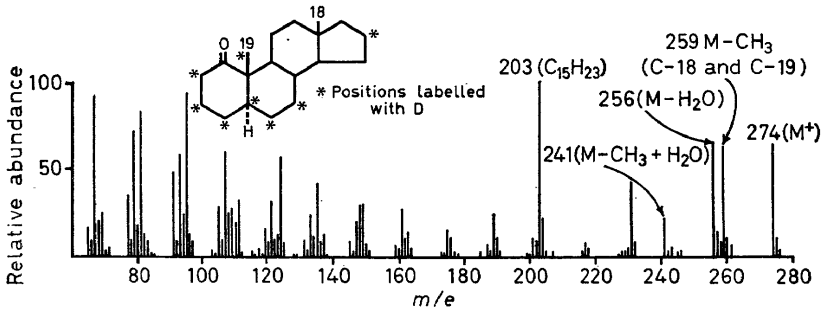


Figure 2. Mass spectrum of 5α-androstan-1-one

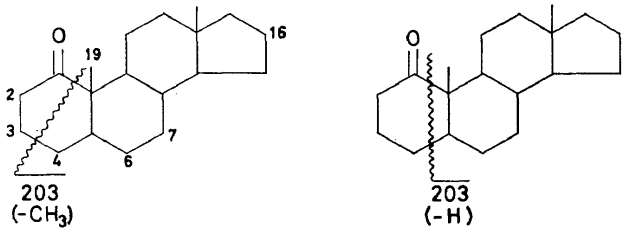
The deuterium labelling results, summarized in *Figure 3*, show that the *m/e* 203 peak is a composite one, approximately 30 per cent containing C–4 and 55 per cent losing this carbon atom.‡ Since the ion does not contain oxygen or carbon atoms 2 and 3, the two major cleavages may be indicated schematically by the wavy lines in *Figure 3*.

We can now examine in further detail (see *Figure 4*) possible mechanisms for the genesis of the two major contributors to the *m/e* 203 ion. If we assume intervention of a molecular ion (VI) in which one of the non-bonding electrons from oxygen has been removed, α-fission of the 1–10 bond would be expected to be a favoured process. Loss of carbon monoxide, ethylene

† Measured with an MS-9 mass spectrometer through the courtesy of Dr R. M. Elliot of Associated Electrical Industries, Ltd., Manchester.

‡ An alternate explanation of the labelling data is that C–4 is retained in all of the *m/e* 203 fragment and that in 55 per cent of the species, both hydrogen atoms attached to C–4 are lost. Such an interpretation is very unlikely, since scission of the 3–4 bond would then be required in all cases and this in turn would necessitate the fission of no less than three bonds connected to the same carbon atom (C–4).

and a methyl group then leads to the *m/e* 203 ion (VIII), which is completely consistent with the labelling results. In particular, it should be noted that all of the C–18 but none of the C–19 methyl group is lost, which is reasonable, since C–10 in the intermediate (VIIa) is already a radical site



d_0	203 (98 % $C_{15}H_{23}$ by high-resolution M.S.)		
2,2-d_2	203		203
3-d_1	203		203
4,4-d_2	205 (30 %)	204 (15 %)	205 (55 %)
6,6-d_2	205		205
7-d_1	204		204
16-d_1	204		204
19-d_1	204		204

Figure 3. Shifts of *m/e* 203 peak of 5α-androstan-1-one upon deuteriation

The data in *Figure 3* showed that 55 per cent of the *m/e* 203 ion arises from fission of the 1-10 and 4-5 bonds together with transfer of one hydrogen atom from the charged portion of the molecule. Deuterium labelling at C–5 demonstrated[6] that approximately 40 per cent of that hydrogen arises from C–5, which implies the operation of the process VI → (VIIb) → (IX) → (X)

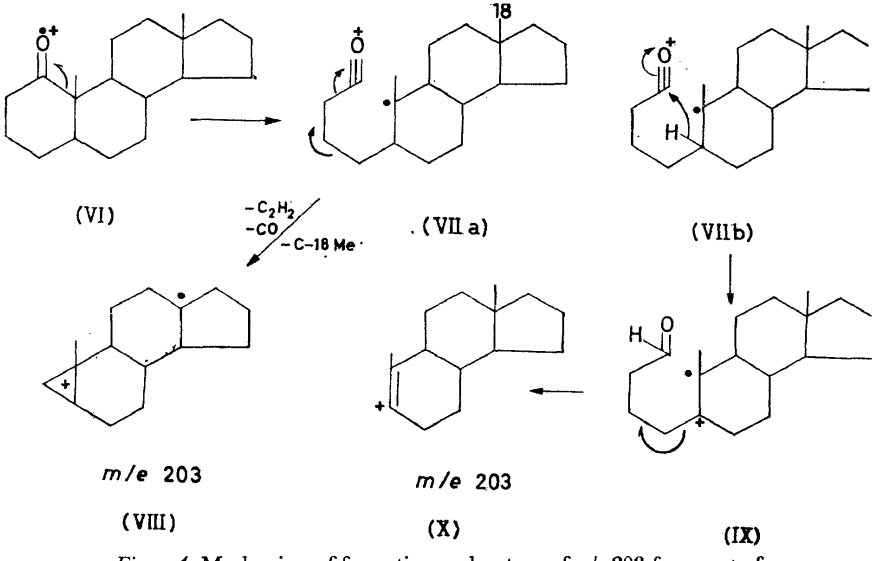


Figure 4. Mechanism of formation and nature of *m/e* 203 fragment of 5α-androstan-1-one

(*Figure 4*). Intermediate (IX), produced through hydrogen transfer in a six-membered transition (VIIb), is a favoured species, since it contains an "ionized double bond"[7]; the further cleavage ((IX) → (X) in *Figure 4*) of the 4-5 bond is less obvious, as it formally represents fission of a vinylic bond. Evidence from other related studies[8] seems to indicate that such a bond fission may not be infrequent.

Other instances where the composite nature of an ion is only detectable by isotope labelling but not by high-resolution mass spectrometry, have been encountered in a recent study[9] of the mass spectrometric behaviour of alicyclic amines.

DEUTERIUM LABELLING

Even the few examples cited should suffice to demonstrate that, for the purpose of gaining proper insight into the fragmentation mechanism of a given substance, isotope labelling is indispensable and clearly the method of choice. Of the various isotopes, deuterium is by far the most useful one, although supplementary or confirmatory information should at times be secured through the use of ^{18}O, ^{13}C or ^{15}N. The two greatest advantages of deuterium labelling are the following: (*a*) wide choice of reagents and methods for the introduction of deuterium[10]; (*b*) deuterium will not only serve as a "label" for certain portions of the molecule, but at the same time it will yield valuable information about the occurrence and origin of hydrogen transfer reactions which are of major mechanistic significance. During the past two years, an extensive programme of deuterium labelling has been undertaken in our laboratory—using simple model compounds[9, 11] as well as complex natural products. Some examples from this latter work will now be presented.

One of the most common features[12] in the mass spectra of C–17 substituted steroids is the loss of ring *D* (carbon atoms 15, 16 and 17) together with one hydrogen atom, the most likely candidates for the transferred hydrogen being those attached[13, 14] to positions 8, 12 or 18; plausible mechanisms are reproduced in *Figures 5* and *7* and it will be noted that in each instance primary ionization is postulated to occur through fission of the 13-17 bond with formation of a tertiary carbonium ion.

A priori, C–8 might be considered to be the most likely locus for the itinerant hydrogen atom (see *Figure 5*). Transfer from C–8 to C–17 would involve a six-membered intermediate (XI) and at the same time, a secondary radical (C–17) would be converted into a tertiary one (C–8). Homolysis of the 14-15 linkage (XII) would then lead to the stable allylic carbonium ion (XIII). For this reason, the synthesis of 8β-d_1-cholestane was undertaken[15] in the manner outlined in *Figure 6* but only a negligible transfer of the C–8 deuterium atom was observed.

A second alternative would be C–12, the hydrogen transfer ((XIV) → (XV), *Figure 7*) involving a seven-membered intermediate, A precedent for the intervention of medium-sized rings in some hydrogen transfer steps has been cited elsewhere[16]. The driving force for this first step would be the generation of an "ionized double bond"[7], while the final step ((XV) → (XVI), *Figure 7*) would then be the usual homolysis of the 14-15 linkage. An analogous sequence (XVII → XVIII → XIX, *Figure 7*) can be postulated

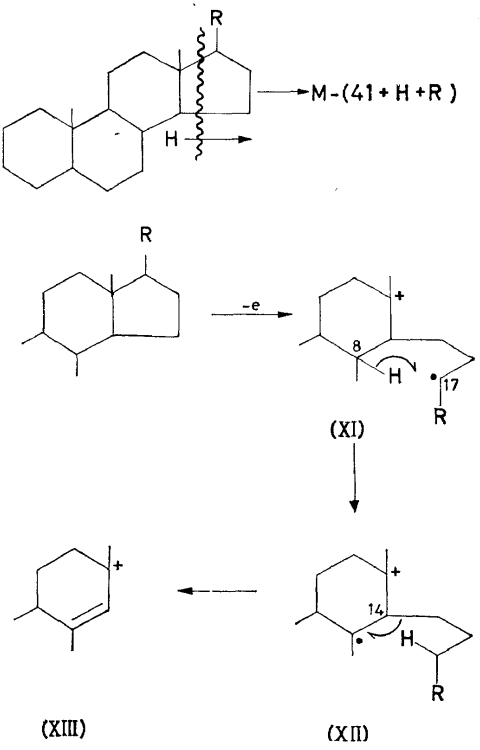


Figure 5. Possible participation of C–8 hydrogen atom in ring *D* fission of steroids

via transfer of the C–18 hydrogen atom. Migration of the C–14 hydrogen atom would be less likely, as this would require scission of two bonds connected to C–14 and the absence of such a transfer could be demonstrated through examination of the mass spectra of 14-d_1-cholestane[17] and 4,4,14α-trimethylcholestane (= lanostane), which still exhibited the usual loss of the side chain together with 42 mass units.

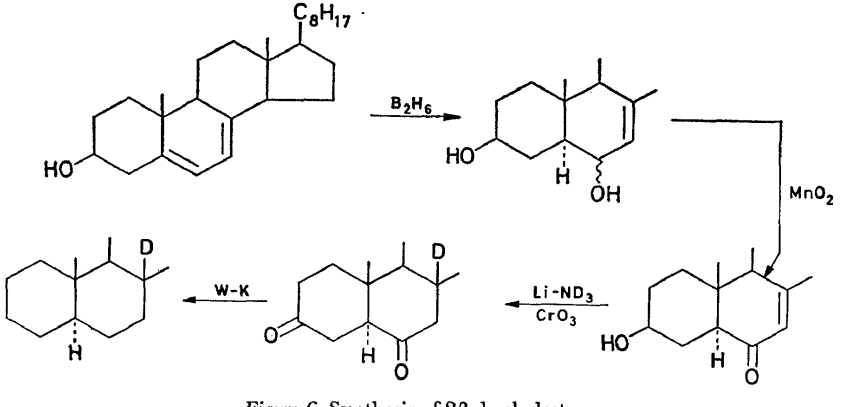


Figure 6. Synthesis of 8β-d_1-cholestane

Involvement of the C–12 hydrogen atom was excluded by the synthesis[18] of 12,12-d_2-5α-pregnane, so that by exclusion the hydrogen atom attached to C–18 (XVII, *Figure 7*) is implicated. Final confirmation through synthesis of 18-deuterated steroids is currently under way in our laboratory.

While the generation of an "ionized double bond"[7] is probably the major energetic driving force for this transfer, it is interesting to speculate why virtually all of it apparently originates from C–18, and practically none from C–12, even though the latter (XIV, *Figure 7*) represents the transfer of a secondary, rather than a primary, hydrogen atom. The most likely explanation is that the free-radical site at C–17 in (XVII) can come very close to the C–18 angular methyl group,† while this is not at all possible with respect to C–12.

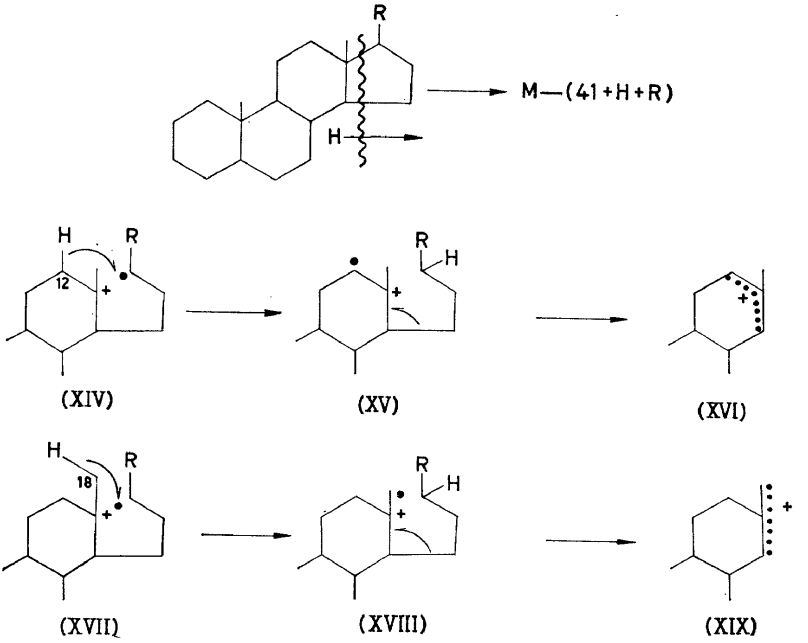


Figure 7. Possible participation of C–12 or C–18 hydrogen atoms in ring *D* fission of steroids

I should now like to turn to another type of hydrogen-transfer reaction, which has been studied in some detail through deuterium labelling and which is of considerable mechanistic importance. I refer to the migration of a γ-hydrogen atom to a carbonyl oxygen with associated β-cleavage—a reaction first postulated by McLafferty[19] and most conveniently visualized[3] through a molecular ion (XX, *Figure 8*) in which the positive charge resides on the oxygen atom. The virtually exclusive involvement of hydrogen in the γ rather than other positions has been demonstrated in 5,5-d_2-hexan-2-one[20], in deuterated long-chain fatty acid methyl esters[21] and

† Equal proximity is feasible with respect to C–7, but there is no obvious driving force for a C–7 → C–17 hydrogen transfer, and this possibility was eliminated by appropriate deuterium labelling.

in ethyl 4,4,4-d_3-butyrate[22]. Finally, by examining the mass spectra[23] of methyl 4-d_1-, 4,4-d_2-, and 4,4,4-d_3-butyrate, it was possible to show that a significant isotope effect occurs in this transfer to the extent of 0·88 atom of deuterium per atom of hydrogen. The question now arises whether such a γ-hydrogen transfer is equally operative in more complex molecules, especially where the carbonyl group forms part of a ring.

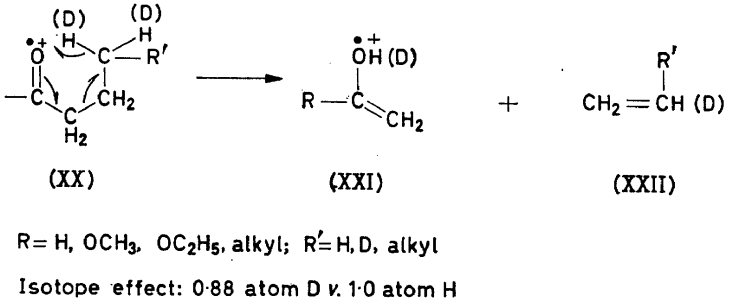


Figure 8. Transfer of γ-hydrogen atom with associated β-cleavage in carbonyl compounds

A relevant case from the alkaloid field is the striking difference in the principal fragmentation of the oxindoles and their pseudoindoxyl isomers. Through substituent and deuterium labelling, it has been shown[24] that the most abundant ion (*m/e* 223) in the mass spectrum of mitraphylline (XXIII, *Figure 9*) may be depicted as (XXIV), no hydrogen transfer being associated with its genesis. In pseudoindoxyls (*e.g.* (XXVI)), on the other hand, the most important peak occurs[25] at *m/e* 222 and thus serves as a convenient criterion of differentiation between isomeric oxindoles (*e.g.* (XXIII)) and pseudoindoxyls (*e.g.* (XXVI)). By labelling rings *A* and *E* with substituents and positions 3, 5, 6 and 14 with deuterium, it was possible to demonstrate[25] unambiguously that the *m/e* 222 ion of ajmalicine pseudoindoxyl (XXVI) encompasses the same atoms as the *m/e* 223 fragment (XXIV) of mitraphylline (XXIII) with the exception of the C–14 hydrogen atom which is transferred in the pseudoindoxyls. By assuming initial localization of the positive charge in the molecular ion at N_b (XXIII, XXVI, *Figure 9*) homolytic fission of the 5-6 bond then becomes a very favourable process because of stabilization with the electrons on nitrogen. The resultant intermediate (XXVII) in the pseudoindoxyl can then undergo transfer of the C–14 hydrogen atom to the carbonyl oxygen in a six-membered transition—a supposition which is confirmed by the appropriate deuterium labels.

The remaining examples will be selected from the steroid field for two reasons. First, ketonic steroids are very common and it is of obvious interest to determine whether hydrogen transfers of the type outlined in *Figure 8* do occur. Second, extensive deuterium labelling of keto steroids in our laboratory has shown that the distance between the carbonyl oxygen and the departing hydrogen atom may play a crucial role.

In 16-keto steroids, such as cholestan-16-one (XXX, *Figure 10*), one of the most significant peaks occurs at *m/e* 259 and is associated with the loss of the side chain, the C–18 angular methyl group and one additional hydrogen

atom. By labelling all of the positions in the side chain with deuterium, it could be shown[16] that only the hydrogen atoms at C–22 were involved in the rearrangement step (XXX → XXXI, *Figure 10*). This constitutes an instructive example[26] of the preference for transfer of a secondary over a primary hydrogen atom, since the C–21 hydrogen atoms were not implicated.

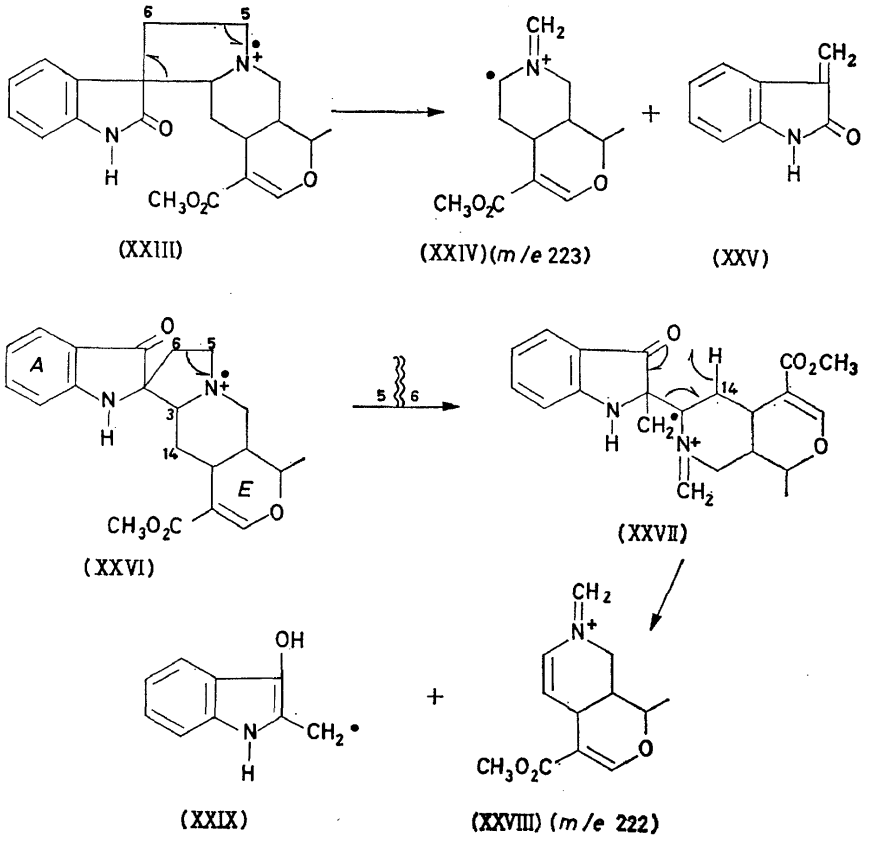


Figure 9. Mechanism of principal fragmentation in mitraphylline (I) and ajmalicine pseudoindoxyl (IV)

In stark contrast to the very specific hydrogen transfer *via* a six-membered intermediate observed among 16-keto steroids (*Figure 10*) is the situation encountered[27] among 11-keto steroids. The base peak in the mass spectrum[28] of 5α-androstan-11-one occurs at *m/e* 164 due to rupture of the 6-7 and 9-10 linkages. While this implies no *net* transfer of hydrogen, extensive deuterium labelling[27] has demonstrated that in fact reciprocal hydrogen transfers to and from the charged species are prevalent. The occurrence of such reciprocal rearrangements, which are of the utmost mechanistic significance, can only be detected by isotope labelling. Since the labelling results[27] demonstrated the loss of the C–8 hydrogen atom from the charged fragment, it seemed likely that the required gain of hydrogen might occur

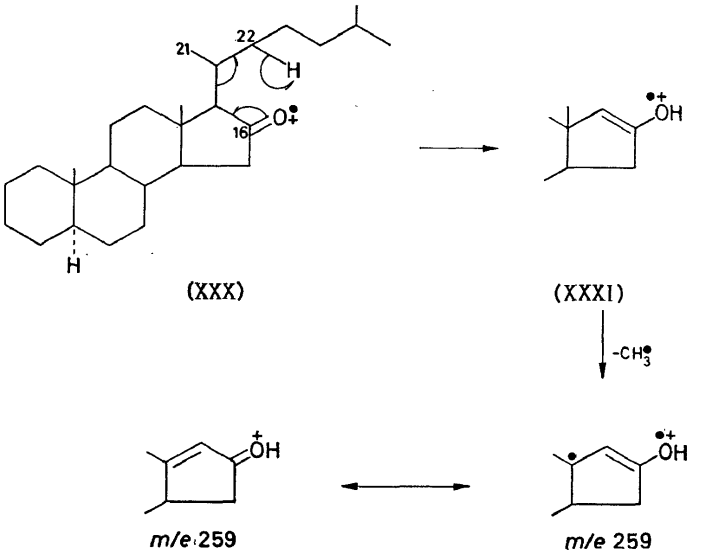


Figure 10. Mechanism of formation and nature of *m/e* 259 fragment of cholestan-16-one

through the usual (see *Figure 8*) six-membered intermediate, in which case C–1 (XXXII → XXXIII, *Figure 11*) or C–19 (XXXIV → XXXV, *Figure 11*) would be the only candidates. In point of fact, labelling[27] of all pertinent positions (1, 2, 3, 4, 5, 6, 19) with deuterium demonstrated that C–1 (XXXII, *Figure 11*) was not at all implicated and C–19 (XXXVI) to only a small extent. Random hydrogen transfer from all other positions was

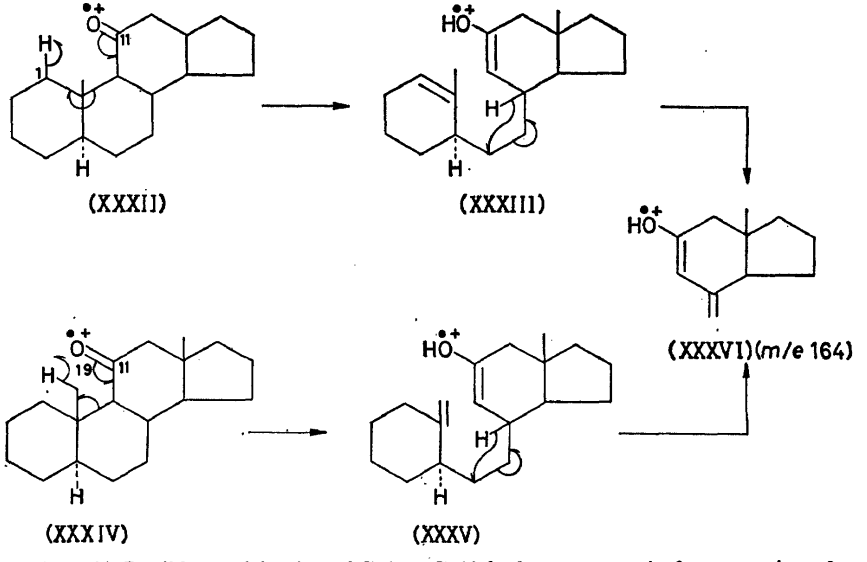
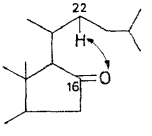
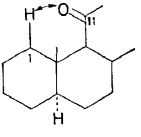
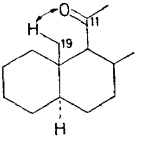
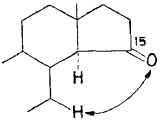
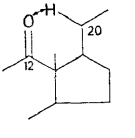


Figure 11. Possible participation of C–1 or C–19 hydrogen atoms in fragmentation of 5α-androstan-11-one

shown to occur, which suggests strongly that 9-10 bond fission is not triggered by the γ-hydrogen transfer with associated β-cleavage (*Figure 8*).

The question now arises whether the apparent anomaly in 11-keto steroids is *sui generis* or of a more general nature. In the aliphatic carbonyl compounds (*Figure 8*), where rearrangement of the γ-hydrogen atom to the carbonyl oxygen had been demonstrated[20-23] originally, there exists no major barrier to close approach of the departing hydrogen to the receptor oxygen. In cyclic systems, the situation is quite distinct. In 16-keto steroids (*Figure 10*), where transfer of the C–22 hydrogen atom to oxygen had been demonstrated[16], the minimum distance between these two atoms is 1·5 Å (see *Table 1*). In 11-keto steroids, this distance (*Table 1*) increases to

Table 1

H⟵O	*Distance* (Å)
	1·5
	1·8
	2·2
	2·3
	3·0

1·8 Å–2·2 Å, depending whether the 1β- (XXXII, *Figure 11*) or 19- (XXXV), *Figure 11*) hydrogen atom is considered. It is quite conceivable that such a value already exceeds the permissible limit for hydrogen rearrangement.

In order to examine this point more closely, 7β-d_1-5α-androstan-15-one was synthesized[29] by the procedure outlined in *Figure 12*. The reason for the selection of this substance was that the most abundant ion in the mass spectrum[29] of 5α-androstan-15-one occurs at *m/e* 97 (*Figure 13*) and that its

formation could well be associated with the transfer of the 7β-hydrogen atom through the standard (*Figure 8*) six-membered intermediate. It should be noted (*Table 1*) that the minimum distance between the C–15 oxygen atom and the 7β-hydrogen atom is 2·3 Å, *i.e.* larger than that existing among 11-keto steroids (*Table 1*). In actual fact, only a very small amount of hydrogen transfer was noted[29] in the 7β-deutero analogue.

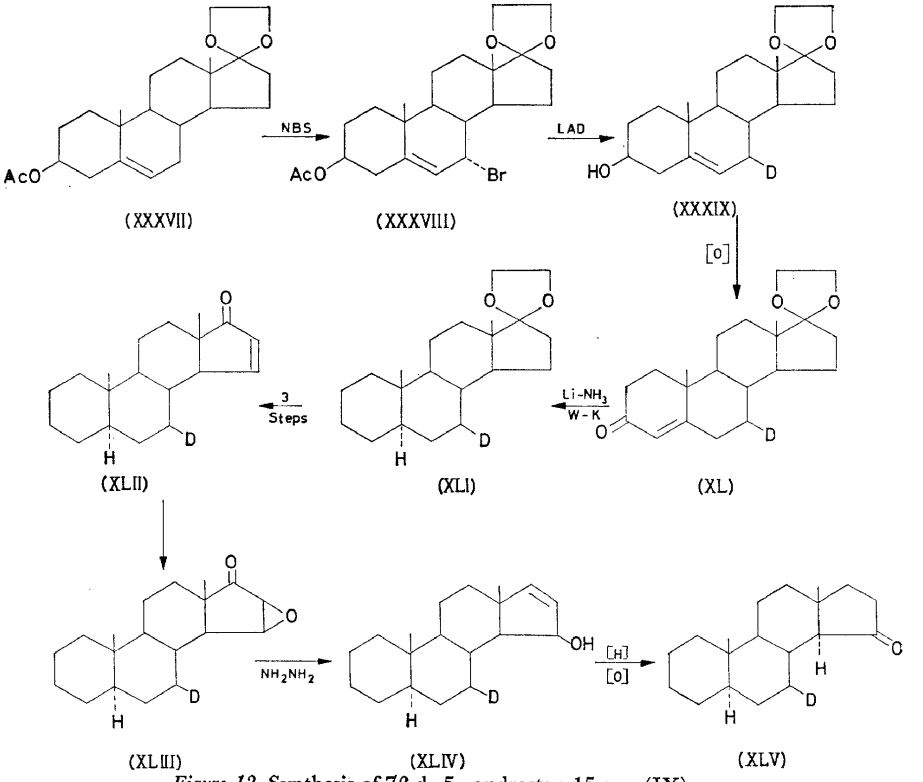


Figure 12. Synthesis of 7β-d_1-5α-androstan-15-one (IX)

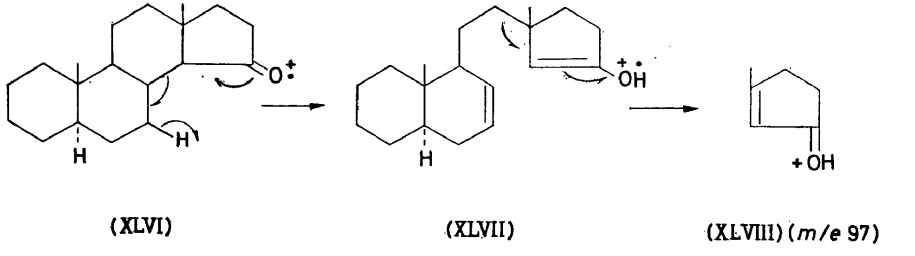


Figure 13. Possible participation of C–7 hydrogen atom in fragmentation of 5α-androstan-15-one

The above results indicate that the hydrogen–oxygen distance plays a very important rôle and that the limit for such a six-membered concerted collapse (*Figure 8*) is somewhere between 1·5–1·8 Å (*Table 1*). With this conclusion in mind, it is particularly instructive to examine some 12-keto

steroids. It has been noted earlier[28] that steroids, which possess a keto group at C–12 and a hydrogen atom at C–20, display in their mass spectra a very characteristic peak, which formally is associated with the loss of ring *D* (carbon atoms 15, 16, and 17) together with one hydrogen atom. It was suggested[28] that the migrating hydrogen originates from C–20 through a six-membered intermediate (XLIX → L, *Figure 14*), subsequent fission of the 14-15 bond being unexceptional. Thus in the mass spectrum[18] of 5α-pregnan-12-one (XLIX, *Figure 14*), the base peak occurs at *m/e* 233 (LI). Inspection of *Table 1* shows that the distance between the C–12 oxygen and the C–20 hydrogen atom is greater (3 Å) than that in any of the other ketones considered and that consequently, no transfer should occur. However, when C–20 was replaced by deuterium, it was found that at least 90 per cent of the deuterium did indeed migrate to the charge-retaining fragment[18]. We believe that this apparent anomaly can actually be rationalized in the light of our accumulated experience.

The concerted rearrangement mechanism (XLIX → L, *Figure 14*) is not accepted, because of the extraordinarily large interatomic distance between the C–20 hydrogen atom and the carbonyl oxygen. Rather,

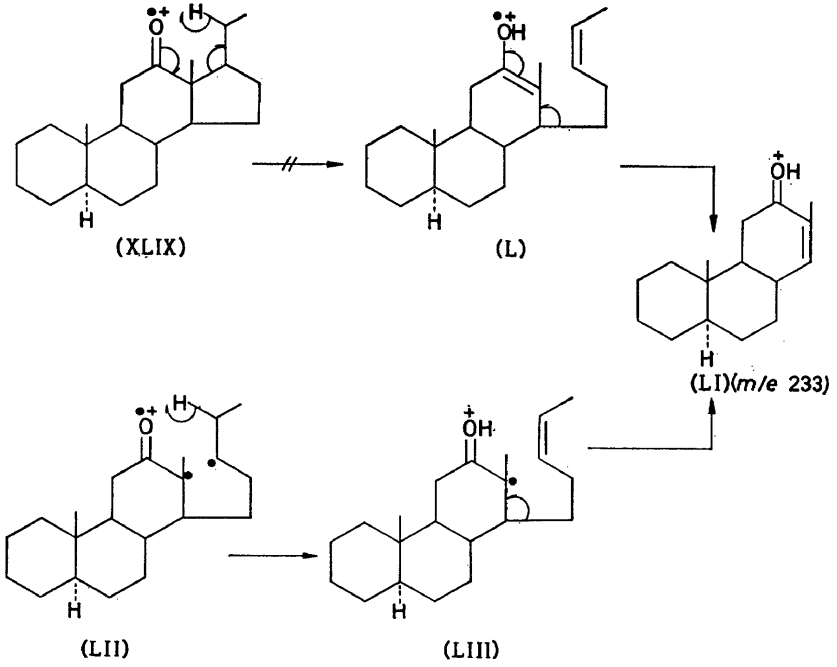


Figure 14. Mechanism of formation and nature of *m/e* 233 fragment of 5α-pregnan-12-one

knowing the propensity of C–17 substituted steroids for 13-17 bond cleavage (*Figures 5* and *7*), we propose homolysis of this bond in the molecular ion (LII, *Figure 14*), followed by transfer of the C–20 hydrogen atom, which is now easily possible since the hydrogen atom can closely approach the receptor oxygen in the ring-opened product. Homolytic scission of the 14-15

linkage (LIII) then completes the fragmentation process leading to the observed *m/e* 233 species (LI).

The few examples cited above illustrate how deuterium labelling among saturated steroid ketones has already led to a considerable refinement in our views of the mechanisms of certain key fragmentation processes. This statement is well supported from other recent studies with deuterium-labelled α,β-unsaturated steroid ketones and ethylene ketals.

For instance, the most intense and characteristic fragment of Δ^1-3-keto steroids after electron bombardment occurs at *m/e* 122 due to fission of the 6-7 and 9-10 bonds[30]. At first sight, no hydrogen transfer appears to be involved, but deuterium labelling[30] demonstrates that a reciprocal hydrogen rearrangement is operative with the hydrogens attached to C–5 and C–8 playing the dominant rôle. These results are easily accommodated in the schemes depicted in *Figure 15*, the primary step being homolysis of the 9-10 bond of the molecular ion (LIV). Migration of the C–8 hydrogen atom to C–10 (LV) results in the generation of an 8-9 double bond (XLXVI), which can now receive the second itinerant hydrogen from C–5 in a six-membered transition state to afford species LIX (*m/e* 122), which may well rearrange to the corresponding aromatic (xylenol) cation. The hydrogen migrations may also proceed in the alternate sequence (LVII → LVIII → LIX, *Figure 15*).

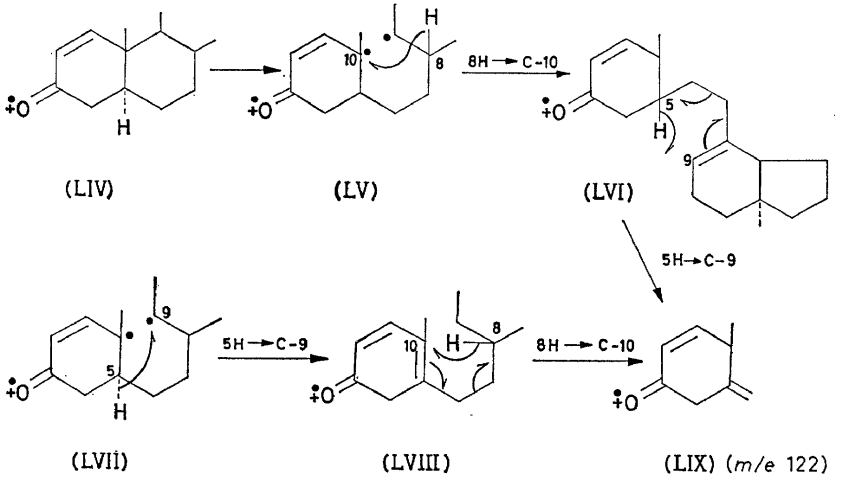


Figure 15. Mechanism of formation and nature of *m/e* 122 fragment of 5α-Δ^1-androsten-3-one

In the isomeric Δ^4-3-keto steroids, the dominant peak† occurs[31, 32] at *m/e* 124. The ruptured bonds (6-7 and 9-10) are identical with those in Δ^1-3-keto steroids (*Figure 15*), but in this case, two hydrogen atoms are transferred during fragmentation process to the charged species. The origin of the migrating hydrogens was demonstrated[30] by deuterium labelling to be C–8 and C–11, which is consistent with one or both of the sequences[30, 33] outlined in *Figure 16*. In each case, the driving force for

† The situation is more complicated in the presence of an 11-keto function (see ref. 30).

the two hydrogen rearrangement steps is the generation of a double bond or a six-membered transition state including one of the double bonds.

In the introduction of this lecture, it was pointed out that one of the important aims of modern mass spectrometry of organic substances is a detailed knowledge of the mechanisms of fragmentation processes. This

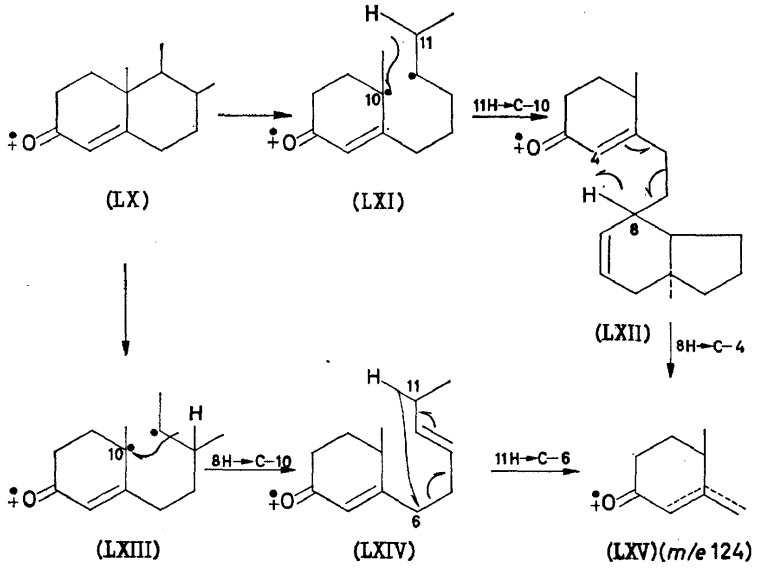


Figure 16. Mechanism of formation and nature of *m/e* 124 fragment of Δ^4-androsten-3-one

should not only aid in the rational interpretation of mass spectra, but also in predicting the influence of given substituents. Directly connected with this aim is the desire to introduce into a molecule substituents, which control the fragmentation process in such a manner that only a few intense peaks of diagnostic significance are produced. One of these functionalities is the ethylene ketal moiety[34, 35] and its dominant imprint upon the fragmentation pattern of the corresponding ketone can be gauged by comparing the mass spectrum[28] (*Figure 17a*) of 5α-androstan-3-one (LXVI) with that[34, 35] (*Figure 17b*) of its ethylene ketal (LXVII). The mass spectrum of the latter displays only three fragment ions (*m/e* 99, 112 and 125) of appreciable intensity, the genesis of each of them being caused by the initial stabilization of the positive charge around the ketal oxygen atoms.

The proposed mechanism[34–36] for the formation of the important *m/e* 99 (LXXI) and *m/e* 125 fragments is depicted in *Figure 18*. The reaction sequence is initiated by abstraction of one of the non-bonding electrons on oxygen followed by the well-known[3] α-fission, which in such an unsymmetrical molecule can proceed in two directions (LXIX or LXXII in *Figure 18*). In each instance, hydrogen migration then occurs, the driving force being the conversion of the primary radical into an allylic one. Subsequent homolysis (LXX → LXXI) then gives rise to the conjugated oxonium ion (LXXI) (*m/e* 99), while repetition of the hydrogen rearrangement (see LXXIV, *Figure 18*) eventually leads to the *m/e* 125 ion.

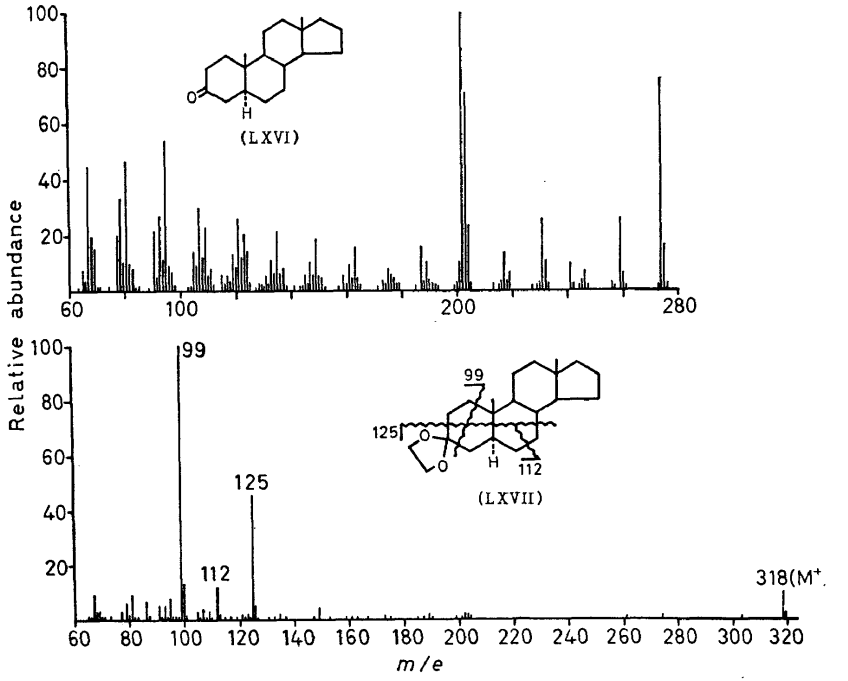


Figure 17. (*a*) Mass spectrum of 5α-androstan-3-one; (*b*) Mass spectrum of 5α-androstan-3-one ethylene ketal

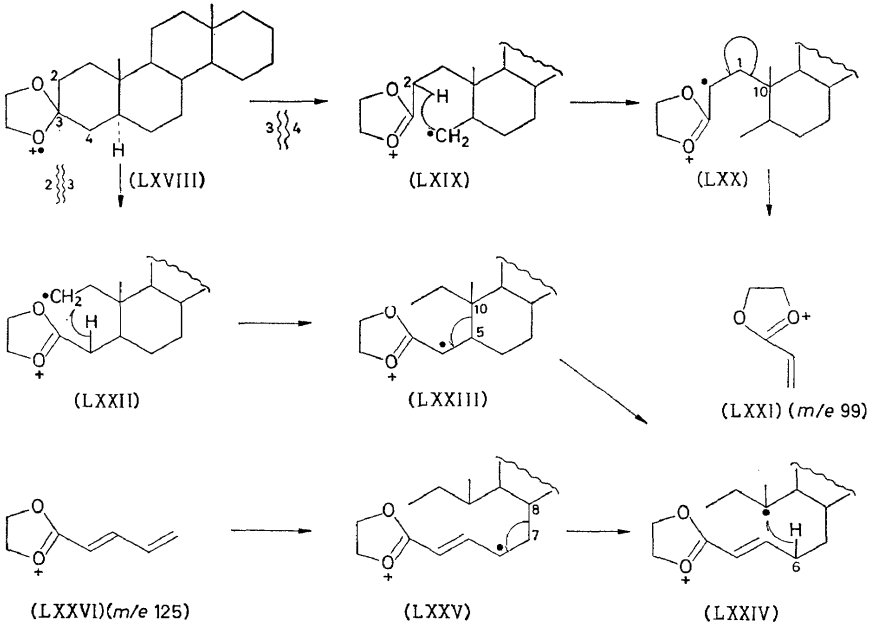


Figure 18. Mechanism of formation and nature of *m/e* 99 and *m/e* 125 fragments of 5α-androstan-one ethylene ketal

The correctness of the proposed mechanisms has been verified[34, 37] by labelling positions 2, 4 and 6 with deuterium and demonstrating the occurrence of the expected migrations (LXIX, LXXII, LXXIV, *Figure 18*) through the appropriate shifts in the mass spectra of the labelled analogues.

The simplification of the mass spectrum of a ketone upon conversion into its ketal has proved to be of considerable structural utility. For instance, it has been observed[37] that the mass spectrum of an ethylene ketal of a 3-keto steroid is not affected by the stereochemistry at C–5 nor the nature of the C–10 angular substituent—the main fragments still occurring at *m/e* 99 and *m/e* 125 (see *Figure 17b*). Consequently, it is a simple matter to determine the site of substitution upon monoalkylation of 3-keto steroids—a vexing problem notably in the 5β-series—by converting the product into the ethylene ketal and observing any movement of the *m/e* 99 (substitution at C–2) or the *m/e* 125 (substitution at C–4) peak.

The mechanism outlined in *Figure 18* for the genesis of the dominant *m/e* 99 peak requires the existence of the partial structure—$CO\text{-}CH_2\text{-}CH_2$— in the ketone. In point of fact, ethylene ketals of ketones of type —$CO\text{-}CH_2\text{-}CH(R)$— also exhibit such an *m/e* 99 ion, although of lower abundance. An example is provided by the mass spectrum[35] (*Figure 19*) of the ethylene ketal of 5α-androstan-7-one, in which the three most prominent peaks occur at *m/e* 125, 99 and 153. The production of the *m/e* 125 ion in *Figure 19* is completely analogous to that (see *Figure 18*) of the C–3 isomer and hence need not be discussed further. Of considerable interest, however, is the presence of the "forbidden" *m/e* 99 species.

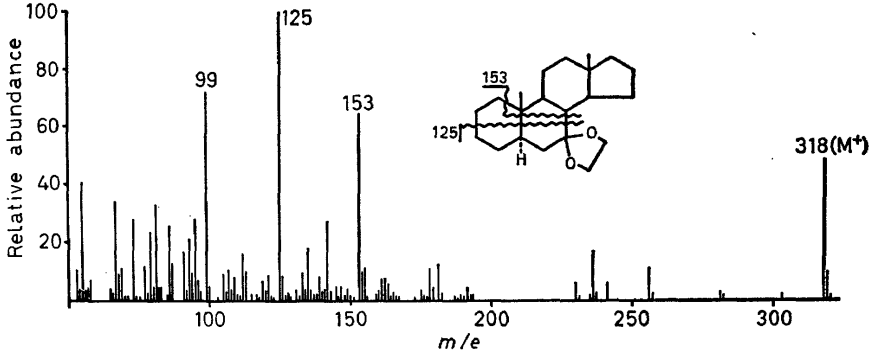


Figure 19. Mass spectrum of 5α-androstan-7-one ethylene ketal

The first hint came from the mass spectrum[37] of 5α-d_1-androstan-3β-ol-7-one ethylene ketal (LXXVII, *Figure 20*), which exhibited a shift of this peak to *m/e* 100, thus demonstrating that it was best represented by structure (LXXVIII). This requires migration of a hydrogen atom to C–5 and the origin of this itinerant atom became clear from the synthesis of 1α-d_1-cholestan-7-one ethylene ketal (LXXIX), the spectrum[37] of which displayed a partial movement from *m/e* 99 to *m/e* 100. The complete sequence for the genesis of the "forbidden" *m/e* 99 species (LXXXV) is reproduced in *Figure 20*, the steps from the molecular ion (LXXX) to the

intermediate (LXXXIII) being unexceptional, since they are completely analogous to those shown in *Figure 18, i.e.* hydrogen rearrangement (LXXXI) to convert primary (LXXXI) radical into allylic radical (LXXXII) followed by homolysis. The key step is the subsequent transfer of the C–1 hydrogen atom to C–5 through a six-membered intermediate with simultaneous formation of a 1-10 double bond. Final stabilization of the ion radical (LXXXIV) then occurs by homolysis of the 4-5 linkage and generation of the *m/e* 99 species.

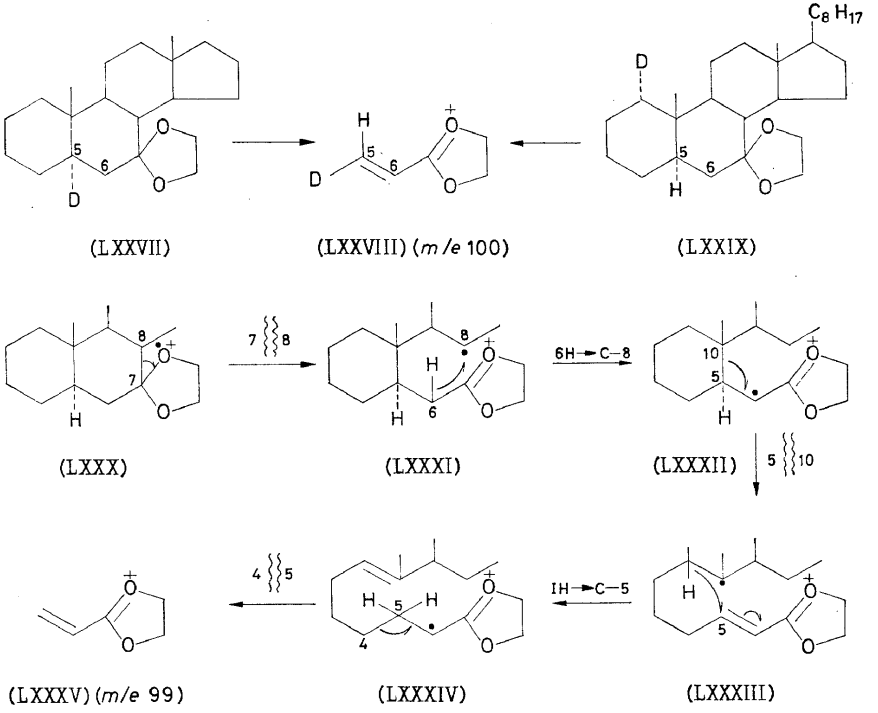


Figure 20. Mechanism of formation and nature of *m/e* 99 fragment of ethylene ketals of 5α-7-keto steroids

In summary, isotope labelling with deuterium is a powerful and frequently indispensable tool for the structure elucidation of mass spectrometric fragment ions and for gaining insight into the bond fission mechanisms which occur after electron bombardment. Fortunately, many convenient methods and reagents are available[10] for introducing deuterium into organic molecules so that it is not unreasonable to expect that deuterium labelling will soon become a *sine qua non* of modern mass spectrometric research.

I am deeply indebted to my associates listed in the bibliography for extensive synthetic studies without which this lecture could not have been presented. A special note of appreciation is due to Drs H. Budzikiwicz and D. H. Williams, who have been associated with many facets—both experimental and conceptual—of the work which I

have discussed. Indispensable financial assistance was provided by the National Institutes of Health of the U.S. Public Health Service.

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