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The Determination of the Position of Double Bond in Unsaturated Steroids by Mass Spectrometry

N.S.Vul'fson and V.G.Zaikin

The possibilities and limitations of the mass-spectrometric method in the solution of problems concerning the location of the double bond in steroids have been demonstrated for a large number of such compounds containing the double bond in various positions in the polycyclic system and in the side chain. The mass spectra of Δ^1-, Δ^2-, Δ^4-, Δ^5-, Δ^7-, Δ^{13}-, Δ^{16}-, Δ^{22}-, Δ^{23}-, and Δ^{24}-steroids, in which the diagnostic fragments are due to the dissociation of at least one allylic bond, are the most characteristic. The double bonds in other positions in the steroid skeleton either do not initiate specific decomposition processes at all, or inhibit processes characteristic of the corresponding saturated analogues, or cause the appearance of fragments the driving force responsible for the formation of which is obscure. A separate section of the review deals with aromatic and diene steroids. The bibliography includes 86 references.

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I. INTRODUCTION

The determination of the position of the double bond in natural unsaturated steroids does not present much difficulty at present, since the steroids from hormones encountered in nature contain double bonds in a limited number of positions in the polycyclic system and in the side chain at $C_{(17)}$. However, this problem constantly faces investigators working in the field of the synthetic chemistry of steroids and also chemists dealing with the determination of the structure of various functional derivatives of natural steroids. In the latter case, when the positions of certain substituents, for example hydroxy-, alkoxy-, or acyloxy-groups, are determined, the problem is significantly facilitated when the location of the double bond formed as a result of the *trans*-elimination of these groups is known. Recently, mass spectrometry has been successfully employed to establish the position of the bond together with other physicochemical methods. The advantage of this method over others consists in its speed and extremely small amounts of substance required for analysis.

Attempts to employ mass spectrometry in order to establish the position of the double bond in aliphatic compounds were unsuccessful. As a rule, owing to the migration of the double bond under the influence of electron impact, peaks of the characteristic ions, the formation of which could be related to the position of the double bond, cannot be observed in the mass spectra of the corresponding compounds. The study of the mass spectra of the products of a transformation of unsaturated aliphatic compounds which fixes the position of the double bond, for example of the corresponding epoxides, is more promising. In cyclic compounds, including steroids, the double bond shows less tendency towards migration under the conditions of mass-spectrometric measurements and frequently initiates specific decompositions which can be used to determine its position. The formation of the majority of fragments arising under the influence of the double bond includes the dissociation of at least one allylic C—C bond. A special case of this process is the decomposition of the type of the retro-diene reaction (RDR) (or the "Diels—Alder retro-reaction"), which takes place with the dissociation of two allylic C—C bonds, and the charge may be located at the diene and or the so called dienophile fragment. Sometimes the process is accompanied by the migration of the hydrogen atom to the charged fragment or away from it. The relative intensities of the peaks corresponding to RDR depend on the stability of the ions formed. Decomposition of the RDR type was first described by Reed[1] and was subsequently analysed in detail by Biemann[2] and Djerassi and coworkers[3] for different types of unsaturated cyclic compounds. This type of fragmentation is interesting because, apart from the determination of the position of the double bond, the mass number of the corresponding ion frequently yields information about the nature of the substituent in the ring.

Among unsaturated steroids, compounds without functional substituents, such as keto-, hydroxy-, alkoxy-, and acyloxy-groups, or additional double bonds, are comparatively rarely encountered. Each of these functional groups can influence the decomposition of the molecular ion under electron impact and the problem of the determination of the double bond position in such cases is closely related to the determination of the nature and position of the substituent.

Numerous studies in which mass-spectrometric data for unsaturated steroids are given and discussed in different degrees of detail have now accumulated. However, there are comparatively few systematic studies in this field and many published investigations suffer from lack of a detailed analysis of the mass spectra quoted.

In the present review an attempt is made to give a systematic account of the accumulated mass-spectrometric determinations of the position of the double bond in unsaturated steroids. Studies in which the mass-spectrometric data quoted are insufficient to infer the influence of the

double bond on fragmentation under electron impact have been excluded from consideration.

One must stipulate beforehand that many of the decomposition mechanisms and ionic structures quoted in the review are of a formal kind and frequently have not been confirmed experimentally. However, such representation of mass-spectrometric processes is now generally adopted in the literature. On the other hand, the proposed mechanisms of decompositions under electron impact frequently find analogies in photochemistry, radiation chemistry, plasma chemistry, electrochemistry of organic compounds, and even solution chemistry. In representing the bond dissociations, we have employed three types of notation: homolytic dissociation is represented by a "hook", an arrow represented heterolytic dissociation, and a wavy line represents any of these dissociations when it is impossible to postulate a reasonably well founded mechanism.

II. STEROIDS WITH A DOUBLE BOND IN RING *A*

1. Δ^2-Steroids

One of the first studies dealing with the effect of the position of the double bond in steroids on the pathways in the decomposition of the molecular ion under electron impact was that by Audier et al.[4], who showed that decomposition via an RDR mechanism is characteristic of the type (A) Δ^2-steroid which they investigated (Table 1):

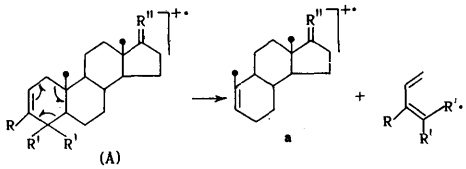
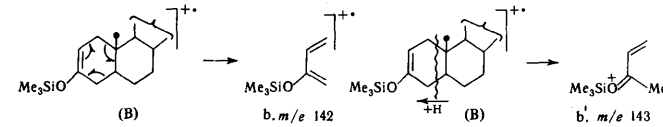


Table 1. The values of *m/e* (intensity expressed as a percentage of the maximum) for the ion a in the mass spectra of type (A) Δ^2-steroids[4]. *

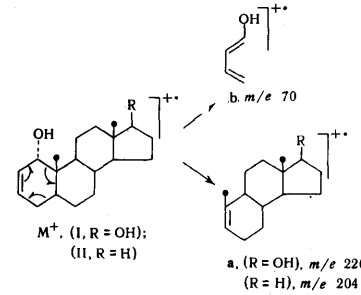
Compound	R	R′	R″	Diene eliminated	*m/e* for ion a
Androst-2-ene	H	H	H_2	Butadiene	$(M-54)^{+\cdot}$,204(91)
Cholest-2-ene	H	H	H, C_8H_{17}	Butadiene	$(M-54)^{+\cdot}$,316(65)
Androst-2-en-17-one	H	H	0	Butadiene	$(M-54)^{+\cdot}$,218(100)
3-Methylcholest-3-ene	Me	H	H, C_8H_{17}	2-Methylbutadiene	$(M-68)^{+\cdot}$,316(81)
Cholest-2-en-3-yl acetate	MeCOO	H	H, C_8H_{17}	2-Acetoxybutadiene	$(M-112)^{+\cdot}$,316(68)
3-Methoxycholest-2-ene	MeO	H	H, C_8H_{17}	2-Methoxybutadiene	$(M-84)^{+\cdot}$,316(38)
4,4-Dimethylcholest-3-ene	H	CH_3	H, C_8H_{17}	2-Methylpenta-2,4-diene	$(M-82)^{+\cdot}$,316(3)

* Audier et al.[4] do not quote the peak intensities of the type b diene ions.

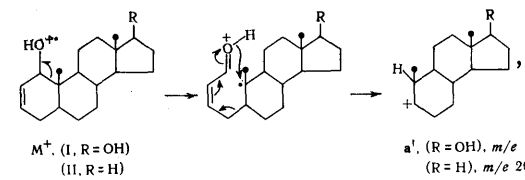
Peaks due to ions caused by the elimination of a diene as a rule predominate in the mass spectra of these compounds, while the ionic peaks of the dienes themselves are low. In the mass spectrum of 4,4-dimethyl-5α-cholest-2-ene the peaks of the dienophile fragment **a** amounts to only 3% of the maximum peak in the spectrum, while the intensity of the peak corresponding to the diene ion (*m/e* 82) is much higher. A similar situation is observed also in the spectrum of lanost-2-ene (4,4,14α-trimethyl-5α-cholest-2-ene).[5] Thus the introduction of a *gem*-dimethyl group in the 4-position partly stabilises the diene fragment. Table 1 shows that the RDR type of decomposition is characteristic also of enol acetates and enolic ethers. In type (B) 3-trimethylsilyloxy-Δ^2-steroids[6], this process leads to the preferential formation of the diene ion **b** with *m/e* 142 to which corresponds a rearranged ion with *m/e* 143 (b′).



In the mass spectra of androst-2-ene-1α,17β-diol (I) [7] and androst-2-en-1α-ol (II),[8,9] there are intense peaks due to ions **b** with *m/e* 70 (in both cases) and also of ions **a** with *m/e* 220 and 204 respectively due to the RDR type of decomposition:



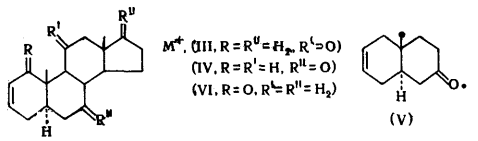
An important characteristic of spectra (I) and (II) is also the presence of peaks with *m/e* 221 and 205 (due to ions a′) respectively, which are much more intense than the peaks due to ions a and are formed with migration of a hydrogen atom to the charged fragment. The mechanism suggested[8,9] for the formation of ions a′,



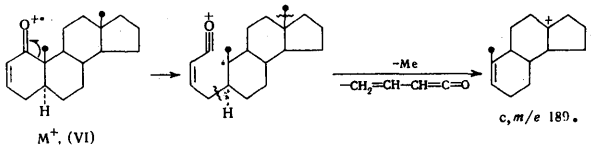
has not been confirmed experimentally.

It is interesting to follow the influence of the number and positions of carbonyl groups in Δ^2-steroids on their capacity to decompose via an RDR mechanism. The effect of carbonyl groups remote from the double bond may be considered for the following two examples. An intense peak due to an ion with *m/e* 218, $(M-54)^+$ formed by RDR in ring *A* accompanied by the ejection of a butadiene molecule, was observed by Djerassi and coworkers[10] in the mass spectrum of 5α-androst-2-en-11-one (III). In contrast there is no peak corresponding to RDR in the spectrum of 5α-androst-2-ene-7,17-dione (IV),[4] while in the spectrum of *trans*-10-methyl-2-octalone (V), which has a similar structure, the peak of the ion due to RDR is present[11]. We believe that this unusual behaviour of (IV) is associated with the possibility of the location of the charge at carbonyl groups remote from the double bond. This factor must be always borne in mind in the interpretation of the mass spectra, since ionisation of molecules "far" from the double bond can either greatly stabilise the molecular ion or can facilitate the occurrence of

competing reactions and thereby inhibit the processes initiated by the double bond:

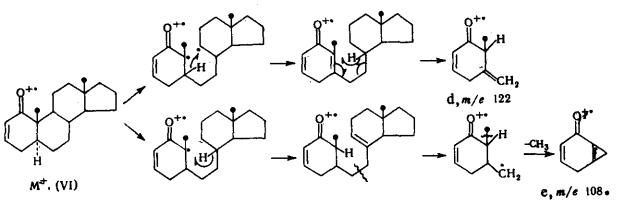


In the study of the decomposition of 5α-androst-2-en-1-one (VI) and its deutero-analogue (labelled at $C_{(2)}$, $C_{(4)}$, $C_{(5)}$, $C_{(6)}$, $C_{(7)}$, and $C_{(16)}$) under electron impact, Djerassi and coworkers[12] showed that the $C_{(2)}$ and $C_{(4)}$ atoms are eliminated from the neutral molecule on formation of an ion with *m/e* 189 (c) from the molecular ion. They proposed the following mechanism for the formation of the ion c:



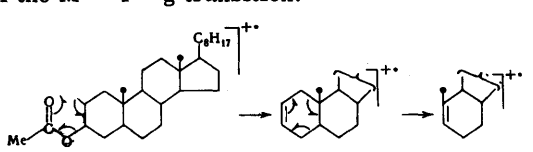
We suggest that the formation of the ion c can be due to an RDR type of decomposition with simultaneous elimination of one of the angular methyl groups.

Moreover, the spectrum of compound (VI) has peaks due to ions with *m/e* 122 (d) and 108 (e) the formation of which is interpreted as follows[12]:



The peak of the ion d with *m/e* 108 is accompanied by the peak of an ion with *m/e* 909 which is formed with migration of two hydrogen atoms, in contrast to the former.

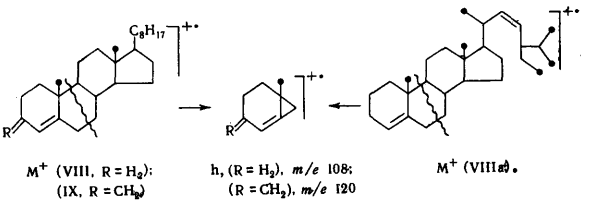
In connection with the discussion of the retro-diene reaction, which is characteristic of Δ^2-steroids, mention should be made of the capacity of such related 3-hydroxysteroids and particularly their acetates to undergo a type RDR decomposition after the elimination of the 3-OH or 3-$OCOCH_3$ groups in the form of water or acetic acid. In most cases the 1,2-elimination of these substituents is associated with a thermal process which leads to the formation of a Δ^2-steroid even before electron impact. Indeed in the spectrum of 5α-cholestan-3β-yl acetate (VII), recorded when the specimen was introduced at 250°C,[13] the peak of the M^+ ion is absent and the peak of the $(M - CH_3COOH)^+$ ion (e), which splits off butadiene as a result of RDR with formation of an *m/e* 316 ion (g), predominates in the mass spectrum. One cannot rule out the possibility that the ion f is formed to some extent also under electron impact. Thus the spectrum of compound (VII), recorded at 80°C,[14] shows all the peaks characteristic of the $M^+ \rightarrow f \rightarrow g$ transition:



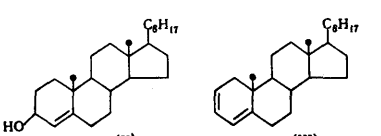
A similar sequence is observed also in the decomposition of the molecular ions from 5α-cholestan-3β-ol, coprostan-3β-ol, its acetate, and 4,4-dimethyl-5α-cholestan-3β-ol.[15] This feature of the fragmentation of saturated 3β-acetoxysteroids has been used[16] to establish the composition of a mixture of plant sterols subjected to preliminary hydrogenation and then acetylated. Butadiene is not split off from Δ^5-hydroxysteroids and their acetates[14], which also form readily $(M - 18)^+$ and $(M - 60)^+$ ions. This phenomenon is associated with the preferential formation of a conjugated 3,5-diene and not its 2,5-isomer.

2. Δ^4-Steroids

Among the simplest Δ^4-steroids without additional functional groups, cholest-4-ene (VIII) [17,18] and stigmasta-4,22-diene (VIIIa) [1] have been investigated. The peak of the ion with *m/e* 108 (h), which is the second most intense in the mass spectrum of compound (VIII), is due to the simple dissociation of the 6—7 and 9—10 allylic bonds[18]. An intense peak due to the ion h is observed also in the spectrum of compound (VIII).[1] A similar dissociation of 6—7 and 9—10 allylic bonds in 3-methylenecholest-4-ene (IX) [18] leads to an ion with *m/e* 120. The peak of this ion in the spectrum of compound (IX) is accompanied by equally intense peaks due to ions with *m/e* 106 and 94 the first of which may be formed as a result of the dissociation of 5—6 and 9—10 bonds:

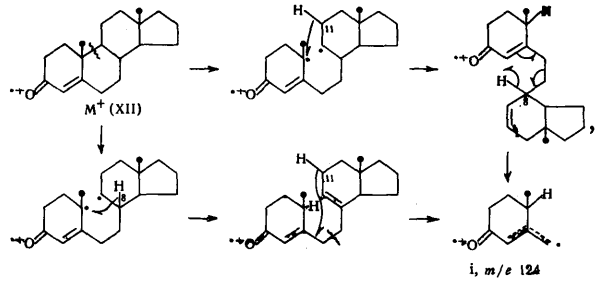


Proceeding now to steroids containing functional groups, we must consider in the first place the mass spectrum of allocholesterol (X).[17-19] The allocholesterol molecule shows an exceptional tendency towards thermal dehydration and therefore its spectrum, recorded using a hot glass inlet system at 200°C, does not contain the peak of the molecular ion and is absolutely identical with the spectrum of cholesta-2,4-diene (XI) (see section VII, 2). When the specimen is introduced directly into the ion source, which makes it possible to reduce the experimental temperature to 90—100°C, a large peak due to the molecular ion and the fragments resulting from its subsequent decomposition appears in the spectrum of compound (X), but a very significant feature of this spectrum is still the decomposition characteristic of compound (XI).

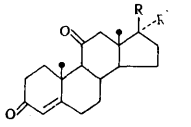


The most extensive and interesting data have been obtained in mass-spectrometric studies on Δ^4-3-ketosteroids. Shapiro and Djerassi[20] were the first to demonstrate for androst-4-en-3-one (XII) an important characteristic of the decomposition of such systems. It was found that the main peak in the mass spectrum of compound (XII) corresponds to the ion i with *m/e* 124. It was shown with the aid of the mass spectra of 2,2,4,6,6-D_5-, 8-D_1, 11,11-D_2-, and 19-D_1-analogues of compound (XII), its 7β-D_1-17β-OH-analogue, and also the

spectrum of cholest-4-en-3-one (XIII) that the ion i contains the *A* ring, the 19-CH_3 group, and a $C_{(6)}$ atom, being formed as a result of the dissociation of 6—7 and 9—10 allylic bonds with transfer of two hydrogen atoms from $C_{(8)}$ and $C_{(11)}$ to the charged fragment, as shown in the mechanism below[20,21]:



An additional piece of evidence of the validity of the mechanism proposed to account for the formation of the ion i is also the finding that in the mass spectra of androst-4-ene-3,11-dione (XIV),[20,22] cortisone (XV),[2,20,23-25], and androst-4-ene-3,11,17-trione (XVI) [24] a peak with *m/e* 122 due to the simple dissociation of the 6—7 and 9—10 bonds without the migration of hydrogen atoms appears instead of the ion i. This can be explained by the absence of hydrogen atoms at $C_{(11)}$ in compounds (XIV)—(XVI).

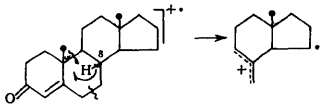


(XIV, R = R' = H);
(XV, R = $COCH_2OH$, R = OH);
(XVI, R + R' = O)

The presence of the peak due to the ion i and its analogues, the mass number of which depends on the substituents in ring *A* and at $C_{(6)}$ and $C_{(19)}$ is common to the mass spectra of all steroid Δ^4-3-ketones and can be used for their identification (Table 2).

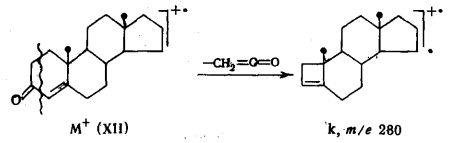
The ion i and its analogues are formed directly from the molecular ion and therefore their peak intensities are related. In the spectra of compounds containing, for example, functional groups which are readily eliminated by electron impact, the molecular ion peaks are small or altogether absent. At the same time there is a significant decrease of the peak due to the ions i. However, the peaks of these ions and their analogues with even mass numbers, can be as a rule reliably detected in the spectrum, since groups of peaks in which odd peaks are much more intense than even peaks are characteristic of this part of the mass scale for steroids and other carbocyclic compounds and one can readily observe even a slight increase in the intensity of the even peaks in the region where the peaks of the ion or its analogues is presumed to be.

Another characteristic of the mass spectra of steroid Δ^4-3-ketones is the presence of peaks due to the dissociation of the same 6—7 and 9—10 bonds but with fixation of the charge on the fragment containing the *C* and *D* rings. It has been shown for androst-4-en-3-one (XII) with the aid of its 8-D_1-analogue[20] that the formation of the corresponding ion j is accompanied by the migration of the hydrogen atom from $C_{(8)}$ to a neutral fragment:

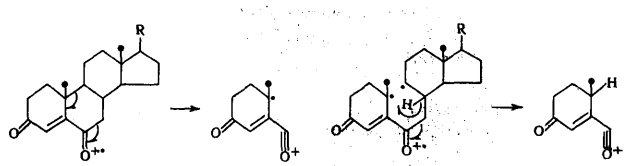


Peaks due to analogous ions have been detected also in the mass spectra of substituted progesterones[9,34]. Although this is a decomposition pathway which in general leads to low-intensity peaks and is not discussed for other Δ^4-3-ketones listed in Table 2, it is also evidently common to all Δ^4-3-ketosteroids.

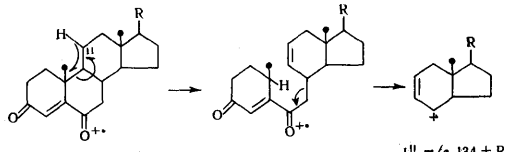
The third specific pathway in the fragmentation of Δ^4-3-ketosteroids is associated with the formation of an $(M - 42)^+$ ion (k). The probable mechanism of its formation involves the ejection of $C_{(2)}$ and $C_{(3)}$ atoms in the form of keten:



The situation changes somewhat in Δ^4-3,6-diketosteroids. Djerassi and coworkers[41] investigated the mass spectra of androst-4-ene-3,6-dione (XVII), pregn-4-ene-2,6-dione (XVIII), and cholest-4-ene-3,6-dione (XIX) and observed in them an intense double peak with *m/e* 136 and 137. The spectrum of compound (XVII) also contains an intense *m/e* 135 peak, the peaks in the triplet decreasing in the sequence 135 < 136 < 137. The peaks of the ions with *m/e* 136 (l) and 137 (l') in the mass spectrum of 2,2,7,7-D_4-(XIX) shift by 2 a.m.u., which indicates the retention of ring *A* in their structures. The involvement of the hydrogen atom at $C_{(8)}$ in the formation of the ion l' is postulated by analogy with Δ^4-3-ketones:



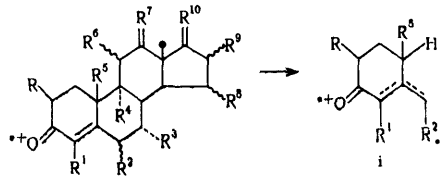
The *m/e* 135 peak (ion l''), which is characteristic of the mass spectrum of compound (XVII), shifts to *m/e* 163 and 247 in the spectra of compounds (XVIII) and (XIX) respectively. The authors suggest that the corresponding ion is formed as a result of a rearrangement involving the hydrogen atom at $C_{(7)}$ similar to the rearrangement described above for Δ^4-3-ketosteroids:



The mass spectra of the Δ^4-3,6-diketones under consideration also contain peaks of $(M - CH_2CO)^+$ ions, the formation of which may be due to both the 3-oxo-and the 6-oxo-group.

In conclusion of the section dealing with Δ^4-steroids, mention may be made also of studies on the mass-spectrometry of Δ^4-6-aza-7-ketosteroids (XX) [42,43] the spectra of which contain, despite the presence of a heteroatom in ring *B*, peaks of m, m', and m'' ions, whose intensity

Table 2. The values of *m/e* (intensity expressed as a percentage of the maximum) for the ion i in the mass spectra of Δ^4-3-ketosteroids.

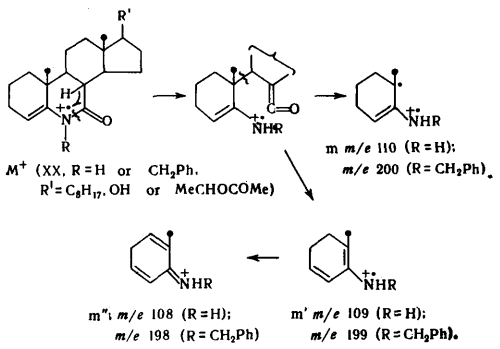


i

Compound	R	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	R^{10}	*m/e* for ion i
Androst-4-en-3-one (XII) (Ref.20)	H	H	H	H	H	Me	H	H_2	H	H	H_2	124 (100)
17β-Hydroxyandrost-4-en-3-one (Refs.14,26)	H	H	H	H	H	Me	H	H_2	H	H	H, β-OH	124 (100)
17β-Hydroxy-10α-androst-4-en-3-one (Ref.26)	H	H	H	H	H	(α-Me)	H	H_2	H	H	H, β-OH	124 (100)
Androst-4-ene-3,17-dione (Refs.17,19)	H	H	H	H	H	Me	H	H_2	H	H	O	124 (80)
19-Norandrost-4-en-3-one (Ref.14)	H	H	H	H	H	H	H	H_2	H	H	H_2	110 (100)
19-Norandrost-4-ene-3,17-dione (Ref.27)	H	H	H	H	H	H	H	H_2	H	H	O	110 (95)
19-Nor-9β-androst-4-ene-3,17-dione (Ref.27)	H	H	H	H	(β-H)	H	H	H_2	H	H	O	110 (35)
6β-Methyl-19-norandrost-4-ene-3,17-dione (Ref.22)	H	H	β-Me	H	H	H	H	H_2	H	H	O	124 (3.4 *)
6β-Ethyl-19-norandrost-4-en-3-one (Ref.22)	H	H	β-Et	H	H	H	H	H_2	H	H	H_2	138 (1.9 *)
15α-Hydroxyandrost-4-ene-3,17-dione (Refs.28,29)	H	H	H	H	H	Me	H	H_2	α-OH	H	O	124 (40)
17α-Hydroxy-17β-methyl-19-norandrost-4-en-3-one (Ref.30)	H	H	H	H	H	H	H	H_2	H	H	α-OH, β-Me	110 (28)
17β-Hydroxy-17α-methyl-19-norandrost-4-en-3-one (Ref.30)	H	H	H	H	H	H	H	H_2	H	H	β-OH, α-Me	110 (48)
17β-Ethyl-17α-hydroxy-19-norandrost-4-en-3-one (Ref.30)	H	H	H	H	H	H	H	H_2	H	H	α-OH, β-Et	110 (30)
17α-Ethyl-17β-hydroxy-19-norandrost-4-en-3-one (Ref.30)	H	H	H	H	H	H	H	H_2	H	H	β-OH, α-Et	110 (25)
11β-Hydroxyandrost-4-ene-3,17-dione (Ref.24)	H	H	H	H	H	Me	β-OH	H_2	H	H	O	124 (60)
17α-Methyltestosterone (Ref.31)	H	H	H	H	H	Me	H	H_2	H	H	α-CH_3, β-OH	124 (40)
19-Nortestosterone (Ref.27)	H	H	H	H	H	H	H	H_2	H	H	H, β-OH	110 (100)
19-Nor-8α-testosterone (Ref.27)	H	H	H	H	H	H	H	H_2	H	H	H, β-OH	110 (25)
19-Nor-9β-testosterone (Ref.27)	H	H	H	H	(β-H)	H	H	H_2	H	H	H, β-OH	110 (100)
Progesterone (Refs.17,19,25,26,32–34)	H	H	H	H	H	Me	H	H_2	H	H	H, β-COMe	124 (100)
10α-Progesterone (Ref.26)	H	H	H	H	H	(α-Me)	H	H_2	H	H	H, β-COMe	124 (100)
17-Isoprogesterone (Ref.33)	H	H	H	H	H	Me	H	H_2	H	H	H, α-COMe	124 (25)
2α-Methylprogesterone (Ref.34)	Me	H	H	H	H	Me	H	H_2	H	H	H, β-COMe	138 (46)
4-Methylprogesterone (Ref.34)	H	Me	H	H	H	Me	H	H_2	H	H	H, β-COMe	138 (31)
6α-Methylprogesterone (Ref.34)	H	H	α-Me	H	H	Me	H	H_2	H	H	H, β-COMe	138 (100)
6β-Methylprogesterone (Ref.34)	H	H	β-Me	H	H	Me	H	H_2	H	H	H, β-COMe	138 (37)
7α-Methylprogesterone (Ref.34)	H	H	H	Me	H	Me	H	H_2	H	H	H, β-COMe	124 (21)
9α-Hydroxyprogesterone (Ref.34)	H	H	H	H	OH	Me	H	H_2	H	H	H, β-COMe	124 (84)
11-α-Hydroxyprogesterone (Refs.28,29,34)	H	H	H	H	H	Me	α-OH	H_2	H	H	H, β-COMe	124 (78)
11β-Hydroxyprogesterone (Refs.28,29,34)	H	H	H	H	H	Me	β-OH	H_2	H	H	H, β-COMe	124 (60)
Progesteron-11α-yl acetate (Ref.34)	H	H	H	H	H	Me	α-OCOMe	H_2	H	H	H, β-COMe	124 (19)
12α-Hydroxyprogesterone (Ref.35)	H	H	H	H	H	Me	H	H, α-OH	H	H	H, β-COMe	124 (20)
15α-Hydroxyprogesterone (Refs.28,29,34)	H	H	H	H	H	Me	H	H_2	α-OH	H	H, β-COMe	124 (75)
15β-Hydroxyprogesterone (Refs.28,29)	H	H	H	H	H	Me	H	H_2	β-OH	H	H, β-COMe	124 (40)
16α-Methylprogesterone (Refs.32–34)	H	H	H	H	H	Me	H	H_2	H	α-Me	H, β-COMe	124 (52)
16β-Methylprogesterone (Refs.33,34)	H	H	H	H	H	Me	H	H_2	H	β-Me	H, β-COMe	124 (43)
16β-Methyl-17-isoprogesterone (Ref.33)	H	H	H	H	H	Me	H	H_2	H	β-Me	H, α-COMe	124 (10)
16α-Hydroxyprogesterone (Refs.28,29,34)	H	H	H	H	H	Me	H	H_2	H	α-OH	H, β-COMe	124 (31)
16β-Hydroxyprogesterone (Refs.28,29)	H	H	H	H	H	Me	H	H_2	H	β-OH	H, β-COMe	124 (40)
17α-Hydroxyprogesterone (Refs.34,35)	H	H	H	H	H	Me	H	H_2	H	H	α-OH, β-COMe	124 (40)
20-Hydroxyprogesterone (Ref.35)	H	H	H	H	H	Me	H	H_2	H	H	H, β-CHOHMe	124 (100)
21-Hydroxyprogesterone (Refs.25,34)	H	H	H	H	H	Me	H	H_2	H	H	H, β-$COCH_2OH$	124 (7)
11α, 16α-Dihydroxyprogesterone (Refs.28,29)	H	H	H	H	H	Me	α-OH	H_2	H	α-OH	H, β-COMe	124 (35)
12β,15α-Dihydroxyprogesterone (Ref. 34)	H	H	H	H	H	Me	H	H, β-OH	α-OH	H	H, β-COMe	124 (43)
6α,16α-Dimethylprogesterone (Refs.22,32)	H	H	α-Me	H	H	Me	H	H_2	H	α-CH_3	H, β-COMe	138 (7.4*)
6α,16β-Dimethylprogesterone (Ref.32)	H	H	α-Me	H	H	Me	H	H_2	H	β-CH_3	H, β-COMe	138 (100)
17α-Hydroxy-16α-Methylprogesterone (Ref.32)	H	H	H	H	H	Me	H	H_2	H	α-CH_3	α-OH, β-COMe	124 (47)
16α,17α-Dimethylprogesterone (Ref.36)	H	H	H	H	H	Me	H	H_2	H	α-Me	α-CH_3, β-COMe	124 (8)
17α-Hydroxy-6β,16α-Dimethylprogesterone (Ref.32)	H	H	β-Me	H	H	Me	H	H_2	H	α-Me	α-OH, β-COMe	138 (53)
Cortisol (Refs.24,25)	H	H	H	H	H	Me	β-OH	H_2	H	H	α-OH, β-$COCH_2OH$	124 (40)
Corticosterone (Ref.25)	H	OH	H	H	H	Me	β-OH	H_2	H	H	H, β-$COCH_2OH$	124 (20)
Cholest-4-en-3-one (XIII) (Refs.14,17,19)	H	H	H	H	H	Me	H	H_2	H	H	H, β-C_8H_{17}	124 (100)
4-Hydroxycholest-4-en-3-one (Ref.22)	H	H	H	H	H	Me	H	H_2	H	H	H, β-C_8H_{17}	140 (2.7*)
6β-Methylcholest-4-en-3-one (Ref.18)	H	H	β-Me	H	H	Me	H	H_2	H	H	H, β-C_8H_{17}	138 (100)
19-Hydroxycholest-4-en-3-one (Ref.35)	H	H	H	H	H	CH_2OH	H	H_2	H	H	H, β-C_8H_{17}	110 (20)
24-Hydroxycholest-4-en-3-one (Ref.35)	H	H	H	H	H	Me	H	H_2	H	H	H, β-$C_8H_{16}OH$	124 (100)
25-Hydroxycholest-4-en-3-one (Ref.35)	H	H	H	H	H	Me	H	H_2	H	H	H, β-$C_8H_{16}OH$	124 (100)
26-Hydroxycholest-4-en-3-one (Refs.35,37)	H	H	H	H	H	Me	H	H_2	H	H	H, β-$C_8H_{16}OH$	124 (100)
3-Oxocholest-4-en-26-yl acetate (Ref.37)	H	H	H	H	H	Me	H	H_2	H	H	H, β-$C_8H_{16}OCOMe$	124 (100)
Methyl 3-oxocholest-4-en--26-oate (Ref.37)	H	H	H	H	H	Me	H	H_2	H	H	H, β-$C_7H_{14}COOMe$	124 (100)
Methyl 7α,12α-Dihydroxy-3-oxochol--4-enoate (Ref.38)	H	H	H	α-OH	H	Me	H	H, α-OH	H	H	H, β-C_4H_8COOMe	124 (100)
Methyl 7α,12α-Dihydroxy-3-oxobisnorchol--4-enoate (Ref.38)	H	H	H	α-OH	H	Me	H	H, α-OH	H	H	H, β-C_2H_4COOMe	124 (100)
Methyl 7α-hydroxy-3,12-dioxochol-4--enoate (Ref.39)	H	H	H	α-OH	H	Me	H	O	H	H	H, β-C_4H_8COOMe	124 (25)
Methyl 7α-hydroxy-3,12-dioxobisnorchol-4--enoate (Ref.39)	H	H	H	α-OH	H	Me	H	O	H	H	H, β-C_2H_4COOMe	124 (90)

* Intensity expressed as a percentage of the total ionic current.

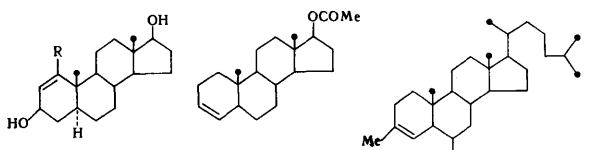
is highest, which are also due to the dissociation of the 6—7 and 9—10 bonds:



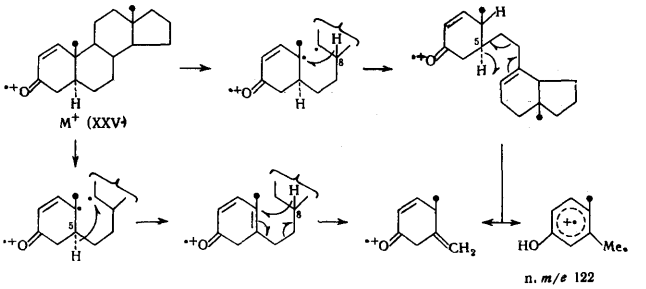
3. Δ^1- and Δ^3-Steroids

Specific fragmentation pathways have not been observed in Δ^1- and Δ^3-steroids without functional groups near the double bond. Thus the most intense peak in the mass spectrum of 1-methyl-5α-androst-1-ene-3β,17β-diol (XXI) [7] is that with m/e 220, the structure of the corresponding ion being most probably similar to that of the ion a. Probably the fragmentation of the molecular ion of compound (XXI) resembles the decomposition of Δ^2-steroids, which hinders their identification. Spitteller and coworkers[8,9] noted that fragments typical of Δ^4-steroids can be detected in the spectrum of compound (XXII), the 1-desmethyl analogue of compound (XXI).

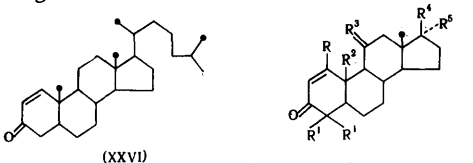
One of the main peaks in the spectrum of androst-3-en-17β-yl acetate (XXIII) [9] is one with m/e 108 (ion h, $C_8H_{12}^+$), which is also characteristic of 4-cholestene (VIII). In the spectrum of 3-methylcholest-3-en-6β-yl acetate (XXIV), [18] there is no molecular ion peak, the dominant intensity being that of the peak due to the $(M - CH_3COOH)^+$ ion, which corresponds to the maximum mass number. The latter subsequently splits off part of ring *A* with formation of an $(M - 60 - 68)^+$ ion corresponding to m/e 314; its formation is a result of the RDR ejection of an isoprene molecule (68 a.m.u.), which is more likely to be typical for the corresponding Δ^2-steroid:

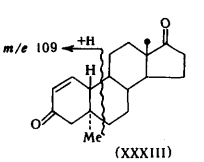


The mass spectra of Δ^1-3-ketosteroids are significantly more characteristic. The decomposition of these compounds resembles that of their Δ^4-3-keto-isomers. The most intense peak in the mass-spectrum of 5α-androst-1-en-3-one (XXV) [20] is that of the m/e 122 ion n. Judging from its mass number, this ion should form as a result of a simple dissociation of the 6—7 and 9—10 bonds without the migration of a hydrogen atom. However, analysis of the mass spectra of the 5α- and 8β-D_1 analogues of compound (XXV) showed that in the formation of the ion n hydrogen atoms move towards each other, that from $C_{(5)}$ migrating to the neutral fragment and that from $C_{(8)}$ to the charged fragment. The following possible mechanism for the formation of the ion n has been proposed on the basis of these results[20,21]:



Intense peaks due to the ions n (m/e 122) are present also in the mass spectra of cholest-1-en-3-one (XXVI), [22] 5α-androst-1-ene-3,11-dione (XXVII), 17β-hydroxy-5α(and 5β)-androst-1-en-3-ones (XXVIII), and 5α(and 5β)-androst-1-ene-3,17-diones (XXIX) [44] and the peaks of the homologous ions with m/e 136, 150, and 108 are present in the spectra of 17β-hydroxy-1-methyl-5α-androst-1-ene-3-one (XXX), 4,4-dimethyl-5α-cholest-1-en-3-one (XXXI), [22] and 19-nor-Δ^1-3-ketosteroids (XXXIIa, b) respectively. In connection with the different behaviour of Δ^1- and Δ^4-3-ketosteroids, it has been suggested[14] that the reason why the ion n cannot be formed from the latter is the absence of a hydrogen atom at $C_{(5)}$, which is necessary for the migration of the two hydrogen atoms towards one another described above. This hypothesis is confirmed by the finding that the mass spectrum of 5α-methyl-19-norandrost-1-ene-3,17-dione (XXXIII), [18] which also lacks a hydrogen atom at $C_{(5)}$, contains virtually no peak with m/e 122, the main peak being one with m/e 109, which probably arises on dissociation of the 5—6 and 9—10 bonds with migration of a hydrogen atom to the charged fragment.





Thus, in contrast to Δ^4-3-ketosteroids, the dissociation of the 6—7 and 9—10 bonds in Δ^1-3-ketosteroids is accompanied only by the migration of hydrogen atoms towards one another and the corresponding characteristic peak of the ion n is smaller by 2 a.m.u. than the peak of the ion i, which is characteristic of Δ^4-3-ketosteroids.

It is striking that the intensity of the peak due to the ion n depends on the configuration at $C_{(5)}$ and is always greater in the 5β-series[44,45].

The presence in the Δ^1-3-ketosteroid molecule of an $\alpha\beta$-unsaturated keto-chromophore is the reason for the elimination of keten from M^+. This process has been noted for the majority of the Δ^1-3-ketones described above and in this respect their decomposition is analogous to that of their Δ^4-3-keto-isomers, which leads to ions of type k. Egger[44] suggested that the absence of the peak of the $(M - 42)^+$ ion from the mass spectrum of the 5β-isomer (XXVIII) be employed to determine the configuration of the

5th centre in steroids of the Δ^1-3-keto-series. However, Schulz[45] did not find a similar difference in the 5α- and 5β-Δ^1-3-ketones which he investigated, and noted the presence of the peak of the $(M - 42)^+$ ion in the spectra of the 5α- and 5β-isomers of compound (XXXIIa) and its absence from the spectra of the 5α- and 5β-isomers of compound (XXXIIb).

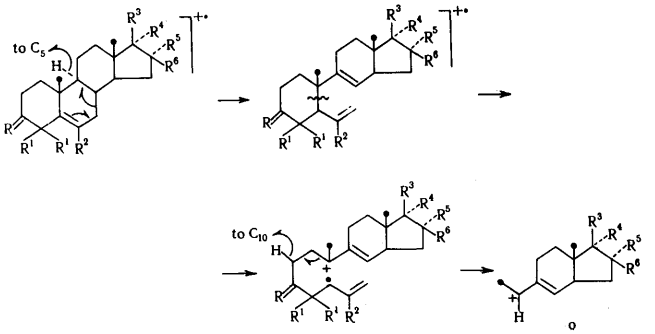
III. STEROIDS WITH A DOUBLE BOND IN RING *B*

1. Δ^5-Steroids

A large number of natural steroids, particularly of plant origin, contain a double bond in the 5—6 position. A combined gas—liquid chromatographic and mass-spectrometric method has been widely used recently to investigate natural steroid mixtures. As a rule, volatile steroid derivatives—acetates and especially trimethylsilyl ethers—are used in such investigations. In many studies of this kind, no data were obtained whereby one could infer the influence of the position of the double bond on the nature of the decomposition of the molecular ion under electron impact. Naturally, these investigations are not considered here.

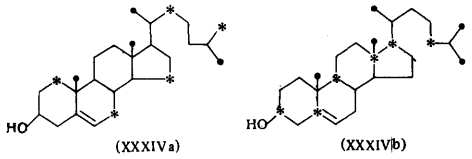
In one of the first mass-spectrometric studies on Δ^5-steroids, Friedland et al.[46] were the first to draw attention to the intense peak due to ions with *m/e* 275, 303, and 177, among the many peaks characteristic of the majority of steroids, in the spectra of cholesterol (XXXIV), β-sitosterol (XXXV), and dehydroepiandrosterone (XXXVI) respectively. They attributed the formation of these ions to the dissociation of the 9—10 bond and of the 5—6 vinylic bond. Somewhat later Ryhage and Stenhagen[15] also published the spectrum of cholesterol but failed to interpret many of the peaks in it, including the *m/e* 275 peak. We undertook a systematic study of the effect of the Δ^5-bond [17,19] in numerous Δ^5-3-hydroxy- and Δ^5-3-keto-steroids. Regardless of the conditions employed during its recording (a heated glass filling system or direct introduction into the ion source), the mass spectrum of cholesterol (XXXIV) shows an intense *m/e* 275 peak, the intensity of which depends on that of the peak due to the molecular ion: in experiments with a hot glass inlet system at 200°C, the intensity of the *m/e* 275 peak decreases together with decrease of that of the molecular ion peak; under milder conditions, when the specimen is introduced directly into the ion source, the size of the *m/e* 275 peak differs little from the molecular ion peak, which predominates in the spectrum.

The mass spectrum of cholest-5-en-3-one (XXXVII),[17,19] recorded on an apparatus with a heated glass filling system at 200°C, is identical with the spectrum of cholest-4-en-3-one (XIII), which indicates the ease of the thermal isomerisation of Δ^5-3-ketones under the conditions of mass-spectrometric measurements. However, when the apparatus with direct introduction of the specimen into the ion source is employed, the mass spectrum of compound (XXXVII) differs significantly from that of compound (XIII), since it contains an intense *m/e* 275 peak (ion o). The *m/e* 275 peak of the ion o is observed also in the spectrum of cholest-5-ene (XXXVIII).[8,9,17] Similarly the spectra of pregn-5-en-3,20-dione (XXXIX) and androst-5-ene-3,17-dione (XL) show intense peaks with *m/e* 205 and 177 respectively, which are absent from the spectra of the isomeric Δ^4-3-ketones. However, it is noteworthy that the spectra of the Δ^5-3-ketones as a rule also show peaks with *m/e* 124 (ion i) and M − 42, which are characteristic of Δ^4-3-ketones. This indicates a partial isomerisation of Δ^5-3-ketones to Δ^4-3-ketones even under mild experimental conditions. Thus one may conclude that the ions o are characteristic of Δ^5-steroids. Since the mass number of the ion o depends on the size of the side chain at $C_{(17)}$, it must contain rings *C* and *D* together with the side chain (Table 3). In view of the fact that the mass number of the ion o does not vary with substitution in ring *A*, the latter must be absent (or only a small portion of it remains at the junction with ring *B*). On the other hand, the $C_{(6)}$ atom does not enter into the composition of the ion o, since in the spectrum of 6-methylcholesterol (XLI) [18,19] its mass number remains unchanged compared with the spectrum of compound (XXXIV). Thus the mechanism of the formation of the *m/e* 275 ion, proposed previously[46], is erroneous and the following mechanism may be proposed for the formation of the ion o:[8,18]



The mechanism shows that the ion o is probably formed directly from the molecular ion, although the corresponding metastable peak was not observed. This hypothesis is confirmed indirectly by the fact that in the spectra of compounds which do not contain the peak of the molecular ion, or its intensity is low, the intensity of the peak due to the ion o is greatly reduced, sometimes to such an extent that it cannot be reliably identified. For example, in the spectra of the acetate (XLII), trifluoroacetate (XLIII), and trimethylsilyl ether of cholesterol (XLIV), of which the first two altogether fail to give rise to the molecular ion peak and the third contains a low-intensity M^+ peak, the peaks of the ion o are small (Table 3).

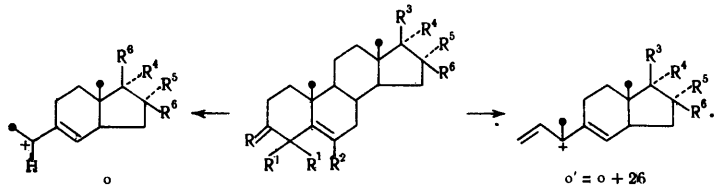
The final proof of the origin of the ion o can be obtained by studying the spectra of labelled compounds. In this connection, Knights[47] put forward an interesting idea. He suggested that one should investigate the mass spectra of cholesterols labelled with ^{13}C in various positions of the steroid skeleton, namely the spectra of compounds (XXXIVa) and (XXXIVb), which may be obtained by biosynthesis from [2-^{13}C]- and [4-^{13}C]-mevalonic acids respectively. However, this has not been achieved experimentally.



(XXXIVa) (XXXIVb)

The mass spectra of Δ^5-3-hydroxysteroids contain, together with peaks of the ions o, peaks with an intensity higher by 26 a.m.u. (o + 26, ion o′), which are not observed in the spectra of Δ^5-3-ketones (Table 3). As a rule, the intensity of these peaks is of the same order of

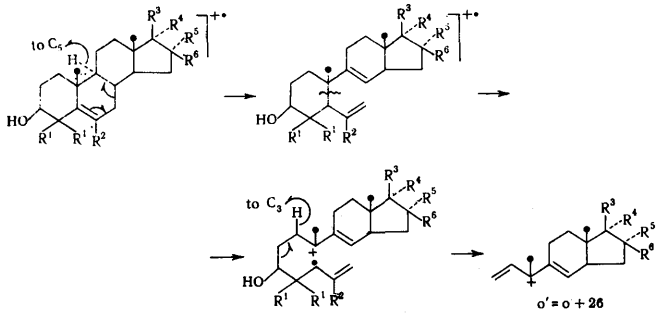
Table 3. The values of *m/e* (intensity expressed as a percentage of the M^+ peak) for the ions o and o′ in the mass spectra of Δ^5-steroids.



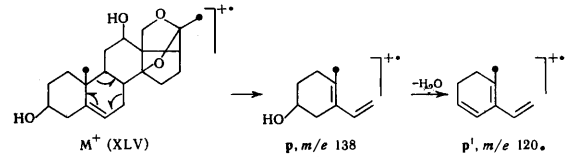
Compound	R	R^1	R^2	R^3	R^4	R^5	R^6	*m/e* for ion o	*m/e* for ion o′
Cholest-5-ene (XXXVIII) (Refs.8,17)	H_2	H	H	C_8H_{17}	H	H	H	275 (22)	301 (25)
Cholesterol (XXXIV) (Refs.17,19,46,47)	H, β-OH	H	H	C_8H_{17}	H	H	H	275 (90)	301 (45)
Cholest-5-en-3-one (XXXVII) (Refs.17,19)	O	H	H	C_8H_{17}	H	H	H	275 (12)	—
Pregn-5-ene-3,20-dione (XXXIX) (Refs.17,19)	O	H	H	$COCH_3$	H	H	H	205 (17)	—
Androst-5-ene-3,17-dione (XI) (Refs.17,19)	O	H	H	$R^3+R^4=O$		H	H	177 (30)	—
β-Sitosterol (XXXV) (Refs.17,46,47)	H, β-OH	H	H	$C_{10}H_{21}$	H	H	H	303 (70)	329 (40)
Stigmasterol (Ref.17)	H, β-OH	H	H	$C_{10}H_{19}$	H	H	H	301 (18)	—
Campesterol (Ref.47)	H, β-OH	H	H	C_9H_{19}	H	H	H	289 (65)	315 (60)
16α,17α-Epoxy-3β-hydroxypregn-5-en-20-one (Ref.17)	H, β-OH	H	H	$COCH_3$	$R^4+R^5=O$		H	219 (16)	345 (20)
4,4-Dimethylcholesterol (Ref.17)	H, β-OH	CH_3	H	C_8H_{17}	H	H	H	275 (8)	201 (5)
4,4-Dimethylcholest-5-en-3-one (Ref.17)	O	CH_3	H	C_8H_{17}	H	H	H	275 (8)	—
6-Methylcholesterol (XLI) (Refs.18,19)	H, β-OH	H	CH_3	C_8H_{17}	H	H	H	275 (25)	—
17β-Hydroxyandrost-5-en-3-one (Ref.17)	O	H	H	OH	H	H	H	179 (30)	—
Androst-5-ene-3β,17β-diol (Ref.17)	H, β-OH	H	H	OH	H	H	H	179 (53)	205 (50)
13-Isoandrost-5-ene-3β,17β-diol (Refs.48,49)	H, β-OH	H	H	OH	H	H	H	179 (40)	205 (20)
13-Isoandrost-5-ene-3β,17α-diol (Refs.48,49)	H, β-OH	H	H	H	OH	H	H	179 (35)	205 (18)
Dehydroepiandrosterone (XXXVI) (Ref.46)	H, β-OH	H	H	$R^3+R^4=O$		H	H	177 (63)	203 (60)
3β-Hydroxyandrost-5-en-16-one (Ref.18)	H, β-OH	H	H	H	H	$R^5+R^6=O$		177 (28)	—
Cholesteryl acetate (XLII) (Ref.47)	H, β-OAc	H	H	C_8H_{17}	H	H	H	275 (4 *)	—
Cholesteryl trifluoroacetate (XLIII) (Ref.47)	H, β-OCCOF$_3$	H	H	C_8H_{17}	H	H	H	275 (6 *)	—
Trimethylsilyl ether of cholesterol (XLIV) (Refs.47,50)	H,β-OSiMe$_3$	H	H	C_8H_{17}	H	H	H	275 (8 *)	301 (4 *)
Trimethylsilyl ether of β-sitosterol (Ref.50)	H,β-OSiMe$_3$	H	H	$C_{10}H_{21}$	H	H	H	303 (10 *)	329 (8 *)
Trimethylsilyl ether of campesterol (Ref.50)	H,β-OSiMe$_3$	H	H	C_9H_{19}	H	H	H	289 (10 *)	315 (8 *)

* Intensity expressed as a percentage of the maximum in the spectrum.

magnitude as that of peaks due to the ions o. The data in Table 3 lead to the conclusion that the ions o′ contain rings *C* and *D* and do not contain the $C_{(4)}$ atom. However, they also incorporate the $C_{(1)}$ and $C_{(2)}$ atoms. A formal mechanism has been proposed for the formation of both the o and o′ ions assuming the retention in the latter of the most probable carbon atoms[8,18]:

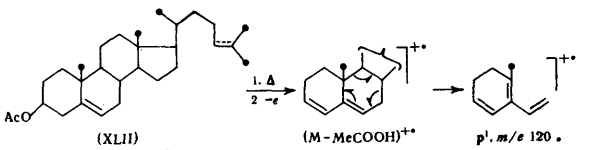


In principle one may expect that Δ^5-steroids should decompose via a type RDR mechanism. One cannot rule out the possibility that in the cases described above this process does occur but only to such a slight extent that the corresponding peaks are difficult to observe against the background of the surrounding peaks. This applies particularly to the peaks of the diene fragments, since their mass numbers are usually smaller than 100 and this region contains fairly intense uncharacteristic peaks typical for all carbocyclic compounds. Nevertheless in the case of certain Δ^5-steroids containing labile functional groups, the spectra of which show low-intensity peaks due to molecular ions (this precludes an unambiguous identification of the peaks due to the ions o and o′), peaks caused by RDR in ring *B* frequently appear. Reichstein and coworkers[51] observed in the spectrum of stapelogenin (XLV) a medium-intensity *m/e* 138 peak (ion p) due to a type RDR decomposition. This ion is accompanied by one formed as a result of dehydration (the ion p′). The origin of the ions has been confirmed by comparison with the spectrum of the di-OD-analogue of compound (XLV):



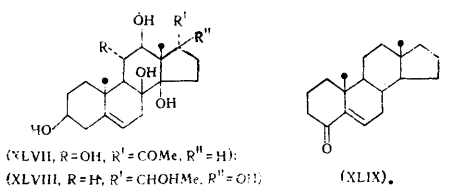
Later Galli and Maroni[52] observed *m/e* 120 peaks of the ion p′ in the mass spectra of cholesteryl acetate (XLII) and cholesta-5,24-dien-3β-yl acetate (XLVI) (with intensities of 30 and 15% respectively), which arise as a result of RDR in the $(M - CH_3COOH)^+$ ion. Probably the latter is formed by the thermal elimination of CH_3COOH, since these spectra do not contain M^+ peaks and the peaks

of the $(M - CH_3COOH)^+$ ions have the highest mass number.



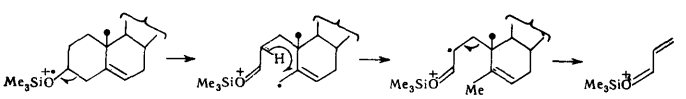
The mass spectra of compounds (XLII) and (XLVI) contain also the peaks of *m/e* 247 and 245 ions, which are probably formed on dissociation of the same bonds (7—8 and 9—10) but with migration of hydrogen and location of the charge on the dienophile fragment.

In a recent mass-spectrometric study of a large number of polyols of the 5-pregnene series, of the type of marsdenin (XLVII) and sarcostin (XLIII), Fukuoka et al.[53] also observed peaks of ions p and p'. They likewise interpreted a series of other peaks produced by a mechanism similar to that of the formation of the ions p and p' but involving a rearrangement of the hydrogen atoms. The presence of peaks due to ions formed by the dissociation of the same bonds (7—8 and 9—10), but involving the fixation of the charge on the dienophile fragment with $m/e = M^+ - 138$ ($M^+ - 180$ for 3-acetoxy-derivatives), was noted in the same investigation.



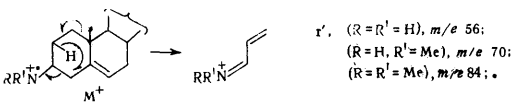
Thus the peaks of the ions p and p' or o and o' are diagnostic in the series of Δ^5-steroids, depending on the stability of the molecular ion and the presence of labile functional groups. It is interesting to note that the mass spectrum of androst-5-en-4-one (XLIX) [54] also contains the peaks of the ion o with *m/e* 163. Von Unruh and Spiteller[55] believe that the peaks of *m/e* 135, 147, and 149 ions are characteristic of compound (XLIX). The first two probably arise in a type RDR decomposition in ring *B* with migration of the hydrogen atom to or away from the charged fragment; it is not clear which half of the molecule is charged under these conditions.

Certain data concerning the capacity of trimethylsilyl ethers of Δ^5-3-hydroxysteroids to give rise to ions of types o and o' in decomposition under electron impact are presented in Table 3. Another characteristic of the decomposition of these derivatives is the ease of formation of the ion with *m/e* 129 (r) whose peak is the most intense in their spectra[50,56-58] (in the spectra of the corresponding saturated analogues, the peak of this ion is insignificant). The ease of formation of the ion r on decomposition of Δ^5-steroids is due to the dissociation of two allylic bonds (3—4 and 1—10), which is confirmed by their comparison with the spectra of deutero-analogues[57]:

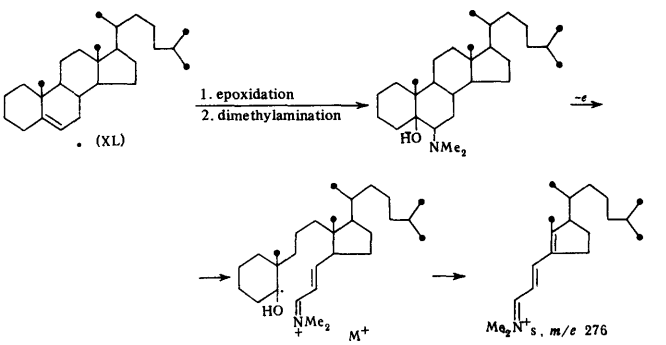


It is noteworthy that the presence of the Δ^5-bond greatly intensifies the elimination of $(CH_3)_3SiOH$ from the molecular ion[57,58], which in all probability results in the formation of the ion of the conjugated 3,5-diene.

In a manner similar to the increase of the intensity of the peak of the ion r on passing from saturated to Δ^5-3-trimethylsilyloxy-derivatives, there is a sharp increase of the intensity of the peak due to the ion r' in the mass spectra of 3-amino-Δ^5-steroids compared with their saturated analogues[59-65]. The mechanism of the formation of the ion r' is evidently similar to that of the formation of the ion r, the dissociation of two allylic bonds (3—4 and 1—10), which intensifies the formation of the ion r', also being responsible for the occurrence of this process:



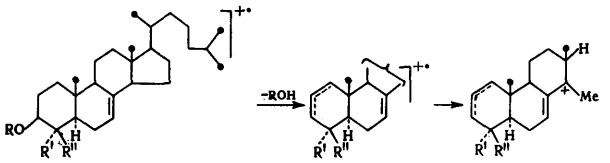
In conclusion of the discussion of Δ^5-steroids, attention should be drawn to the interesting method of determining the position of the double bond in these compounds, proposed by Reed[66]. Although the principle of this method has been demonstrated only for 5-cholestene (XL), it is presumably of more general validity. Its essential feature is that the test compound is epoxidised, the epoxide treated with dimethylamine, and the resulting α-aminoalcohol analysed mass-spectrometrically. The presence in the mass spectrum of the peak due to the ion s serves as evidence of the presence of a Δ^5-bond in the olefin investigated:



2. Δ^7-Steroids

Compounds containing the double bond in the 7—8 position occupy an important place among plant sterols. A number of mass-spectrometric studies have been made on hydroxysteroids of this type, particularly their ethers, trimethylsilyl ethers, and acetates. Gustavsson et al.[58], who considered the mass spectra of the trimethylsilyl ethers of 5α-cholest-7-en-3β-ol (L) and 4α-methyl-5α-cholest-7-en-3β-ol (LI), noted the insignificant intensity of the peaks of the ions r. In addition, they pointed out the presence in the spectra of these compounds of peaks due to ions with *m/e* 229 and 243, the nature of which they failed to establish. Galli and Maroni[52] also observed peaks due to ions with *m/e* 229, 243, and 257 in 5α-cholest-7-en-3β-yl (LII), 4α-methyl-5α-cholest-7-en-3β-yl (LIII), and 4,4-dimethyl-5α-cholest-7-en-3β-yl (LIV) acetates respectively. Having investigated a large number of acetate and trifluoroacetate esters and trimethylsilyl ethers of Δ^7-3-hydroxysteroids, Knights[47] concluded that the formation of the ions t as a result of the elimination of the substituents at $C_{(3)}$ together with a hydrogen atom from the side chain and part of ring *D* is most characteristic of these compounds.

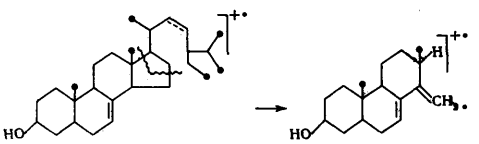
The formation of the ion t can be represented in a general form as follows:



M^+, (L, R = Me_3Si, R' = R'' = H);
(LI, R = Me_3Si, R' = Me, R'' = H);
(LII, R = COMe, R' = R'' = H);
(LIII, R = COMe, R' = Me, R'' = H);
(LIV, R = COMe, R' = R'' = Me)

t, (R' = R'' = H), *m/e* 229;
(R' = Me, R'' = H), *m/e* 243;
(R' = R'' = Me), *m/e* 257.

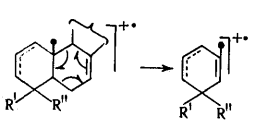
In a study of the mass spectra of stigmast-7-en-3β-ol (LV) and stigmasta-7,22-dien-3β-ol (LVI), Clark-Lewis and Dainis[67] drew attention to the *m/e* 246 fragment (t') which they also regard as characteristic of Δ⁷-steroids:



M^+ (LV)
(LVI: Δ²²)

t, *m/e* 246

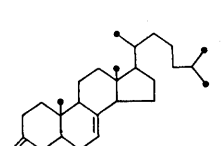
Without denying that the formation of the ions p is characteristic of the decomposition of Δ⁷-steroids under electron impact, Galli and Maroni[52] nevertheless believe that type RDR decomposition in ring *B* is more important. They detected peaks with *m/e* 94, 108, and 122 (the ions u) in the mass spectra of compounds (LII), (LIII), and (LIV) respectively, which are probably formed as a result of RDR after the elimination of the functional group at $C_{(3)}$ with an additional hydrogen atom:



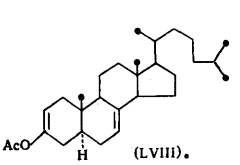
$(M-ROH)^{+\cdot}$ (LII-LIV)

u, (R' = R'' = H), *m/e* 94;
(R' = Me, R'' = H), *m/e* 108;
(R' = R'' = Me), *m/e* 122.

We believe that the ion u is difficult to employ for the identification of the Δ⁷-bond, since the spectra of all steroids contain groups of peaks of approximately equal intensity with *m/e* 91—96, 105—110, and 119—124 against the background of which it is not always possible to isolate the required peak with certainty. Moreover, the process cannot be regarded in principle as specific to the steroid olefins under consideration. For example, in the spectrum of cholest-7-en-3-one (LVII) [18] there is no peak corresponding to the ion u, but there are peaks due to ions of types t and t' (both contain the C=O group). In 5α-cholesta-2,7-dien-3β-yl acetate (LVIII),[68] RDR in ring *B* is not observed either but the peak due to the fragment caused by type RDR decomposition in ring *A*, characteristic of Δ²-steroids, is very intense (75% of the maximum peak in the spectrum).



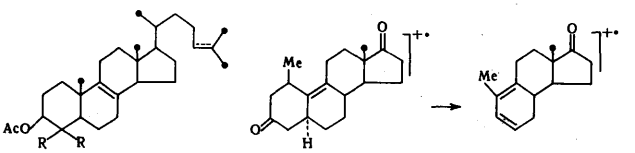
(LVII)



(LVIII).

3. Δ⁸- and Δ⁹-Steroids

In a study of the mass spectra of cholesta-8,24-dien-3β-yl (LIX), cholest-8-en-3β-yl (LX), and 4,4-dimethylcholest-8-en-3β-yl (LXI) acetates, Galli and Maroni[52] failed to observe the peaks of characteristic ions the formation of which might have been due to the Δ⁸-bond.



(LIX, R = H, Δ²⁴);
(LX, R = H);
(LXI, R = Me)

M^+ (LXII)

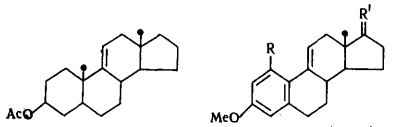
v, *m/e* 216.

The mass spectrum of 1β-methyl-19-norandrost-9-en-3,17-dione (LXII) [18] is fairly characteristic, but on the basis of this single example it is hardly possible to conclude that the decomposition pathways considered are typical for all Δ⁹-steroids. After the molecular ion peak, the most intense peak in the spectrum of compound (LXII) is that of the ion with *m/e* 216 (v), which is most probably formed as a result of the ejection of the 1-CH_3 group and the cyclopropanone moiety (atoms $C_{(2)}$ – $C_{(4)}$) from ring *A*, although one cannot rule out the possibility that this ion can be formed from the $(M-15)^+$ ion with scission of part of ring *D* (atoms $C_{(15)}$ – $C_{(17)}$).

IV. STEROIDS WITH A DOUBLE BOND IN RING *C*

1. Δ⁹⁽¹¹⁾-Steroids

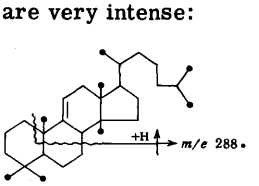
Despite the fact that the double bond in the 9—11 position might initiate type RDR decomposition, the mass spectra of androst-9(11)-en-3β-ol (LXIII) and derivatives of Δ⁹⁽¹¹⁾-dehydro-oestrone (LXIV, a—c) [3,69] do not contain the corresponding peaks:



(LXIII) (LXIV)

(a, R = H, R' = H, OH);
(b, R = OMe, R' = O);
(c, R = Me, R' = O)

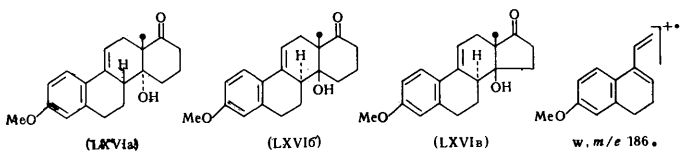
This is in full agreement with Reed's observation[11] that the peak of the *m/e* 204 ion (produced as a result of a type RDR decomposition) has only a slight intensity in the spectrum of lanost-9(11)-ene, while the peak of the $(M-15)^+$ ion (evidently produced by the ejection of the 19-CH_3 group) and the peak of the *m/e* 288 ion formed as a result of the dissociation of three allylic bonds (1—10, 5—10, and 7—8) with transfer of a hydrogen atom to the charged fragment, are very intense:



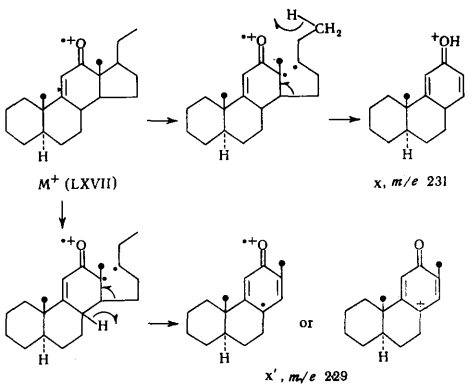
(LXV)

In contrast, in the mass spectra of the methyl ethers of Δ⁹⁽¹¹⁾-dehydro-14α-hydroxy-D-homo-oestrone (LXVIa), Δ⁹⁽¹¹⁾-14β-hydroxy-8α-D-homoiso-oestrone (LXVIb), and Δ⁹⁽¹¹⁾-dehydro-14β-hydroxy-8α-iso-oestrone (LXVIc) the dominant peaks correspond to ions with *m/e* 186 (w).[70,71]

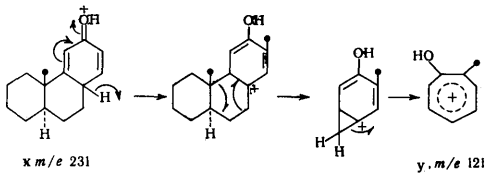
Although medium-intensity peaks with the same value of m/e are present in the mass spectra of almost all oestrone derivatives without the 9—11 double bond, their sharp increase in the spectra of compounds (LXVI, a—c) is apparently evidence that in this case the ions w are formed via a type RDR mechanism:



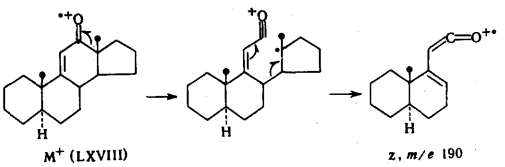
Tökes and Djerassi[72] made a mass-spectrometric study of $\Delta^{9(11)}$-12-keto-steroids and found that the mass spectrum of 5α-pregn-9(11)-en-12-one (LXVII) contains, together with the intense m/e 231 peak (the ion x), due to the elimination of ring D and the migration of a hydrogen atom to the charged moiety (the composition typical for 12-ketosteroids), there is also a peak due to an ion with m/e 229 (x′) formed as a result of an analogous process with migration of a hydrogen atom from the 8β-position in the charged species:



The maximum peak in the spectrum of compound (LXVII) with m/e 121 (the ion y) is due to further decomposition of the ion x:



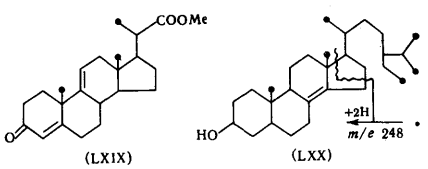
In contrast to compound (LXVII), in the mass spectrum of 5α-androst-9(11)-en-12-one (LXVIII) there are virtually no peaks due to the ions x and y. It was also suggested[72] that this is due to the absence from compound (LXVIII) of a substituent at $C_{(17)}$, which initiates the dissociation of the 13—17 bond and serves as a donor of a hydrogen atom from the 20-position in the course of the formation of the ion x. The peak of the m/e 147 ion ($C_{10}H_{11}O^+$), which contains, according to the mass-spectrometric data for the 8,11-dideutero-analogue of compound (LXVIII), the $C_{(8)}$ and $C_{(11)}$ atoms, predominates in the spectrum of compound (LXVIII). Moreover, the spectrum of this compound contains a medium-intensity peak due to an ion with m/e 190 (z) caused by the dissociation of the 8—14 and 12—13 bonds. In compound (LXVII) this process is less characteristic:



Ions of types x, y, and z are also present in the mass spectrum of the ester of 3,12-dioxobisnorchola-4,9(11)-dienoic acid (LXIX).[18]

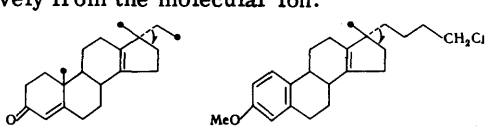
2. $\Delta^{8(14)}$-Steroids

The mass spectra of only two examples of compounds of this type have been described, namely of stigmast-8(14)-enol (LXX) and its acetate. Clark-Lewis and Dainis[57] believe that the peaks of ions with m/e 248 and 234, are characteristic of both compounds; the first is evidently formed as a result of the dissociation of the 13—17 and 15—16 bonds accompanied by the migration of two hydrogen atoms to the charged fragment. Naturally this one example is insufficient to assess whether or not this process is characteristic of all $\Delta^{8(14)}$-steroids.



V. STEROIDS WITH A DOUBLE BOND IN RING D

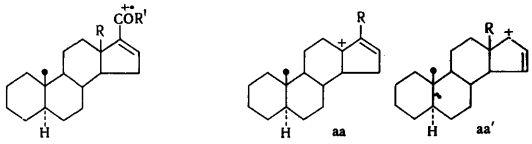
The presence of a double bond in the 13—14 position in the steroid molecule stimulates the dissociation of the allylic 17—20 bond with elimination of the substituent at $C_{(17)}$ and therefore the mass spectra of 17α-ethyl-17β-methyl-18-norandrosta-4,13-dien-3-one (LXXI) and 17α-(γ-chloropropyl)-3-methoxy-17β-methyl-18-nor-oestra-1,3,5(10),13-tetraene (LXXII) show[73] very intense peaks associated with the elimination of C_2H_5 and C_3H_6Cl respectively from the molecular ion:



Peterson[32] quotes mass-spectrometric data for a group of Δ^{16}-steroids of the pregnane series, a characteristic feature of which is the absence of peaks due to the elimination of ring D from the molecular ion with formation of the so called "tricyclic fragment" containing rings A, B, and C. Generally speaking, this process should be inhibited in all compounds containing a double bond in any position in ring D, since its occurrence requires the dissociation of at least one double bond.

Tökes and Djerassi[72] confirmed the absence of the peak due to the "tricyclic fragment" from the spectrum of 5α-pregn-16-en-20-one (LXXIII) and noted that the most intense peaks in the spectrum are those of the $(CH_3CO)^+$ and $(M - CH_3CO)^+$ ions with m/e 43 and 257 respectively, due to the dissociation of the 17—20 double bond, i.e. a

process which is extremely unfavourable on energy grounds. To account for this anomaly, the authors suggested that this process involves a rearrangement accompanied by the migration of the CH_3 group from the 13-position to the 17-position and the formation of a stable allyl carbonium ion **aa**. On the basis of this single instance, the authors conclude that the dissociation of the double bond may be facilitated if it is associated with a simultaneous rearrangement. From our point of view, the formation of the $(M - CH_3CO)^+$ ion may be due to a preliminary migration in the molecular ion of the double bond to the 15—16 position followed by the dissociation of the 17—20 bond, which has become allylic, and the formation of the ion aa′:



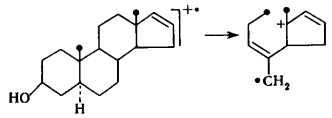
M^+ (LXXIII, R = R′ = Me); (LXXIIIa, R = CD_3, R′ = Me); (LXXIIIб, R = Me, R′ = CD_3)

aa *m/e* 257 (R = Me); *m/e* 260 (R = CD_3); aa′ *m/e* 257 (R = Me).

Studies of the mass spectra of trideuteromethyl analogues of compounds (LXXIIIa and b) showed[72] that, in contrast to saturated pregnanes, in which the elimination of the CH_3 group from the 13-position does not occur, in the mass spectrum of compound (LXXIII) the peak of the $(M - 15)^+$ ion has been generated to the extent of 82% as a result of the loss of this particular methyl group.

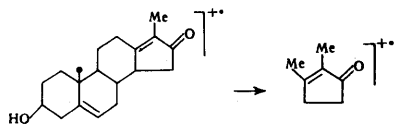
It is noteworthy that the elimination of the side chain from the $C_{(17)}$ atom is believed by many investigators[74-76] to be typical also for $\Delta^{13(17)}$-steroids, although they did not consider the mechanism of this process. Probably in the present case too there is a preliminary migration of the double bond to the 13—14 position with subsequent dissociation of the 17—20 bond, which has become allylic.

The peak of the ion with *m/e* 148, to which structure **bb** was ascribed, was detected in the mass spectrum of 5α-androst-16-en-3β-ol (LXXIV).[9] Von Unruh and Spiteller[55] believe that this ion is common to all steroids in which the structures of rings *B*, *C*, and *D* are analogous to those of compound (LXXIV):



M^+ (LXXIV) bb, *m/e* 148.

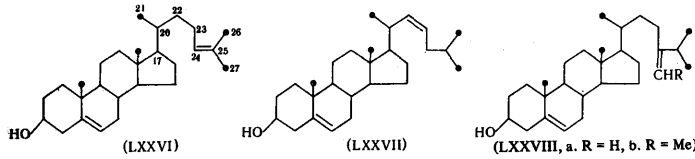
An interesting example of the effect of the double bond in ring *D* on decomposition under electron impact is provided by the mass spectrum of 3β-hydroxy-17-methyl-18-norandrosta-5,13(17)-dien-16-one (LXXV),[18] which shows a medium-intensity peak with *m/e* 110 (the ion **bb**) due to the dissociation of two allylic bonds 8—14 and 11—12 with migration of two hydrogen atoms to the charged fragment. Probably, as in the formation of the ion **i** (section II, 2), the driving force of this process in compound (LXXV) is provided by the αβ-unsaturated ketone grouping in ring *D*:



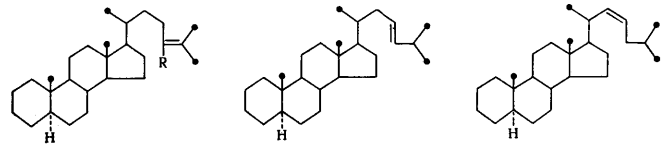
M^+ (LXXV) u, *m/e* 110.

VI. STEROIDS WITH A DOUBLE BOND IN THE SIDE CHAIN AT $C_{(17)}$

Wyllie and Djerassi[77] demonstrated for a large number of steroids with a double bond in different positions in the side chain at $C_{(17)}$ that the scission of the side chain is accompanied by the migration of two hydrogen atoms from the charged fragment. This distinguishes the above type of steroids from their saturated analogues which lose the side chain as a result of simple dissociation of the 17—20 bond. Thus the spectra of cholesta-5,24-dien-3β-ol (LXXVI), cholesta-5,22-dien-3β-ol (LXXVII), 24-methylenecholesterol (LXXVIIIa), and 24-ethylidenecholesterol (LXXVIIIb) show peaks with *m/e* 271, while in those of 5α-cholest-24-ene (LXXIXa), 5α-ergost-24-ene (LXXIXb), 5α-cholest-23-ene (LXXX), and 5α-cholest-22-ene (LXXXI) the mass numbers of the above peaks are displaced to *m/e* 257.

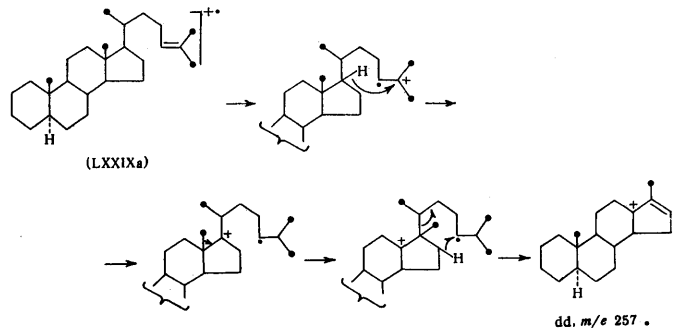


(LXXVI) (LXXVII) (LXXVIII, a. R = H, b. R = Me)



(LXXIX, a. R = H, b. R = Me) (LXXX) (LXXXI).

Analysis of the mass spectra of 17,22,22-D_3-, 15,15,17-D_3-, 12,12-D_2-, 14-D_1, and 16,16-D_2-analogues of (LXXIXa) showed that the rearrangement leading to the formation of the *m/e* 257 ion (**dd**) involves the hydrogen atom at $C_{(17)}$ to the extent of 100%, while the second hydrogen atom migrates from $C_{(12)}$ (35%), $C_{(14)}$ (10%), and $C_{(16)}$ (25%). The mechanism proposed[77] for the formation of the ion **dd** includes also the migration of the 18-CH_3 group to the $C_{(17)}$ atom, which leads to an allylically stabilised ion:



(LXXIXa)

dd, *m/e* 257.

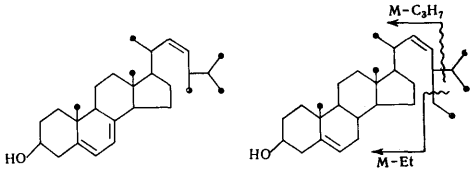
In Δ^{22}-steroids, the hydrogen atoms at $C_{(17)}$ (100%) and $C_{(14)}$ (55%) are involved in the formation of the ion **dd**, which has also been confirmed by the corresponding shifts in the mass spectra of the deutero-analogues[77].

The presence in the mass spectrum of the steroid of the peak due to the ion **dd** is evidence that it contains an unsaturated side chain at $C_{(17)}$ but does not provide information about the location of the double bond in it. As the position of the double bond is altered, the intensity of the peak due to the ion **dd** changes too, which has been recommended[77] as a feature suitable for diagnostic purposes. However, the mass spectra of steroids with

an unsaturated side chain contain many characteristic peaks, which reflect more rigorously the location of the double bond.

1. Δ^{22}-Steroids

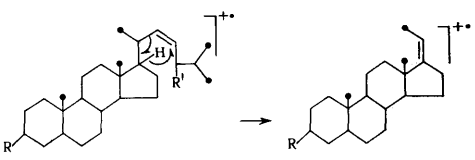
A characteristic feature of this group of steroids, together with the peaks of the ion **dd**, is the presence in the mass spectra of intense peaks caused by the elimination of the side chain at $C_{(17)}$ from the molecular ion without the migration of hydrogen atoms. The driving force of this process is provided by the dissociation of the 17—20 allylic bond. Thus the most intense peaks correspond to *m/e* 271 in the spectrum of ergosterol (LXXXII),[23,78] *m/e* 273 in the spectra of stigmasterol (LXXXIII)[47,77] and 7,8-dihydroergosterol (24-methylcholesta-5,22-diene-3β-ol),[77] and *m/e* 259 in the spectrum of 5α-cholest-22-ene (LXXXI).[77] In the mass spectra of the above compounds, all the peaks are present as doublets with those due to the ions **dd** differing from the peaks described above by 2 a.m.u.



(LXXXII) (LXXXIII).

In addition, the spectra of stigmast-22-en-3β-yl acetates (LXXXIV)[79] and (LXXXIII)[77] contain peaks due to the $(M - C_2H_5)^+$ and $(M - C_3H_7)^+$ ions produced as a result of the elimination of part of the side chain owing to the dissociation of other allylic bonds.

The mass-spectra of compounds (LXXXI)[77] and (LXXXIV)[79] also show peaks due to ions with *m/e* 344 and 286 (ee), caused respectively by the dissociation of the 20—22 double bond with transfer of a hydrogen atom from the charged species. It has been noted that for low ionisation energies the *m/e* 286 peak in the spectrum of compound (LXXXIV) becomes a maximum. Naturally when the molecule contains an acetoxy-group [for example, as in compound (LXXXIV)] the peak of the ion ee is accompanied by that of the $(ee - CH_3COOH)^+$ ion:



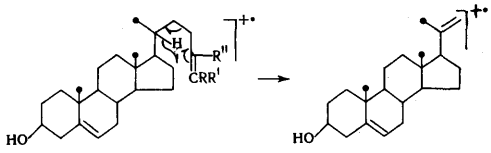
M^+ (LXXXI, R = R' = H); (LXXXIV, R = OCOMe, R' = Et) ee (R = H), *m/e* 286; (R = OCOMe), *m/e* 344

2. Δ^{23}-Steroids

The mass spectrum of 5α-cholest-23-ene (LXXX)[77] contains, together with the intense peak of the ion **dd** (*m/e* 257), an appreciable peak due to the ion with *m/e* 287 produced by the dissociation of the allylic 20—22 bond. When the ionising potential is reduced, the peak of the ion **dd** becomes dominant in the spectrum.

3. Δ^{24}- and 24-Alkylidene-derivatives of Steroids

In the mass spectra of compounds of this type, the peak of the ion **dd** is one of the most intense. On the other hand, the presence of very intense peaks of ions of type **ff**, generated by the dissociation of the allylic bond 22—23 with the elimination of a hydrogen atom, probably from the 20-position, is frequently characteristic of such compounds, for example (LXXVIIIa and b),[77] (LXXIXa and b),[77] desmosterol (LXXXV),[47] and many other 24-alkylidene-derivatives discussed by Knights and coworkers[47,80,81] and Benveniste et al.[82] Peaks of ions of type **ff** are characteristic only of the spectra of Δ^{24}- and 24-alkylidene-derivatives of steroids:

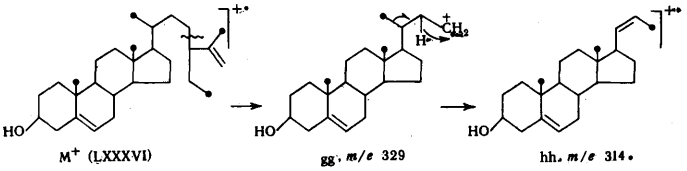


M^+ (LXXVIIIa, R = R' = H, R'' = C_3H_7); (LXXV, R = R' = Me, R'' = H) **ff**, *m/e* 314.

Galli and Maroni[52] noted that the most intense peaks in the spectra of cholesta-5,24-dien-3β-yl (XLVI) and cholesta-8,24-dien-3β-yl (LIX) acetates are those of the $(CH_3)_2C{=}\overset{+}{C}HCH_2$ ion with *m/e* 69, produced by the dissociation of the allylic 22—23 bond.

4. Δ^{25}-Steroids

The only example of a Δ^{25}-steroid, namely stigmasta-5,25-dien-3β-ol (LXXXVI), has been described by Wolfgang[83], who pointed out the presence in the spectrum of *m/e* 329 and 314 peaks, but did not discuss the question of their origin. We believe that the first (the ion **gg**) is due to the dissociation of the allylic 23—24 bond, while the second (the ion **hh**) may be formed as a result of the subsequent loss by the ion **gg** of one of the methyl groups, for example at $C_{(20)}$:[18]



M^+ (LXXXVI) **gg**, *m/e* 329 **hh**, *m/e* 314.

In conclusion of this section, we may note that in the majority of the studies quoted the decrease of the intensities of the characteristic peaks following the introduction of additional double bonds into the nucleus was considered. On the other hand, according to the observations of Knights[47], the appearance of a double bond in the side chain hinders certain processes characteristic of the decomposition of Δ^5- and Δ^7-steroids.

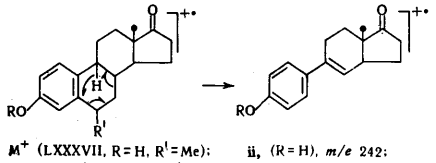
VII. AROMATIC AND DIENE STEROIDS

Certain aromatic and diene steroids were frequently mentioned in the previous sections but as a rule these were compounds with unsaturated centres fairly remote from one another which have little mutual influence and do not alter the fundamental decomposition pathways characteristic of each (see sections II, 2; III, 3; IV, 1; VI; VI, 1, 3, and 4). Aromatic and diene steroids containing conjugated or very close unsaturated centres, which

sometimes alters radically the fragmentation pattern characteristic of the corresponding monoenes, constitute a special group.

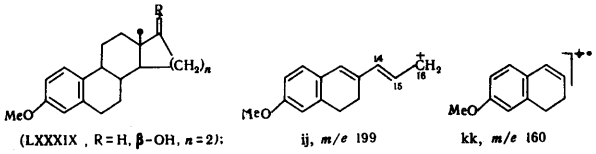
1. Aromatic Steroids

Djerassi et al.[69] investigated the mass spectra of many steroids with one and two aromatic rings, belonging to the group of oestrone and equilenin. The presence of the aromatic ring in these compounds greatly stabilises the molecular ion, the peak of which is as a rule dominant in the spectrum. In the high-molecular-weight region of the spectrum of 6-methyloestrone (LXXXVII) and the methyl ether of oestrone (LXXXVIII), there are intense peaks due to the $(M - C_2H_4)^+$ and $(M - C_3H_6)^+$ ions caused respectively by the elimination of the $C_{(6)}$ and $C_{(7)}$ atoms with formation of the ion ii:



M^+ (LXXXVII, R = H, R' = Me); (LXXXVIII, R = Me; R' = H) ii, (R = H), *m/e* 242; (R = Me), *m/e* 256

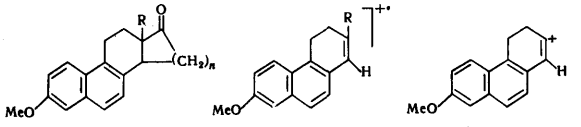
The peak of the *m/e* 199 ion (jj), for the formation of which a complex mechanism has been proposed involving the loss of the $C_{(11)}$–$C_{(13)}$ and $C_{(17)}$ atoms, is one of the most characteristic in the spectra of compound (LXXXVIII) and its stereoisomers. The peak is observed also in the spectra of the stereoisomers of the methyl ether of oestradiol (LXXXIX) [27,70] and the methyl ether of D-homo-oestrone (XC).[84]



(LXXXIX, R = H, β-OH, *n* = 2); (XC, R = O, *n* = 3) ij, *m/e* 199 kk, *m/e* 160

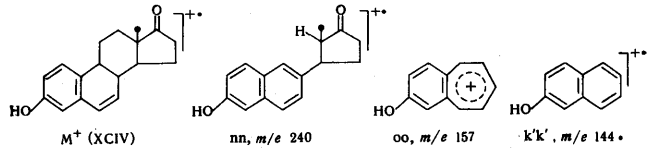
The mass spectra of all the compounds considered above, and also of their 14-hydroxy-derivatives (LXVI, a–c) [71] contain intense peaks due to *m/e* 160 ions (kk) formed as a result of the dissociation of the 8–14 and 9–11 bonds and the ion w with *m/e* 186 (see section IV, 1).

In the mass spectra of the methyl ether of equilenin (XCI),[69] its 18-nor-13-ethyl-analogue (XCII),[69] and the methyl ether of D-homoequilenin (XCIII),[84] there are also intense peaks due to molecular ions. On the other hand, the characteristic peaks are those of the "tricyclic ions" ll and mm, the formation of which involves the dissociation of bonds in ring *D*:



(XCI, R = Me, *n* = 2); (XCII, R = Et, *n* = 2); (XCIII, R = Me, *n* = 3) ll, (R = Me), *m/e* 224; (R = Et), *m/e* 238 mm, *m/e* 209

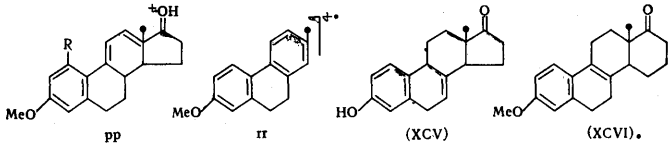
In the spectrum of Δ^6-dehydro-oestrone (XCIV) [69] there is an intense M – 28 peak (the ion mm), the formation of which probably involves the elimination of an ethylene molecule from ring *C*. The most intense peaks in the spectrum of compound (XCIV) with *m/e* 157 and 144 most probably correspond to the ions oo and k'k' respectively:



M^+ (XCIV) nn, *m/e* 240 oo, *m/e* 157 k'k', *m/e* 144.

The mass spectrum of equilin (XCV) [69] differs from that of compound (XCIV) only by a lower intensity of the peak due to the ion oo.

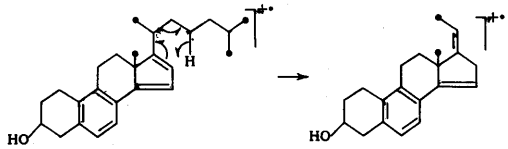
The peaks due to $(M - 15)^+$ ions, which may have the structure pp, are dominant in the mass spectra of the methyl ether of $\Delta^{9(11)}$-dehydro-oestrone and its 1-methyl analogue (LXIVc) (see section IV, 1).[69] Another characteristic feature of the spectra of these compounds is the presence of an M – 57 and M – 58 double peak (the ion rr) due to the loss of ring *D*:



pp rr (XCV) (XCVI).

The characteristic ions derived from the methyl ether of Δ^8-dehydro-D-homo-oestrone (XCVI),[27] as for the methyl ether of equilenin (XCI), are those formed as a result of the dissociation of bonds in ring *D*, namely the 6,7-di-hydro-analogues of the ions ll and mm.

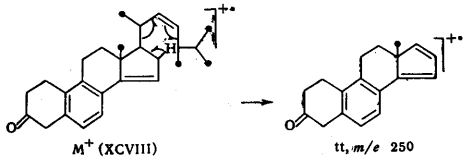
The peak of the ion corresponding to the loss of part of the side chain at $C_{(17)}$ with migration of a hydrogen atom to the charged species ss has been detected by Brown and Turner[85] in the mass spectrum of cholesta-5(10),6,8,14,16-pentaen-3β-ol (XCVII). Presumably the driving force in this process comes from the dissociation of the allylic 20–22 bond accompanied by the migration of the double bond and the transfer of a hydrogen atom via a six-membered transition state:



M^+ (XCVII) ss, *m/e* 280.

However, on the basis of this one example, it is impossible to say whether the process is determined by the Δ^{16}-bond alone or whether it can occur only in the presence of a conjugated system of double bonds.

In ergosta-5(10),6,8,14,22-pentaen-3-one (XCVIII),[85] the side chain is eliminated as a result of the rupture of the allylic 17–20 bond accompanied by the elimination of a hydrogen atom from the charged species (the ion tt).



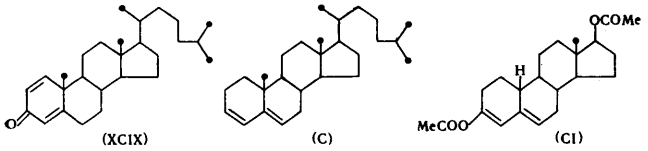
M^+ (XCVIII) tt, *m/e* 250

Apparently the elongation of the conjugation chain provides the driving force for the formation of the ion tt.

2. Diene Steroids

The intense peak of the ion with m/e 122 is the most characteristic of the mass spectrum of cholesta-1,4-dien-3-one (XCIX).[22] It is probably formed via a mechanism similar to that of the formation of the ion i (see section II, 2) from which it differs by two mass units, as expected.

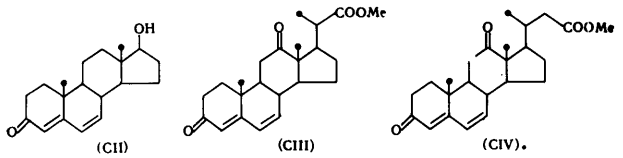
Galli and Maroni[52] believe that the most characteristic type of fragmentation of cholesta-3,5-diene (C) involves the formation of the ion p' with m/e 120 (see section III, 1) as a result of type RDR decomposition in ring B. The peak of an analogous ion with m/e 122 is observed also in the spectrum of 19-norandrosta-3,5-diene-3,17β-diyl diacetate (CI).[18] When the molecular ion of compound (CI) decomposes, a molecule of keten is initally eliminated from the 3-CH_3COO group (a process characteristic of enol acetates) and the resulting radical-ion undergoes a further type RDR decomposition). Moreover, ions with m/e 147 (tt) and 161 (uu) are formed from the $(M-CH_2=C=O)^+$ ion.



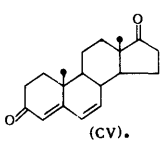


The mass spectrum of cholesta-2,4-diene (XI) (see section II, 2) is not very characteristic[18] but in the region of low mass numbers it contains a very specific triplet of peaks with m/e 105–107. The formation of ions responsible for these peaks probably involves the dissociation of the 6–7 and 9–10 bonds and the location of the charge on ring A.

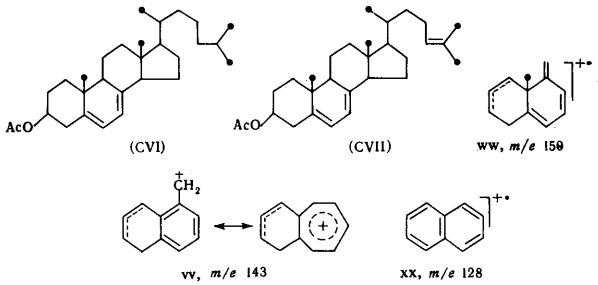
In the mass spectrum of 17β-hydroxyandrosta-4,6-dien-3-one (CII),[9,23] there are intense peaks due to ions with m/e 136 and 157, the origin of which has not, however, been elucidated. These peaks are also observed in the mass spectra of the methyl esters of 3,12-dioxobisnorchola-4,6-dienoic (CIII) [38,39] and 3,12-dioxonorchola-4,6-dienoic (CIV) [38] acids:



Recently Orr and Broughton[86] also observed the peak of the m/e 136 ion in the mass spectrum of androsta-4,6-diene-3,17-dione (CV) and explained its origin by the dissociation of the 9–10 and 7–8 bonds and the migration of two hydrogen atoms to the charged fragment containing ring A and part of ring B. When the charge is located in the other part of the molecule with migration of a hydrogen atom away from it, an ion with m/e 149 is formed with the dissociation of the same bonds, its peaks being approximately three times less intense than that of the first ion:



Galli and Maroni[52] have analysed the mass spectra of cholesta-5,7-di-en-3β-yl (CVI) and cholesta-5,7,24-trien-3β-yl (CVII) acetates. The authors noted intense peaks due to ions with m/e 143 (vv) and 158 (ww) and a small peak with m/e 128 (xx).



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Thus the entire data analysed above demonstrate that the mass spectra of steroids with double bonds in the 1–2, 2–3, 4–5, 5–6, 7–8, 13–14, 16–17, 22–23, 23–24, and 24–25 positions are very characteristic which as a rule makes it possible to infer unambiguously the location of the double bond in the steroid nucleus or in the side chain by analysing the mass spectra. The double bonds in other positions either do not initiate specific decomposition pathways of the molecular ions or give rise to processes which are difficult to interpret from the standpoint of the current hypothesis of the preferential dissociation of the weakest bonds. In certain cases the insufficient number of examples investigated precludes a generalisation. Steroids with several double bonds remote from one another decompose via pathways initiated by each separately. The inclusion of double bonds in the conjugated system gives rise to special decomposition pathways which are not characteristic of compounds with the corresponding isolated double bonds, and the stability of the molecular ion increases appreciably.

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Shemyakin Institute of the
Chemistry of Natural
Products, USSR Academy
of Sciences