

# APPLICATIONS OF MASS SPECTROMETRY† IN THE STEROID FIELD

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## ABSTRACT

Among the various physical methods enjoying wide use by organic chemists, mass spectrometry is the most recent and probably also the most spectacular addition. It is not surprising, therefore, that it has also found rapid acceptance in the steroid field. Indeed, many of the recent generalisations about mass spectral fragmentation mechanisms of organic molecules were first established by model studies in the steroid series.

The present lecture deals with the following aspects by employing androstanes, sterols and steroidal sapogenins as suitable illustrations: (a) Use of isotopically labelled steroids for elucidation of mass spectral fragmentation mechanisms. (b) Use of molecular ion peak determinations in the sterol field. (c) Use of specific and characteristic mass spectral fragmentation processes for structure determination of unknown steroids.

## INTRODUCTION

Mass spectrometry is the most recent physical method which has found wide acceptance in the steroid field. Whereas ten years ago it was difficult to find any papers in the steroid literature citing the use of mass spectrometry, it is equally difficult in 1969 to encounter articles dealing with steroid chemistry in which mass spectrometry is not at least used for analytical purposes and frequently for partial or even complete identification purposes.

The literature up to 1964 dealing with the role of mass spectrometry in the steroid field has been completely reviewed in a book from our laboratory<sup>1</sup> but since then a veritable flood of articles has appeared in which the use of mass spectrometry in the steroid area has been emphasized. Unfortunately, a summary or review of this research since 1964 is sorely lacking.

The scope of mass spectrometric applications among steroids can be well illustrated by simply selecting a few pertinent papers from the last two volumes (1968) of *Steroids*. In them, mass spectrometry was employed to determine the sterol composition of human faeces<sup>2</sup>, of algae<sup>3, 4</sup>, of human metabolites of the oral contraceptive norgestrel,<sup>5</sup> and to study the typical fragmentation patterns of such diverse steroid types as 3,6,20-trioxygenated pregnanes<sup>6</sup>, unsaturated ( $\Delta^4$  and  $\Delta^5$ ) ketals<sup>7</sup>, and various adrenal cortical steroids<sup>8</sup>. Another illustration of the actual and potential importance of mass spectrometry in the clinical area is afforded by the very substantial

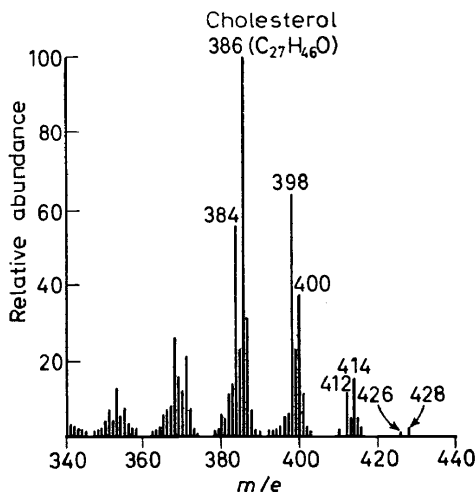
† Part 191 in the Stanford series 'Mass Spectrometry in Structural and Stereochemical Problems'.

number of articles<sup>9</sup> dealing with the combined use of gas liquid chromatography and mass spectrometry of trimethylsilyl derivatives of steroids.

Even this very cursory coverage of the contents of one single journal for one year yields a potpourri which defies adequate coverage within the confines of one lecture. Therefore, I have limited myself largely to a description of some recent research in our laboratory which illustrates the methodology and basis on which ultimately most applications of mass spectrometry in organic chemistry in general, and in the steroid field in particular, and must rest.

### DETERMINATION OF EMPIRICAL FORMULA

The best known and most easily comprehensible use of mass spectrometry pertains to the information extractable from the molecular ion region of a mass spectrum. Thus, high resolution mass spectral measurements will yield the precise empirical formula, which in the sterol field can never be obtained unambiguously by microanalysis (e.g. Calcd. for  $C_{27}H_{46}O$ : C, 83.87; H, 11.99. Calcd. for  $C_{28}H_{48}O$ : C, 83.93; H, 12.08). Even more striking is the fact that such information can be deduced with equal facility from an impure sample and that mass spectrometry is frequently the tool *par excellence* for the analysis of mixtures on a sub-milligram scale. A typical example is shown in *Figure 1*, which contains the high mass range of the spectrum



*Figure 1.* Partial mass spectrum (70 ev) of sterol mixture from marine source.

obtained on a sample of a crude sterol mixture from a marine organism<sup>10</sup>. The principal component is cholesterol (mol. wt. 386), but higher homologues with one (mol. wt. 400), two (mol. wt. 414) and even three (mol. wt. 428) methylene groups can be discerned easily as well as a similar homologous series of sterols possessing an additional degree of unsaturation (mol. wt. 384, 398, 412 and 426). Since fragments of mass 14( $CH_2$ ) or 16( $CH_4$  or O) are rarely ejected from molecular ions, it can be concluded safely that the peaks identified numerically in *Figure 1* are molecular ion peaks of separate

constituents of the mixture rather than fragment ions derived from one single parent ion (e.g. of mass 428).

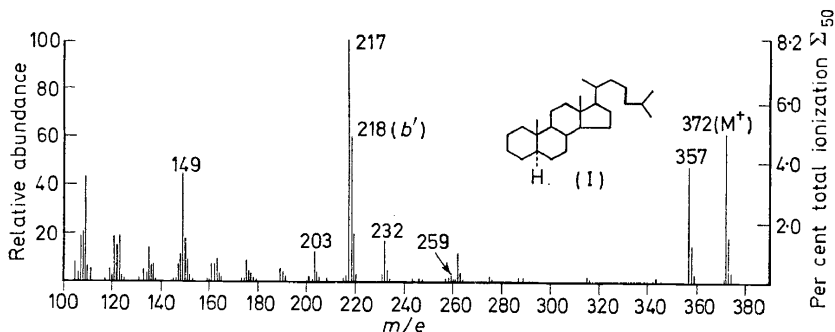
## STRUCTURE ELUCIDATION OF STEROIDS

Of greater relevance to the remainder of my lecture is the more sophisticated use of mass spectrometry, notably for the structure elucidation of unknown steroids. For such applications, it is indispensable that as firm a foundation as possible be laid for establishing the ground rules of mass spectral fragmentation mechanisms so that they may be employed with a fair degree of assurance in a predictive fashion when considering substances of partially or totally unknown constitution. The general approach and methodology to this problem in organic chemistry have been summarized by us in great detail<sup>11</sup> and the remainder of this presentation will be limited to three specific examples which are of fundamental importance in evaluating the mass spectral behaviour of more complicated or highly substituted steroids.

Clearly, if we wish to interpret the mass spectrum of a given steroid, we ought to know how the fundamental skeleton, unencumbered by heteroatomic substituents, behaves after electron impact. For historical reasons, 5 $\alpha$ -cholestane (I,R = C<sub>8</sub>H<sub>17</sub>) rather than 5 $\alpha$ -androstane (I,R = H) was first subjected to close scrutiny and as can be seen from *Figure 2*, aside from the M-CH<sub>3</sub> ion (*m/e* 357), the two most intense fragment peaks occur at *m/e* 217 and 218. The nature of the fragmentation sequences leading to these two ions, which happen to be of great diagnostic as well as mechanistic interest, has been completely elucidated<sup>12</sup> through extensive deuterium labelling of nearly all carbon atoms in either 5 $\alpha$ -cholestane (I,R = C<sub>8</sub>H<sub>17</sub>) or 5 $\alpha$ -pregnane (I,R = C<sub>2</sub>H<sub>5</sub>) and since this and earlier relevant work has been covered recently in great detail<sup>12,13</sup> only those facets will be mentioned briefly that are needed as background for the subsequent discussion.

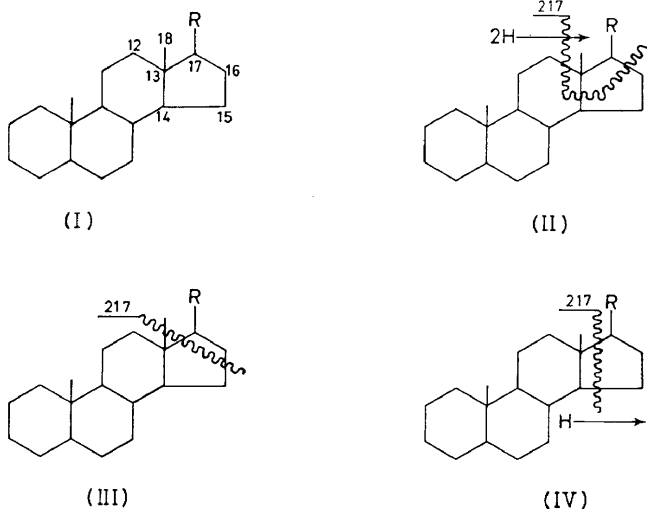
### Use of labelled steroids for elucidation of mass spectral fragmentation mechanisms

Initially, the origin of the *m/e* 217 peak in *Figure 2* was depicted graphically in terms of (II)<sup>14</sup>, and subsequently revised on intuitive grounds to (III)<sup>15</sup> and (IV)<sup>16</sup>. Aside from mechanistic differences, which are implicit in the



*Figure 2.* Mass spectrum (70 ev) of 5 $\alpha$ -cholestane.

requirements of two (II), zero (III) or one (IV) hydrogen transfers, there exists an even more fundamental distinction among these three fragmentation proposals which are of obvious consequence in structural work. Inspection of structures (II), (III) and (IV) will immediately show that different parts of the molecule are retained in the fragment ion. For instance, the ion of mass 217 produced by fragmentation (II) retains carbon atoms 15 and 16, whereas that generated by fragmentation (III) contains C-15, but not C-16. The species resulting from fragmentation (IV) retains neither one of these carbon atoms, but does contain the angular methyl group attached to C-13, whereas this substituent is lost in (II) and (III).

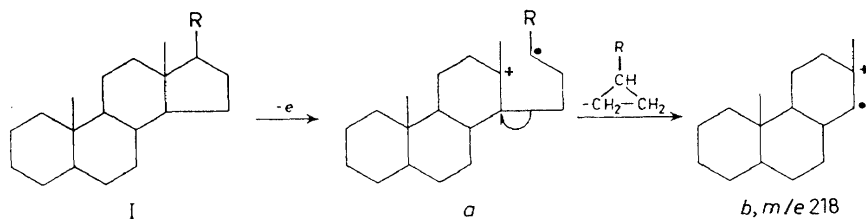


An unambiguous differentiation between these three proposals was accomplished<sup>12</sup> by isotopic labelling experiments, which demonstrated that (IV) constituted the correct schematic representation and that the hydrogen atom attached to C-14 was principally involved in the hydrogen transfer. The mechanistic implications of this observation have already been discussed elsewhere<sup>12, 13</sup> in detail and will not be repeated here. Suffice it to state that if an unknown cholestane derivative were encountered with a substituent at position 15, 16 or 18, totally incorrect structural deductions would be made on the basis of (II) and (III) if the mass spectrum of the unknown sterol possessed an intense peak at  $m/e$  217.

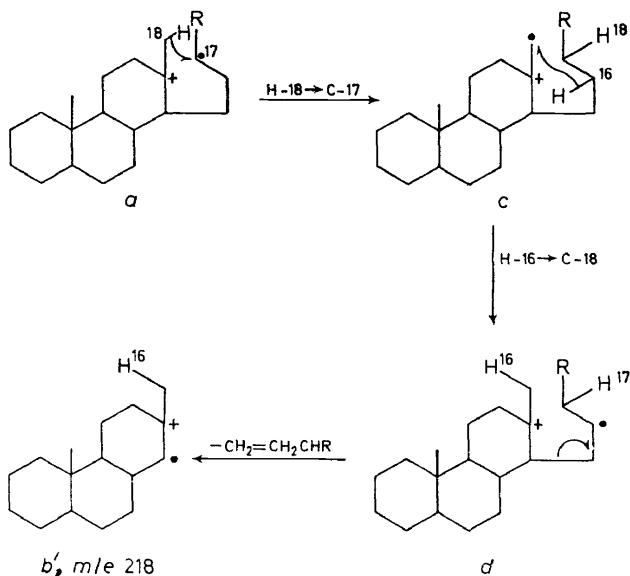
As noted in *Figure 2*, the second most intense fragment peak at 70  $eV$  (actually becoming the most intense one<sup>12</sup> at lower electron voltages) occurs at  $m/e$  218 and would appear to be most readily interpretable<sup>17</sup> in terms of the sequence  $I \rightarrow a \rightarrow b$ , the ejected neutral fragment being a substituted cyclopropane.

In point of fact, the deuterium labelling results<sup>12</sup> showed that while the ejected carbon atoms did indeed correspond to the postulated<sup>17</sup> sequence ( $I \rightarrow b$ ), the reaction path was actually more complicated and involved an exchange between the hydrogen atoms originally attached to C-16 and

C-18. Mechanistically, the sequence, therefore, needs to be rewritten in terms of  $a \rightarrow c \rightarrow d \rightarrow b'$ , the net consequence being that a neutral olefin rather than a cyclopropane is ejected. Such reciprocal hydrogen transfers, though



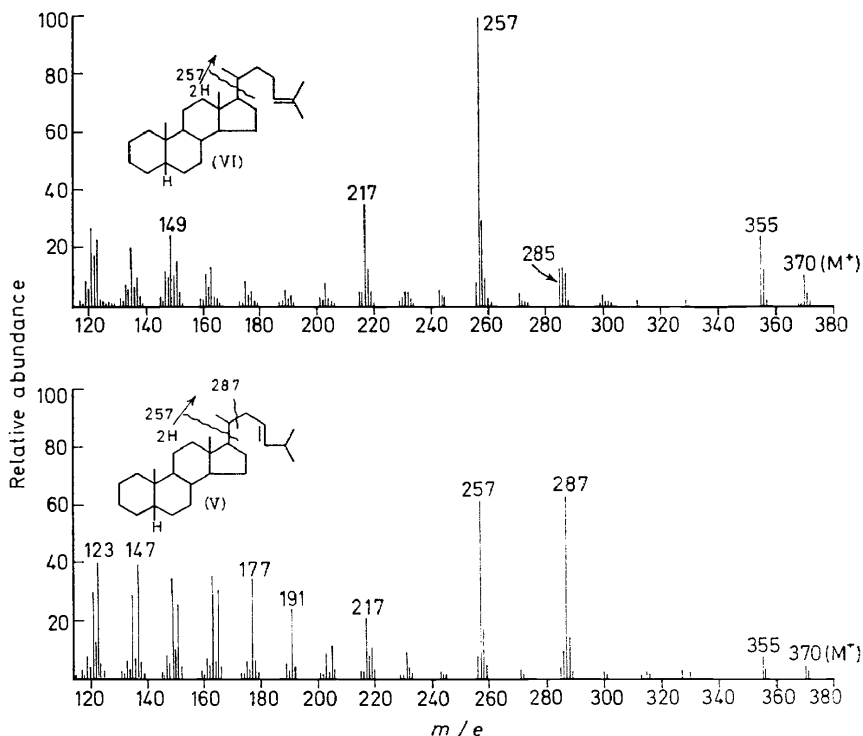
not causing misleading structural conclusions, are of considerable mechanistic interest and, as will be shown below in connection with the mass spectrum (Figure 5) of 5 $\alpha$ -androstande, they are of more common occurrence than might initially be imagined. Obviously, such hydrogen transfers can only be detected by deuterium labelling.



In summary, we are now justified in stating that the fragmentation leading to the intense  $m/e$  217 and 218 peaks in the mass spectra (e.g. Figure 2) of steroid hydrocarbons such as cholestane or pregnane can be very useful in determining the size of the side chain and the presence of substituents in ring D. However, it is necessary to introduce immediately an important reservation by pointing out that the presence of double bonds can drastically change the course of the main fragmentations. This is not unreasonable if we consider that in the saturated hydrocarbons (I), ionization will plausibly lead to a species such as *a* (tertiary carbonium ion and secondary radical), which is responsible for triggering the subsequent fragmentations, whereas

in the presence of unsaturated centres alternative ionized species might intervene, which could lead to completely different fragment ions. Two examples of this type will suffice.

The first refers to introduction of sites of unsaturation in the side chain. As shown in *Figure 3*, the presence of a  $\Delta^{23}$  (V) or  $\Delta^{24}$  (VI) double bond does not eliminate the ring D fragmentation leading to the  $m/e$  217 and 218 peaks, so typical (*Figure 2*) of cholestane, but an alternative bond fission



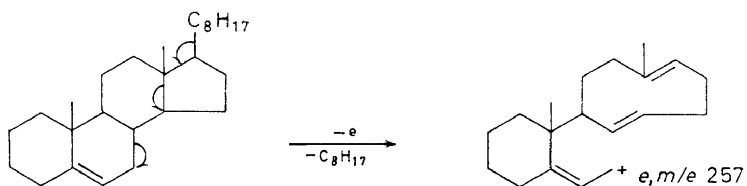
*Figure 3.* Mass spectra (70 eV) of  $\Delta^{23}$ -(V) and  $\Delta^{24}$ -(VI)  $5\beta$ -cholestenes.

leading to an ion of mass 257 now predominates. This cleavage results in loss of the side chain together with two hydrogen atoms, whose origin has been determined precisely by means of deuterium labelling<sup>18</sup>. As shown elsewhere<sup>18</sup>, this new process can be rationalized most readily by assuming initial ionization at the double bond site. The two double bond isomers themselves can be distinguished by the presence of another important bond fission in the  $\Delta^{23}$ -isomer (V), which is due to allylic fission (see  $m/e$  287 in *Figure 3*) and which is very small in the  $\Delta^{24}$  analogue (VI).

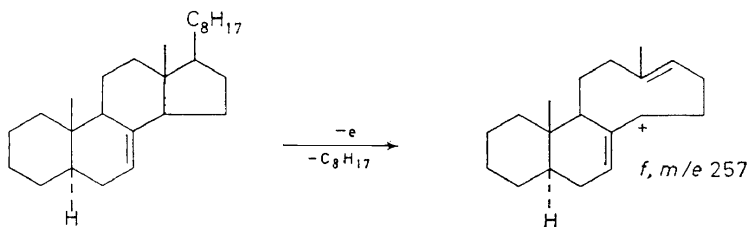
The second example refers to nuclear unsaturation and the mass spectra<sup>19</sup> of three pertinent examples, the isomeric  $\Delta^5$ -(VII),  $\Delta^7$ -(VIII) and  $\Delta^{14}$ -(IX) cholestenes are reproduced in *Figure 4*. Most notable is the appearance of a peak at  $m/e$  257 associated with the loss of the C-17 substituent—a bond fission which is essentially absent (see  $m/e$  259) in the mass spectrum (*Figure 2*) of cholestane (I, R =  $C_8H_{17}$ ). In the case of the  $\Delta^5$ -(VII) and  $\Delta^7$ -(VIII)

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isomers, such loss of the C-17 side chain can be easily visualized as being triggered by allylic fission of the nuclear double bonds leading to species *e* and *f* respectively.

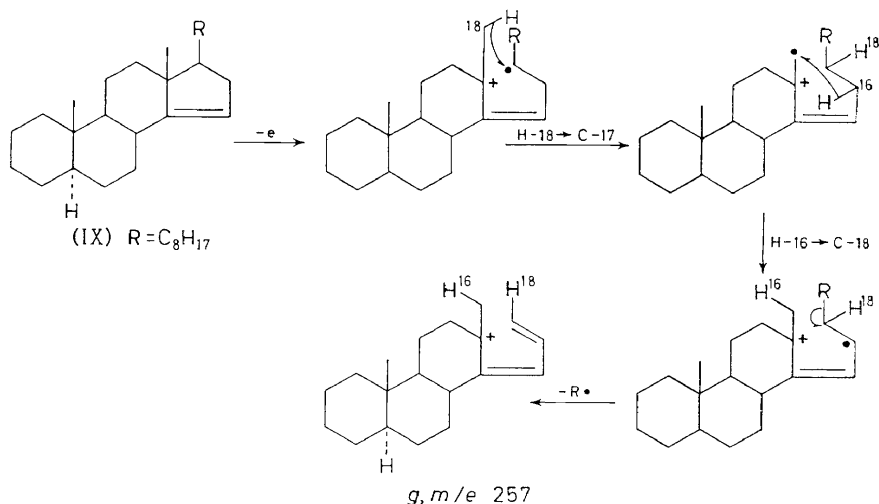


(VII)



(VIII)

Such a supposition is clearly not feasible in  $\Delta^{14}$ -cholestene (IX) and yet this is the substance where such side chain loss occurs to the greatest extent, virtually overshadowing all of the other fragment peaks and especially the molecular ion peak (see *Figure 4*). While deuterium labelling would not be of any assistance in this instance, it seems reasonable to propose the occurrence of a reciprocal hydrogen transfer between positions 16 and 18—



completely analogous to the sequence  $a \rightarrow c \rightarrow d \rightarrow b'$  employed previously to rationalize the genesis of the ion of mass 218 in cholestane—thus leading to the highly stabilized dienic tertiary carbonium ion g.

Another interesting feature of the three mass spectra reproduced in Figure 4 is the  $m/e$  215–216 group, which should correspond to  $m/e$  217–218

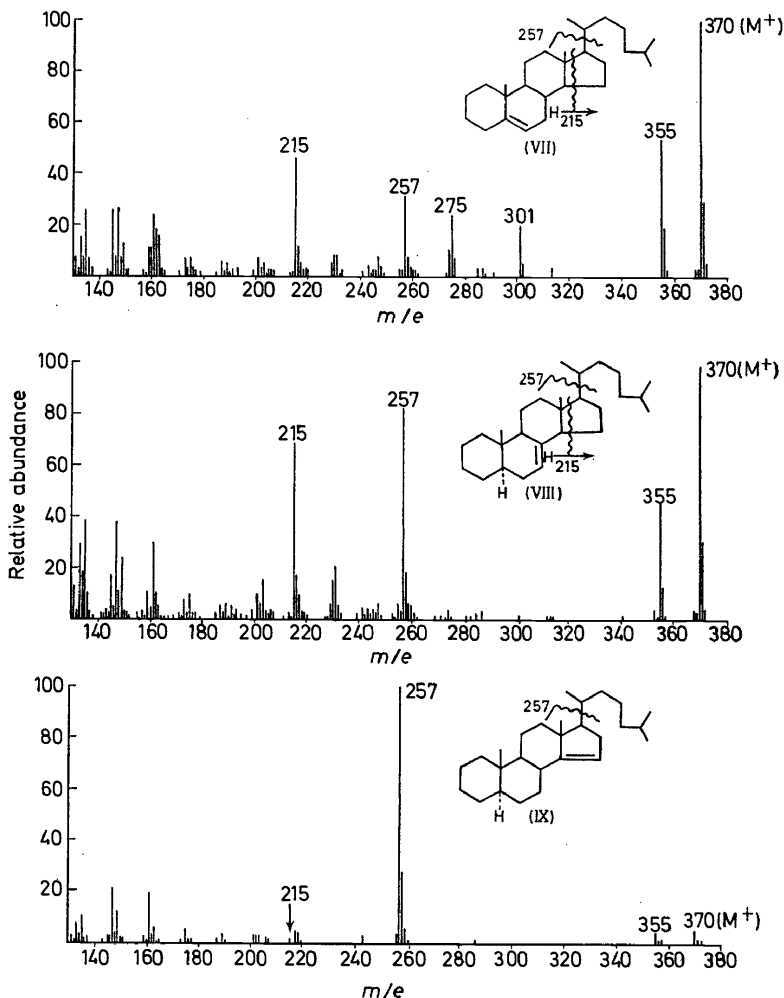


Figure 4. Mass spectra (70 eV) of  $\Delta^5$ -cholestene (VII),  $\Delta^7$ -5 $\alpha$ -cholestene (VIII) and  $\Delta^{14}$ -5 $\alpha$ -cholestene (IX).

in the mass spectrum (Figure 2) of cholestane (I). The complete absence of such peaks in the mass spectrum (Figure 4) of  $\Delta^{14}$ -cholestene (IX) is not surprising, since the double bond effectively blocks the loss of ring D. The  $m/e$  215 peak in (VII) and (VIII) probably corresponds schematically, if not mechanistically (no deuterium labelling has been performed in such unsaturated cholestenes), to the  $m/e$  217 peak in cholestane, but it will be

noted that in terms of per cent contribution to the total ionization, its abundance is considerably reduced. The counterpart ( $m/e$  216) of the  $m/e$  218 peak (ion *b*) in the cholestane spectrum (Figure 2) is virtually absent in the mass spectra (Figure 4) of the unsaturated cholestenes (VII) and (VIII) at both 70 and 12 ev. This is in marked contrast to the situation existing in cholestane, where the intensity of the  $m/e$  218 peak rises greatly upon lowering of the electron voltage.

In summary, it can be stated that the presence of a double bond markedly affects the mass spectrum and competes effectively with the 13–17 bond scission in the saturated C-17 substituted hydrocarbons (I,R = alkyl), such as cholestane and pregnane, in which the most significant fragmentations appear to be triggered by a molecular ion of type *a*, presumably because such 13–17 bond rupture relieves the strain inherent in a substituted *trans*-hydrindane system and at the same time leads to the most highly substituted ion radical possible in a steroid (tertiary carbonium ion and secondary radical site). If one now inspects the mass spectrum (Figure 5) of the C-17 unsubstituted hydrocarbon 5 $\alpha$ -androstane (I,R = H)<sup>20</sup> one encounters,

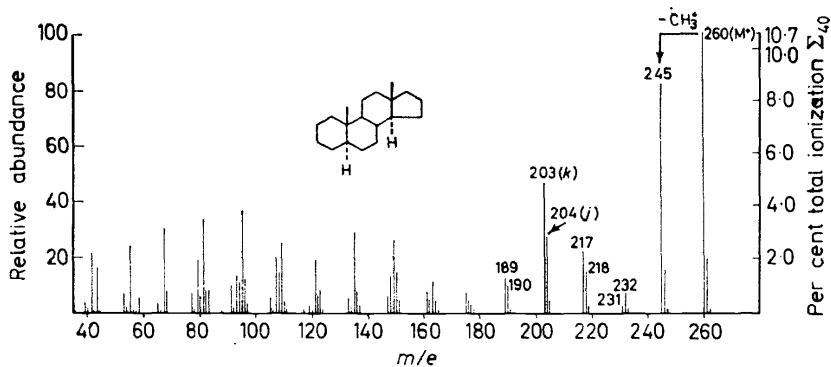


Figure 5. Mass spectrum (70 ev) of 5 $\alpha$ -androstane.

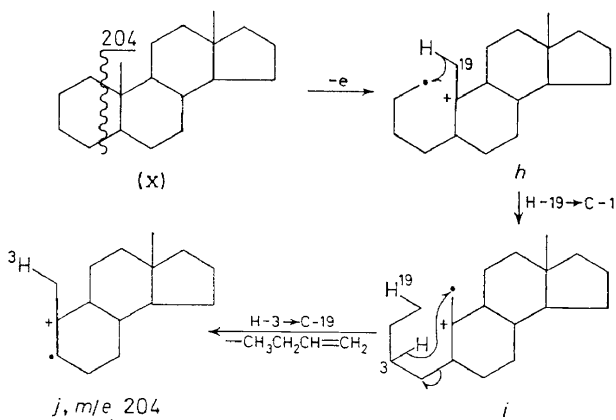
aside from an intense  $M-CH_3$  peak, a series of fragment peaks, none of which is exceptionally abundant or characteristic. This can be rationalized readily, since the absence of a C-17 substituent does not any more favour molecular ion *a* to the extent encountered in cholestane (Figure 2). Recent detailed deuterium labelling experiments<sup>21</sup> with 5 $\alpha$ -androstane have shed considerable light upon the nature of the fragmentation processes in this simplest of all steroid skeletons and attention will be drawn briefly to the most salient conclusions which are of some mechanistic interest.

The first point of interest is the origin of the methyl group lost in the genesis of the  $M-CH_3$  peak ( $m/e$  245 in Figure 5). In 5 $\alpha$ -androstane (I,R = H)<sup>21</sup>, the site of the expelled methyl group is distributed between C-18 and C-19 in a ratio of 3:2, whereas in a C-17 substituted steroid such as 5 $\alpha$ -pregnane (I,R =  $C_2H_5$ )<sup>12</sup> this ratio is reversed to 1:4. Such a dramatic change is readily understandable if one recalls that the preferred loss of the C-19 angular methyl group in 5 $\alpha$ -pregnane was rationalized<sup>12</sup> by the predominance

of molecular ion *a* in C-17 substituted steroids, which would preclude removal of the C-18 methyl group. Since in 5 $\alpha$ -androstande, the C-18 methyl function is ejected preferentially, this can be taken as additional evidence in favour of the earlier postulate that the absence of a C-17 substituent results in a diminished importance of a molecular ion of type *a* and hence a removal of the restrictive factor inhibiting loss of the C-18 methyl group.

A second corollary of this postulate is the considerably reduced intensity of the *m/e* 217, 218 peak group (cf. *Figure 2 vs. Figure 5*), whose origin is again associated with the intervention of molecular ion *a*. Mechanistically, their formation in 5 $\alpha$ -androstande follows the same paths as proposed for cholestane (e.g.  $1 \rightarrow a \rightarrow c \rightarrow d \rightarrow b'$ ) as demonstrated by deuterium labelling<sup>21</sup>.

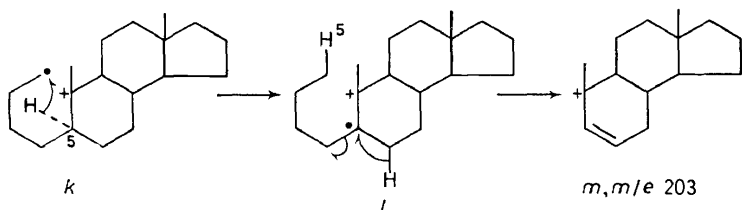
A third feature of the 5 $\alpha$ -androstande spectrum (*Figure 5*) worth commenting upon refers to the peaks encompassed by *m/e* 203 and 204. The labelling data<sup>21</sup> demonstrated that these two ions owe their formation predominantly to fission in ring A by mechanisms which closely parallel the ring D fission of cholestane and pregnane<sup>12</sup>. Thus the ion of mass 204, formally corresponding to simple loss of ring A (see schematic representation in X), actually is generated by a sequence of steps ( $X \rightarrow h \rightarrow i \rightarrow j$ ) which involves the site-specific reciprocal hydrogen transfer from positions 3 and 19, which is completely analogous to the reciprocal hydrogen transfer between positions 16 and 18 demonstrated<sup>12</sup> in cholestane and pregnane, and postulated (though not proved) in  $\Delta^{14}$ -cholestene (IX).



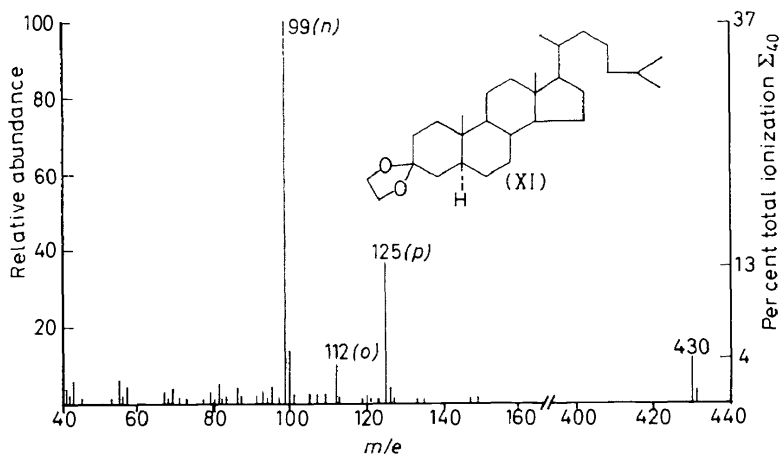
Similarly, the hydrogen transfer accompanying loss of ring A with formation of the ion of mass 203 (see *Figure 5*) involves principally the *a priori* least likely site, namely C-5, which again is completely analogous to the loss of the C-14 hydrogen atom<sup>12</sup> accompanying the expulsion of ring D in cholestane (see *m/e* 217 in *Figure 2*). This apparent rupture of two bonds connected to one carbon atom (C-5) is most readily accommodated by an internal hydrogen migration, a typical example being transfer from C-6 ( $l \rightarrow m$ ).

The discussion to this stage has centred on the role which alkyl substituents and double bonds can play in the electron impact induced fragmentation of steroid hydrocarbons and on the mechanistic information which can be extracted from hydrogen transfer reactions. Attention will now be drawn to

the effect of certain hetero atomic substituents, in particular of ketal groupings.



The dominant effect of the ethylene ketal grouping upon the mass spectrum of steroids has been emphasized numerous times<sup>22</sup>. Thus a comparison of the relatively complicated mass spectrum (*Figure 2*) of 5 $\alpha$ -cholestane (I, R = C<sub>8</sub>H<sub>17</sub>) with that (*Figure 6*) of 3,3-ethylenedioxy-5 $\alpha$ -cholestane (XI) demonstrates the tremendous simplification of the fragmentation pattern

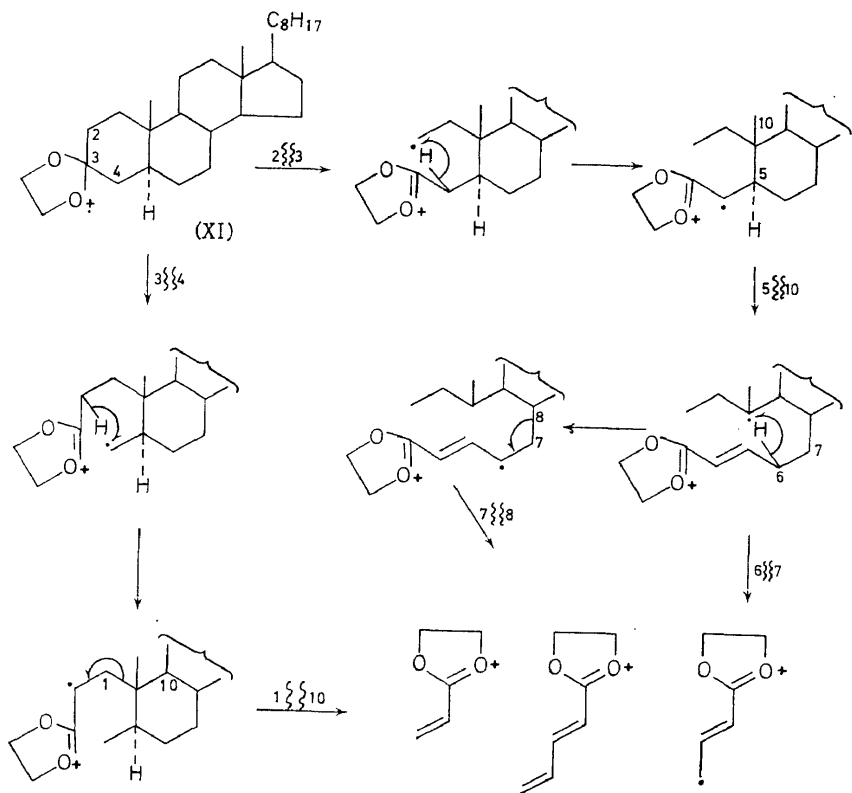


*Figure 6.* Mass spectrum (70 ev) of 3,3-ethylenedioxy-5 $\alpha$ -cholestane (XI).

leading essentially to only three fragment ions of mass 99 (*n*), 112 (*o*) and 125 (*p*), whose origin can be rationalized<sup>22</sup> readily in the following manner, the appropriate hydrogen transfers having been established in each instance by deuterium labelling.

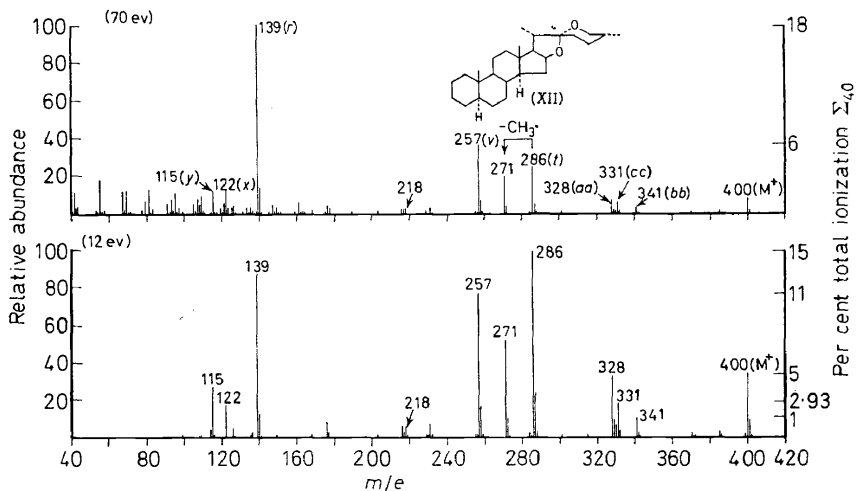
Particularly noteworthy is the absence of any peaks associated with ring D fission (the peaks corresponding to *m/e* 217 and 218 in cholestane (*Figure 2*) would have appeared at *m/e* 275 and 276 in *Figure 6*) at either 70 or 12 ev, which demonstrates that the ring D fission triggered by a molecular ion of type *a* cannot compete energetically with bond ruptures in ring A of the ketal (XI). This conclusion is of some importance as background material for the subsequent discussion.

Among naturally occurring steroids, there is one important group—the steroidal sapogenins—which possesses a ketal grouping as part of its fundamental skeleton. The simplest representative is 3-deoxytigogenin (XII),



*n, m/e* 99    *p, m/e* 125    *o, m/e* 112

whose mass spectrum is reproduced in Figure 7. Even a cursory comparison between this spectrum and that (Figure 2) of cholestane ( $I, R = C_8H_{17}$ )



demonstrates immediately that one is dealing with a completely different fragmentation pattern and that the characteristic ring D fission of cholestane leading to the important peaks at  $m/e$  217 and 218 is practically absent in the sapogenin spectrum (Figure 7).

The only attempt at a detailed analysis of the mass spectral fragmentation paths of steroidal sapogenins dates back to 1962<sup>23</sup> and is based on a comparison of the mass spectra (70 ev) of differently substituted sapogenins without having taken recourse to deuterium labelling. Recent unpublished work<sup>24</sup> in our laboratory on analogues of 3-deoxytigogenin (XII) labelled with deuterium in positions 11, 12, 14, 15, 16, 17, 20, 21, 23, 24, 25, 26 and 27 coupled with low voltage measurements, has shed considerable light on the mode of fragmentation of such steroidal sapogenins and some of the results will be described briefly. In order to evaluate some of the contributions of nuclear substituents the spectra of the 3-(XIII) and 12-(XIV) keto analogues are also reproduced (Figures 8 and 9).

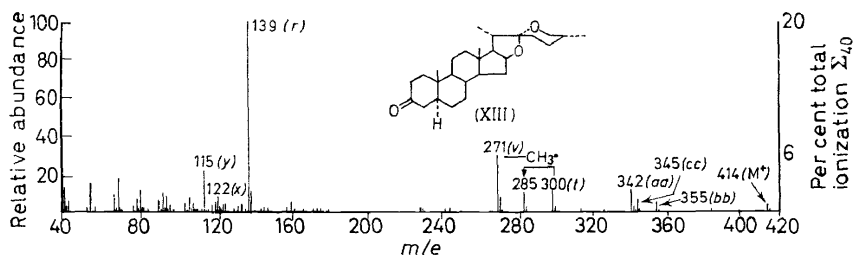


Figure 8. Mass spectrum (70 ev) of tigogenone (XIII).

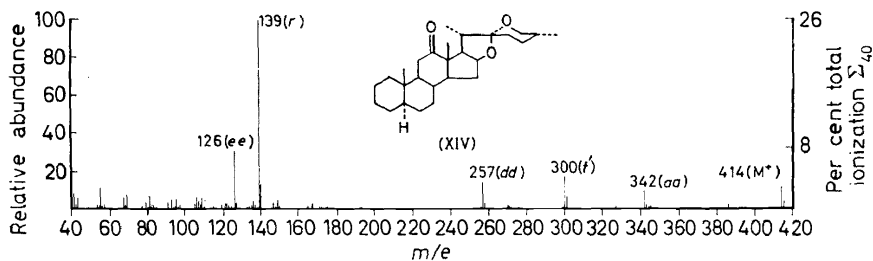
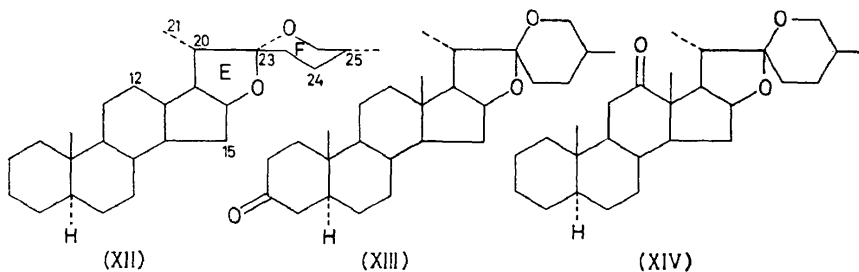


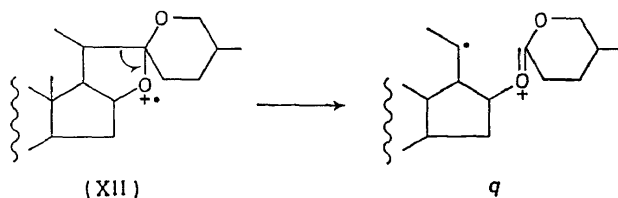
Figure 9. Mass spectrum (70 ev) of 3-deoxyhecogenin (XIV).

### Use of molecular ion peak determinations

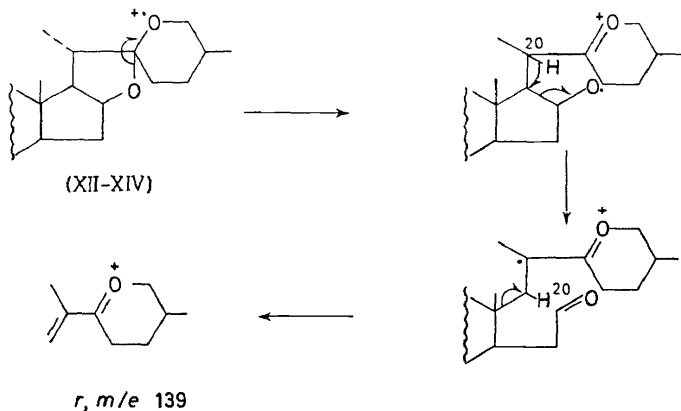
We have already called attention elsewhere<sup>25</sup> to the fact that the spiroketal rings E and F in the steroidal sapogenins (e.g. XII) occupy perpendicular planes, which make certain bond fissions of molecular ions with the charge on one of the oxygen atoms more favourable than others due to orbital overlap. This appears to be one of the reasons why fragmentations initiated by fission of the 20-22 bond (XII  $\rightarrow$  q) are relatively unfavourable and that, therefore, several of the decomposition modes associated with the spiroketal grouping are mechanistically quite distinct from those encountered among simple ethylene ketals (XI). This may also be one of the reasons why



charge localization around the spiroketal oxygens in (XII) is not as pronounced as in (XI) and why some of the hydrocarbon peaks ( $m/e$  286, 271, 257 and 122) in the spiroketal spectrum (Figure 7) gain in importance at low electron voltage, whereas this is not true of the ethylene ketal (XI) (Figure 6).

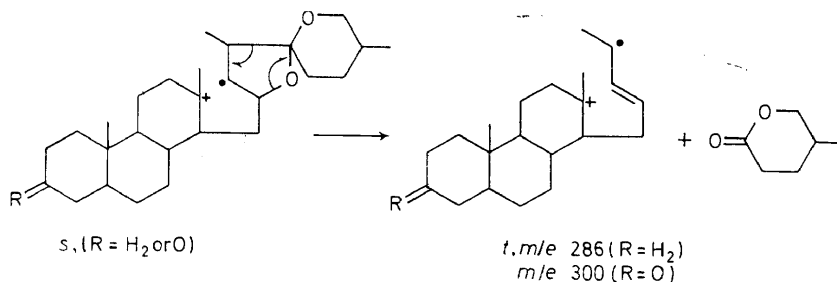


The most characteristic peak of steroidal sapogenins (Figures 7-9) and one that remains intense at low voltages (see Figure 7) is the one at  $m/e$  139. All of the recent deuterium labelling results<sup>24</sup> are consistent with the originally proposed<sup>23</sup> mechanism leading to expression  $r$  for the ion of mass 139. The 1,2-shift of the C-20 hydrogen atom to position 17 is, of course, not amenable to experimental proof by deuterium labelling and is invoked only in order to rationalize what would otherwise constitute a fission of two bonds connected to C-17.

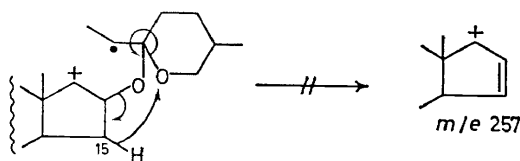


This particular fragmentation process is not affected by substituents in the nucleus (cf. *Figures 8* and *9*) nor by some of the more common substituents in the spiroketal system, such as the replacement of the ring F oxygen by nitrogen<sup>26</sup> or the attachment of a hydroxyl group at C-27.<sup>27</sup>

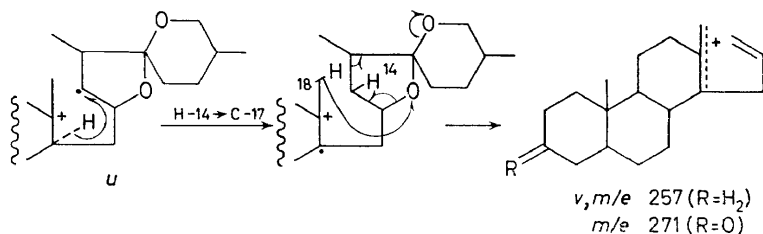
The three peaks at  $m/e$  257, 271 and 286 in *Figure 7* are of special interest because none of them encompasses the spiroketal oxygen atoms and because their abundance increases greatly at reduced voltage. The most important member of this trio, indeed the most important peak of the entire low-voltage spectrum (*Figure 7*), occurs at  $m/e$  286 and all labelling data are consistent with the sequence  $s \rightarrow t$ , the responsible molecular ion  $s$  being completely analogous to the ion  $a$  in the cholestane series. No definite structure can be proposed for the next lower homologue ( $m/e$  271), since the labelling results indicate that this peak corresponds to at least two different species, one (40 per cent) being associated with the loss of the C-21 methyl group from  $t$  ( $m/e$  286) and the remainder (60 per cent) with expulsion of either the C-18 or C-19 functionality. As expected, both peaks ( $m/e$  286 and 271) in deoxytigogenin (*Figure 7*) are shifted by 14 mass units to  $m/e$  300 and 285 in the spectrum (*Figure 8*) of tigogenone (XIII).



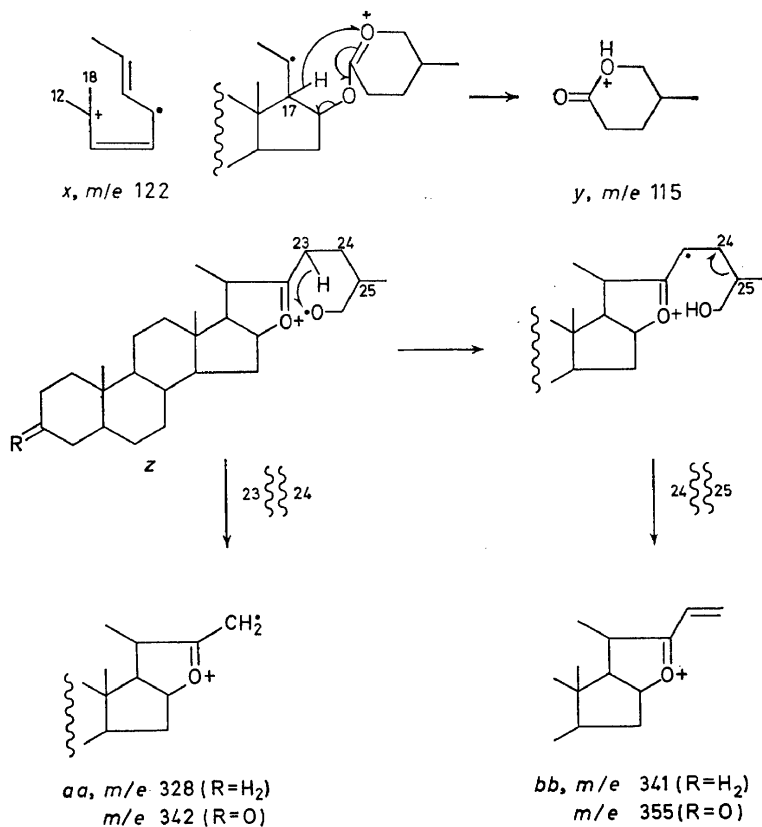
The hydrocarbon nature of the  $m/e$  257 peak had already been noted in the original paper<sup>23</sup> and the following mechanism postulated to account for its formation.



The recent deuterium labelling results<sup>24</sup> definitely eliminate such a path from further consideration, since deuterium attached at C-15 was retained in this fragment. Another conceivable candidate for the hydrogen transfer is C-18, which has not been labelled because of synthetic difficulties. The following path, though purely speculative, has the virtue of being triggered first by an internal transfer from C-14 in the molecular ion  $u$ —a sequence actually documented<sup>12</sup> through deuterium labelling in the genesis of the  $m/e$  217 peak (*Figure 2*) from molecular ion  $a$  in the cholestane series—and leading eventually to the allylic carbonium ion  $v$ .

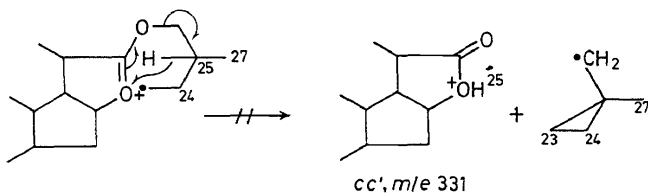


There remain five other peaks which are of potential structural or mechanistic interest and where the deuterium labelling<sup>24</sup> resulted in considerable clarification of their origins. In the low mass range of *Figure 7*, there are encountered two peaks at  $m/e$  115 and 122, which are unchanged in the tigogenone (XIII) spectrum. The labelling studies<sup>24</sup> showed that the ion of mass 122 contains carbon atoms 12–18 as well as C-20 and C-21, but has lost one of the C-15 hydrogens and gained one hydrogen from an undetermined source. A plausible structure for the ion may be *x*. The ion of mass 115, on the other hand, retains both oxygen atoms and is most appropriately depicted by *y*. Three peaks associated with opening of ring F are the ones at  $m/e$  341, 331 and 328 in *Figure 7*, which suffer the expected

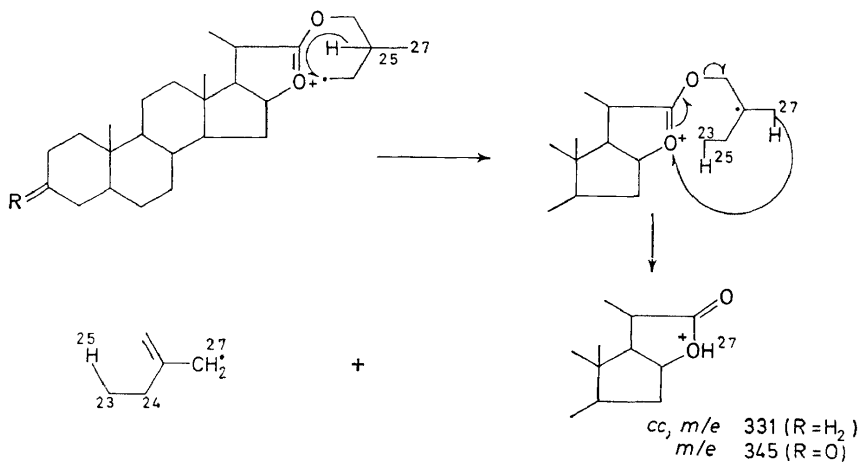


14-mass unit shift in the tigogenone spectrum (*Figure 8*). Unambiguous fragmentation modes can be postulated for them on the basis of the deuterium labelling results<sup>24</sup>. Fission of the F ring with charge retention on the C-16 oxygen atom yields a molecular ion species *z*, which can either suffer direct cleavage of the 23–24 linkage to give *aa* (*m/e* 328) or else undergo initial hydrogen transfer of the C-23 hydrogen atom with concomitant 24–25 bond scission to yield *bb* (*m/e* 341).

Of particular mechanistic interest is the ion of mass 331, for which the following plausible mechanism had been proposed<sup>23</sup> involving transfer of the C-25 hydrogen atom through a six-membered transition state.



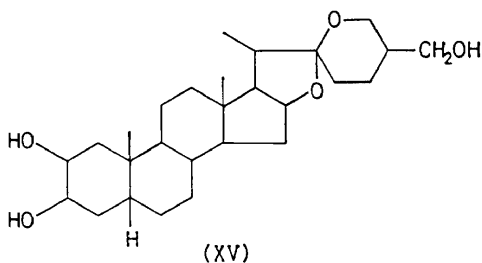
In point of fact, the labelling experiments<sup>24</sup> demonstrated that, for the most part, the hydrogen atom attached to C-27 rather than C-25 had to be transferred, which leads to species *cc* rather than *cc'* for the *m/e* 331 peak, the only difference being the origin of the shifted hydrogen atom. Apparently, the driving force for this alternative path is the production of an allylic radical rather than a cyclopropane-containing radical as the neutral species.



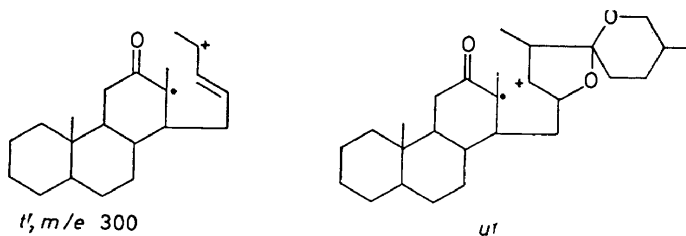
### Use of specific and characteristic mass spectral fragmentation processes

With the availability of plausible structural and mechanistic proposals for the principal characteristic fragment ions associated with the steroidal sapogenin ring system typified by deoxytigogenin (XII), one can now examine the applicability of these results. In theory, a given substituent can

be localized fairly precisely in certain portions of the molecule by determining the occurrence of appropriate peak shifts in certain peaks and not in others. Such an approach works well in a compound such as tigogenone (XIII) and even in as highly substituted a sapogenin as igagenin (XV)<sup>27</sup>, but fails partly in as simple an analogue as the 12-ketone (XIV). Its spectrum (Figure 9) does indeed show the characteristic peak at  $m/e$  139 ( $r$ ) associated solely with ring F and three additional carbon atoms as well as peaks at  $m/e$  300 and 342, which correspond to the  $m/e$  286 ( $t$ ) and 328 ( $aa$ ) species in the 3-deoxytigogenin (XII) spectrum (Figure 7). However, there are certain significant differences to which attention should be called.



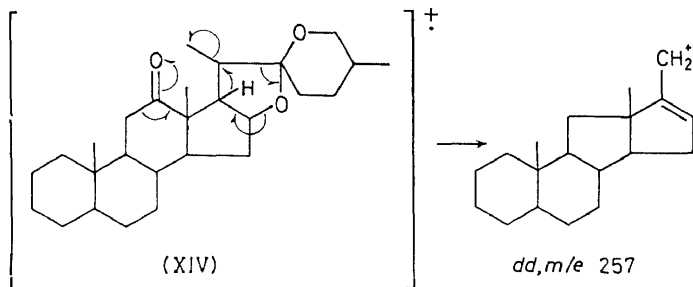
One difference is the absence in Figure 9 of an  $m/e$  115 peak and of appropriately shifted analogues ( $m/e$  136, 271, 285, 345 and 355) of the  $m/e$  122 ( $x$ ), 257 ( $v$ ), 271, 331 ( $cc$ ) and 341 ( $bb$ ) peaks in the deoxytigogenin (XII) spectrum (Figure 7). In at least two cases— $m/e$  122 ( $x$ ) and  $m/e$  257 ( $v$ )—such absences may be rationalized by the undesirability of placing a carbonyl group next to a positive charge (at C-13), but such an objection should also apply to the  $m/e$  286 species ( $t$ ), yet its counterpart ( $m/e$  300) is clearly discernible in Figure 9. This apparent discrepancy can, perhaps, be accommodated by attributing the much more favourable canonical form  $t'$  to the ion of mass 300 in (XIV), a similar alternative representation not being possible in the 12-keto analogue of  $v$ , because the initial trigger provided by the C-14 hydrogen migration to a radical site at C-17 in  $u$  would not be feasible in  $u'$ .



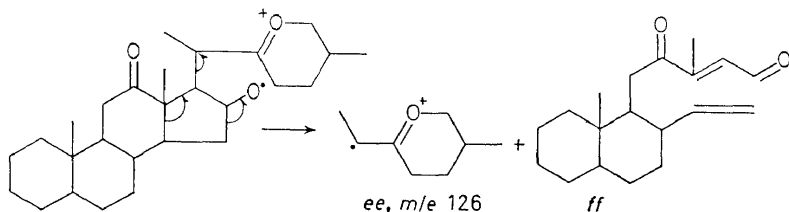
Even more interesting and potentially misleading is the existence of a peak at  $m/e$  257\* in 3-deoxyheecogenin (XIV) (Figure 9), since it might be confused with the  $m/e$  257 peak in the deoxytigogenin (XII) spectrum (Figure 7). Obviously, these two species of identical mass cannot possibly

\*As expected, this peak appears at  $m/e$  273 in the spectrum of heecogenin (XIV with 3 $\beta$ -hydroxyl group).

possess the same structure and exact mass measurements<sup>24</sup> confirmed the hydrocarbon composition ( $C_{19}H_{29}$ ) of this ion derived from (XIV). Since C-11 is retained, as demonstrated by deuterium labelling,<sup>24</sup> the elements of CO must have been ejected from ring C. The simplest representation, therefore, is *dd*, which requires the postulate of an internal 1,2-hydrogen shift (from C-17 to C-20) for which there seems to exist some precedent in the genesis of the important  $m/e$  139 peak (see XII $\rightarrow$ r).



One other peak, which is much more intense in the 12-keto series (XIV as well as hecogenin) and hence may be of diagnostic significance, is the one at  $m/e$  126 (Figure 9). Exact mass measurements<sup>24</sup> confirmed its composition as  $C_8H_{14}O$ , which essentially requires that it be derived from ring F. The simplest formulation is one leading to *ee*, which, however, raises the question why such a species is not equally important in sapogenins lacking a 12-keto function (see Figures 7 and 8)\*. A possible explanation may be the increased stability in the presence of a 12-keto group of the accompanying neutral fragment which may be formulated as the diene-dione *ff*.



One final word of caution is indicated. Significant changes in the conventional fragmentation pattern (see Figures 7 and 8) of the steroidal sapogenins may not be caused only by certain structural alterations, such as the introduction of a 12-keto function, but may also be promoted by stereochemical factors. A striking example is provided by the 70 and 12 ev spectra (Figure 10) of 20-isodeoxytigogenin (XVI), which were measured on the same day under completely identical conditions to those utilized for the spectrum (Figure 7) of deoxytigogenin (XII). The spectra of these two isomers do not only differ quantitatively, but even qualitatively, a striking example being the appearance of a new intense peak at  $m/e$  181 associated

\*It is somewhat more noticeable in igagenin (XV)<sup>27</sup>, where it is shifted to  $m/e$  142 due to the 27-hydroxyl group.

with a ring D cleavage which was not encountered in any of the other saponinins. Evidently, its production can be attributed in some way to relief of the severe steric strain caused by the close proximity of the C-18 and C-21 methyl groups in (XVI).

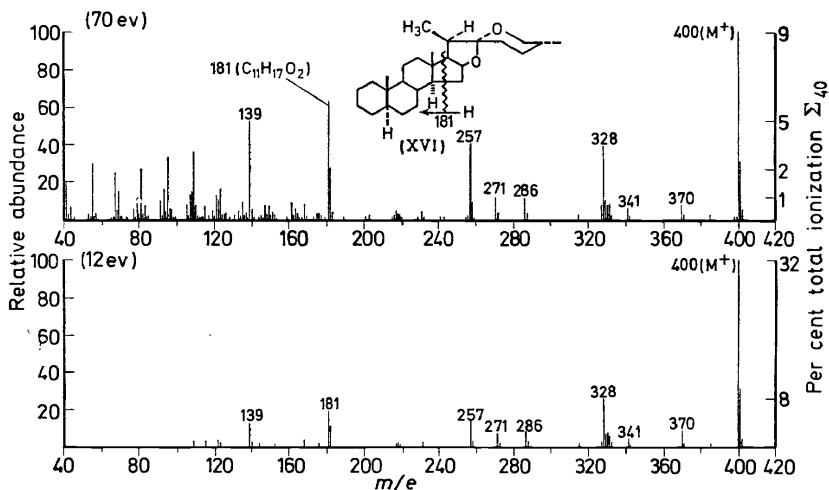


Figure 10. Mass spectra (70 and 12 ev) of 20-iso-3-deoxytigogenin (XVI).

The results of the present lecture can be summarized by stating that detailed information about the fragmentation of a given steroid skeleton is essential before the effect of various superimposed substituents or stereochemical changes can be properly evaluated. Obviously much work still needs to be done before far-reaching generalizations about the mass spectral interpretation of many steroid types are justified, but much progress has been made in recent years and the overall prognosis is clearly promising.

### Acknowledgement

The conclusions described in this lecture rest heavily on extensive, and often also synthetically difficult, deuterium labelling for which I am greatly indebted to the various collaborators listed in the bibliography, notably Dr. Laszlo Tökes and Mr. William H. Faul. Grateful acknowledgement is also due to Dr. Alan M. Duffield and Mr. Robert Ross for many mass spectral measurements and to the National Institutes of Health (grants No. AM-12758 and AM-04257) for financial assistance.

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