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RECENT ADVANCES IN THE MASS SPECTROMETRY OF STEROIDS

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Abstract - Mass spectrometry plays a crucial role in the structure elucidation of new sterols, notably those of marine origin. Secure interpretation of the mass spectra requires an adequate understanding of the mechanism of the key fragmentation processes of steroids and the present paper summarizes recent work from the author's laboratory dealing with the elucidation of the fragmentation mechanism of the most important peaks of the following classes of steroids: (a) saturated steroid hydrocarbons with the 14α and 14β stereochemistry; (b) Δ^{7}- and $\Delta^{8(14)}$-unsaturated sterols; (c) Δ^{4}-, $\Delta^{1,4}$- and $\Delta^{4,6}$-unsaturated 3-ketosteroids; (d) sterols with unsaturated side chains. The techniques of deuterium labelling and of metastable defocussing proved to be indispensable in these studies.

INTRODUCTION

No conference of natural product chemists needs any argument about the enormous utility of mass spectrometry in their chosen field. This method and nuclear magnetic resonance have become the two essential components of the instrumental arsenal of any chemist concerned with the structure elucidation of natural products. In the steroid field, structure proof of new substances from natural sources had slowed down greatly by the middle 1960's, but in recent years has suddenly encountered a spectacular renaissance through the search by many European, Japanese and American investigators for new sterols from marine sources. While it has been possible, utilizing a variety of separation methods (1), to demonstrate the presence of nearly 50 steroids in a single marine animal, the quantities are frequently so small as to permit only a single gas chromatography-mass spectral measurement on which the subsequent structure determination must rest.

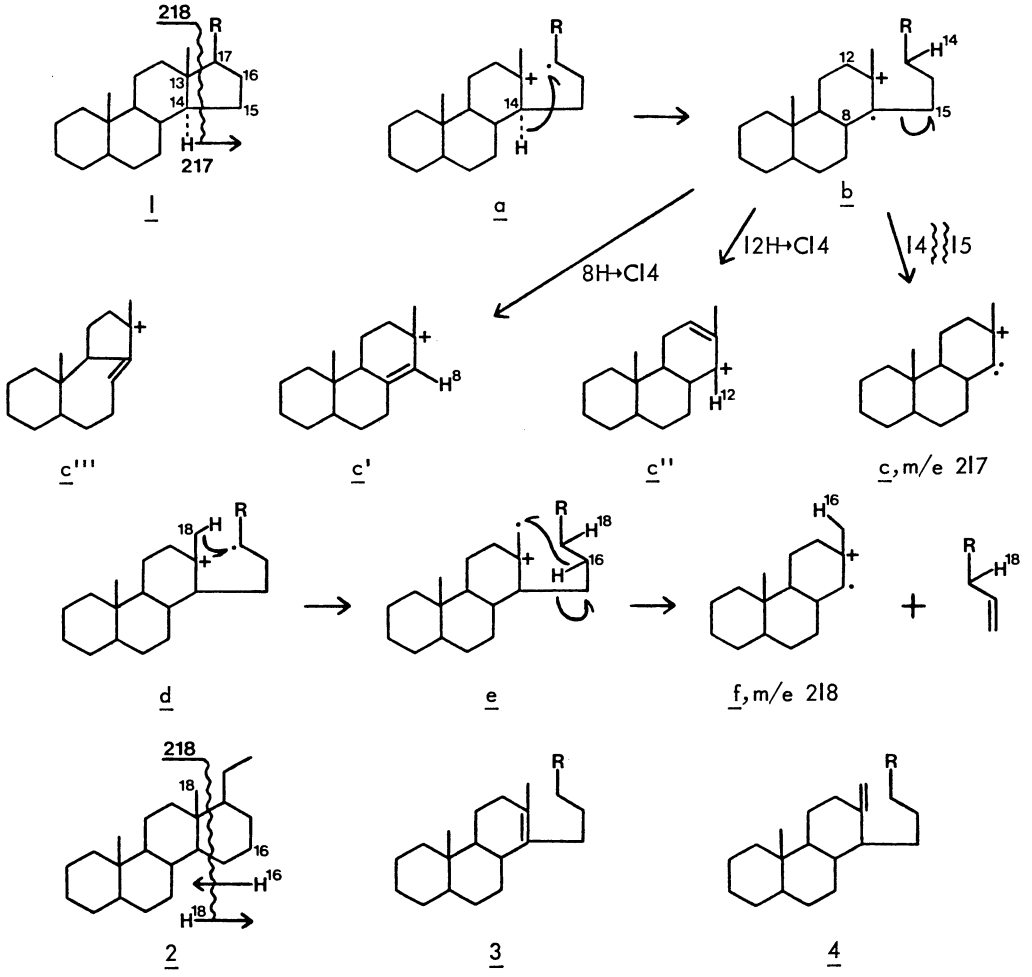
Intelligent and confident interpretation of such mass spectra requires some knowledge of the mechanism of the most significant fragmentation processes. In this regard, modern organic mass spectrometry started around 1960 and steroids were among the first classes of organic compounds to be subjected to serious scrutiny in this regard. This is demonstrated by the appearance of a book on this subject (2) as early as 1964, while continuing interest and efforts in this subject are summarized in a more recent book published in 1976 (3). These compendia cover the literature adequately and the present report deals solely with recent and largely unpublished work from our own laboratory. Most of our present work on mass spectral fragmentation mechanisms is prompted by our concurrent investigations of new sterols from marine sources (1) and centers on those fragmentations which have proved to be of diagnostic utility in the marine steroid field.

RING D FRAGMENTATION OF SATURATED STEROLS AND STERANES

As illustrated in Fig. 1 for 5α-cholestane (1, R = C_8H_{17}), the two most characteristic fragmentation peaks of saturated steroids bearing a C-17 substituent occur at m/e 217 and 218 (shifted appropriately in the presence of certain nuclear substituents such as the ubiquitous 3-hydroxyl group) and have proved to be of great diagnostic use in structure elucidation work. Many rationalizations have been offered (reviewed in ref. 4) for these two processes, but the correct overall picture emerged only from an examination of a variety of deuterium labelled substrates synthesized in our laboratory (4). This work, which was completed nearly a decade ago, demonstrated that the genesis of the m/e 217 peak in Fig. 1 is correctly represented graphically as shown in structure 1 and that the principal origin of the migrating hydrogen atom is C-14. While the transfer of a hydrogen atom from C-14 of the molecular ion species a is eminently plausible, since it leads to the ionized equivalent b of the most highly substituted (tetrasubstituted) olefin (i.e. 3) possible under these circumstances, the subsequent fission of the 14-15 bond was not anticipated since it involves

the a priori unexpected cleavage of two bonds connected to one carbon atom. The resulting ion would then be an ionized carbene of type c or the isoelectronic vinyl carbonium ion - a species which from solution chemistry is known (5) to be reasonably stable. Alternative possibilities would be the allylic carbonium ion c', c'' or c''' resulting from a 1,2-shift of the C-8 hydrogen, a 1,3-shift from the C-12 hydrogen (C-18 hydrogen migration would give a related ion) or a shift of the 8,9 bond respectively. Isotopic labelling can, of course, not differentiate among those possibilities.

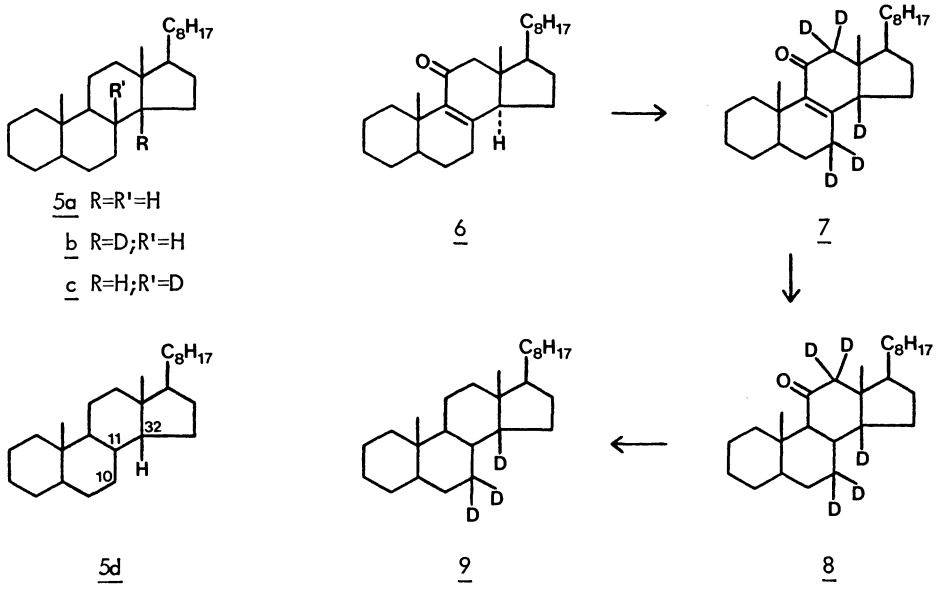
The m/e 218 peak seemed to be the result of simple fission of the 13-17 and 14-15 bonds (see 1) leading to the ionized equivalent of an olefin and ejection of a neutral cyclopropane encompassing carbon atoms 15,16 and 17. The earlier mentioned (4) isotopic labelling studies disproved this simple picture and showed that a reciprocal hydrogen transfer involving the hydrogens attached to C-18 (d) and C-16 (e) had to be invoked leading to the ionized olefin f and a neutral olefin. The driving force for this intriguing reciprocal hydrogen transfer is the desire to expel a neutral olefin rather than a cyclopropane and the generality of this process was further demonstrated (6) with suitably labelled analogs of the D-homopregnane 2 in which transfer of the C-18 and C-16 hydrogens was again documented. The plausibility of these representations was also supported by the synthetic work of Mammato and Eadon (7) with labelled analogs of the phenanthrene hydrocarbons 3 and 4 (R = CH_3).



The indication that ionized carbenes (c) or the isoelectronic vinyl carbonium ions might be important intermediates in mass spectral fragmentations appeared to us to be of sufficient intrinsic importance so as to warrant further work. The possibility of intermediates of type c' and c''' could be examined by investigating appropriately labelled Δ^7-unsaturated steroids (see below). From a synthetic standpoint (8), the preparation of a 14-d-Δ^7-unsaturated sterol is much simpler in the 14β than in the 14α series. While the mass spectra of 14α- (1) and 14β-cholestane (5a) are qualitatively similar (Fig. 1), it was necessary to show that for mechanistic work of the ring D fragmentation of Δ^7-unsaturated steroids, 14α-

and 14β-isomers can be used interchangeably. Consequently, we synthesized (9) 14β-d-5α-cholestane (5b) and found that only 32% of the hydrogen was transferred from C-14, in contrast to the 75% (4) in the 14α-series (1). While a number of mass spectral fragmentation processes exhibit sensitivity to stereochemical features (10), such a large difference between a 14α- and a 14β-steroid was totally unanticipated and clearly warranted additional synthetic efforts (11).

The first labelled analog to be made (11) was 8-d-14β-cholestane (5c), since transfer from that position would lead to highly favored, tertiary allylic carbonium ion (cf. c'), but only 11% migration was encountered from that position (compared to 8% in the 14α-series). Labelling of C-7 (for synthetic route see 6 → 7 → 8 → 9) showed that 10% of the migrating hydrogen (Note a) originated from that center, compared to the 6% encountered (4) in the 14α-series. The percentage of hydrogen migrating from identified sites in 14β-cholestane (5a) is summarized in 5d and accounts for only about half. Further labelling is underway and it is tempting to speculate that position 12 (4% in 14α-series) is an important candidate. In any event, inspection of Dreiding models does not offer any convincing argument why abstraction of hydrogen from C-14 in the 14α-series should be so favored on stereochemical grounds.



RING D FRAGMENTATION OF Δ^{7}- AND $\Delta^{8(14)}$-CHOLESTENE

Our interest in studying (12) the mechanism of the ring D fragmentation of Δ^{7}- (10a) and $\Delta^{8(14)}$- (15a) cholestene was prompted by two reasons. First, Δ^{7}-unsaturated sterols are very common among marine sources, notably starfish, and any diagnostic features associated with their mass spectra are of practical utility. The second and more fundamental reason is that Δ^{7}-cholestene (10a), the parent hydrocarbon in this series, displays in its mass spectrum (13) a significant m/e 215 peak, which is the counterpart of the m/e 217 peak (c) associated with the intensely studied ring D cleavage of saturated steroids. As noted above, two formulations for this ion, namely c' and c''', involving internal migration of a C-8 hydrogen atom or of the 8-9 bond would not be feasible in Δ^{7}-cholestene (10a). Therefore, if it can be demonstrated that migration of the C-14 hydrogen atom is also involved in the genesis of the mass 215 ion of Δ^{7}-cholestene (10a), then this would provide very strong support in favor of an ionized carbene (or vinyl carbonium ion) such as c or else of the intervention of internal 1,3-hydrogen shifts (e.g. c'').

In view of the apparent sensitivity of the ring D fragmentation to the stereochemistry of the C/D fusion, all labelling work had to be performed in the 14α-series (10). The most direct route (12) to 14α-d-Δ^{7}-cholestene (10b) involved deuterioboration of the 7,14-dien-3β-ol 11 followed by removal of the 3β,15α-diol functions of 12 by metal-ammonia reduction of the

Note a. All values are corrected for the alternate m/e 217 fragmentation (loss of carbon atoms 16,17 and 19 - see ref. 4) as established by labelling of C-15.

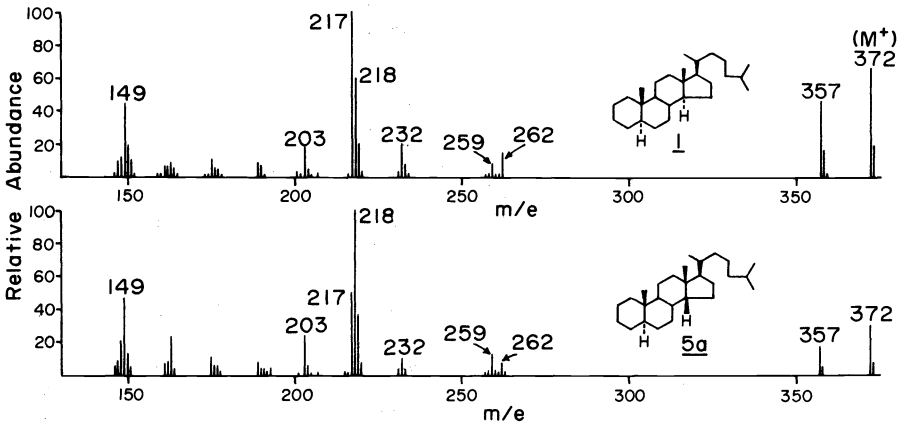


Fig. 1 Mass spectra (70 eV) of 5α-cholestane (1) and 5α,14β-cholestane (5a)

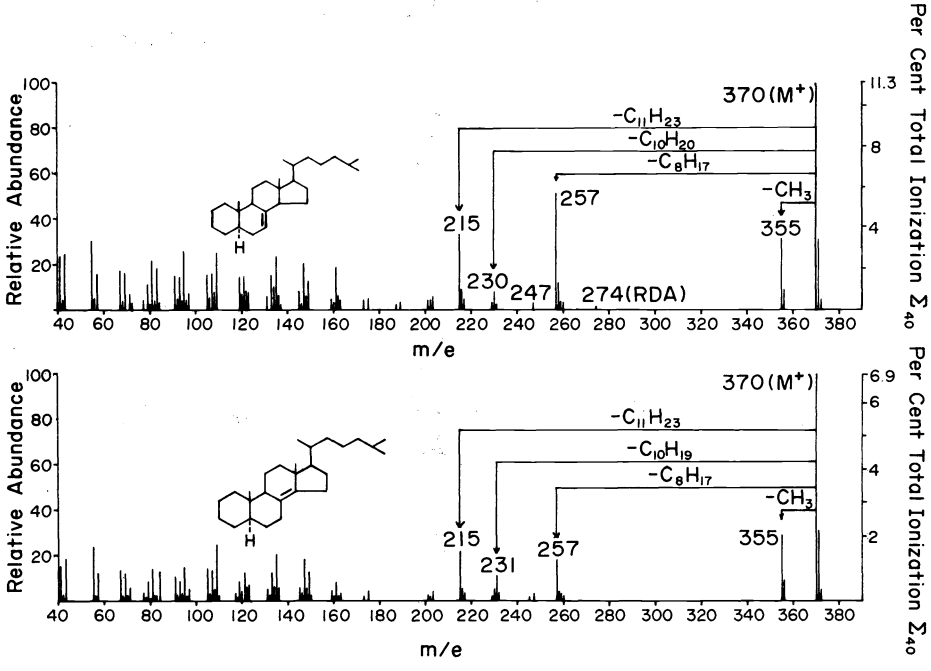
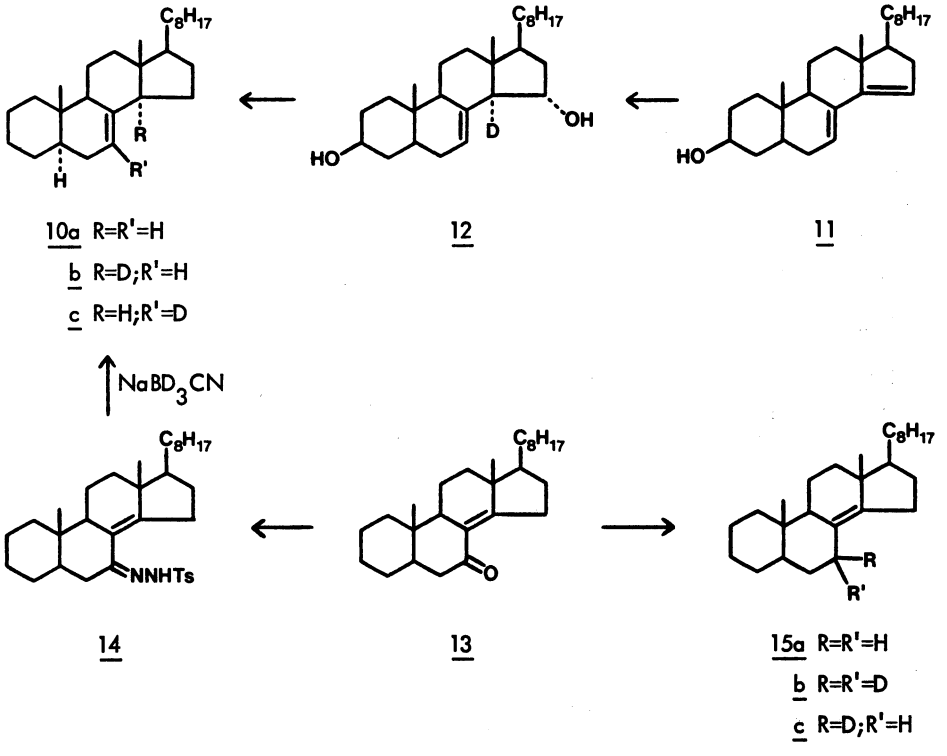


Fig. 2 Mass spectra (70 eV) of Δ^{7}-5α-cholestene (10a) and $\Delta^{8(14)}$-5α-cholestene (15a)

phosphorodiamidate. The mass spectrum indicated the transfer of only 27% of hydrogen from that position and hence necessitated labelling of various other positions to locate the source of the remaining hydrogen that migrates in this fragmentation. To our surprise, we observed that 29% originated from the a priori least likely site, the vinylic C-7 position as shown by the synthesis of 7-d-Δ^{7}-cholestene (10c), which was obtained by sodium cyanoborodeuteride reduction (14) of the tosylhydrazone 14. Since the mass spectra (Fig. 2) of the Δ^{7}- and $\Delta^{8(14)}$-cholestenes are virtually identical, thus suggesting a common precursor after ionization, 7,7-d_2-$\Delta^{8(14)}$-cholestene (15b) was synthesized (12) from $\Delta^{8(14)}$-cholesten-7-one (13) by an adaptation of the method of Cunningham and Overton (15). The observation that 74% of the migrating hydrogen in 15a originates from C-7 is readily rationalized by considering the intermediacy of the molecular ion g in which the C-7 hydrogen atoms are not only easily accessible but also allylically labilized. The resulting ionized butadiene i (in its resonance form i') is then simply the unsaturated counterpart of ion b in the saturated steroids. Homolytic cleavage of its 14-15 bond would yield the expected mass 215 ion, which could again be represented as the ionized carbene j or else as the butadienyl cations j' or j'' if one assumed 1,3-hydrogen shift from C-12 or C-18 respectively. These results with $\Delta^{8(14)}$-cholestene (15a) and its 7,7-d_2 analog 15b now offer a ready rationalization for the mass spectral behavior of Δ^{7}-cholestene (10a) by assuming electron impact-induced double bond migration of the latter (10a) to the former (15a). If correct, the 14α-d-Δ^{7}-cholestene (10b) would first isomerize to 7-d_1-$\Delta^{8(14)}$-cholestene (15c) and thence fragment via species h. The latter would also be the key intermediate in the fragmentation of 7-d-Δ^{7}-cholestene (10c) after initial double bond migration. If this is so, then the 7- and 14-labels would always be equally available for abstraction from C-7 via intermediate g and the fact that nearly identical amounts are transferred from these two positions (27% from C-14 vs. 29% from C-7) is supportive of the view that 56 ± 2% of the hydrogen transfer must actually originate from C-7 in the isomerized steroid. Since the labelling results with 15b showed that in $\Delta^{8(14)}$-cholestene (15a) 74% of the hydrogen migrating to the expelled ring D fragment originates from C-7, it follows that at least 76% of the Δ^{7}-cholestene (10a) isomerized to $\Delta^{8(14)}$-cholestene (15a) in the mass spectrometer in order to achieve a 56 ± 2% transfer from C-7.



10a R=R'=H
b R=D;R'=H
c R=H;R'=D

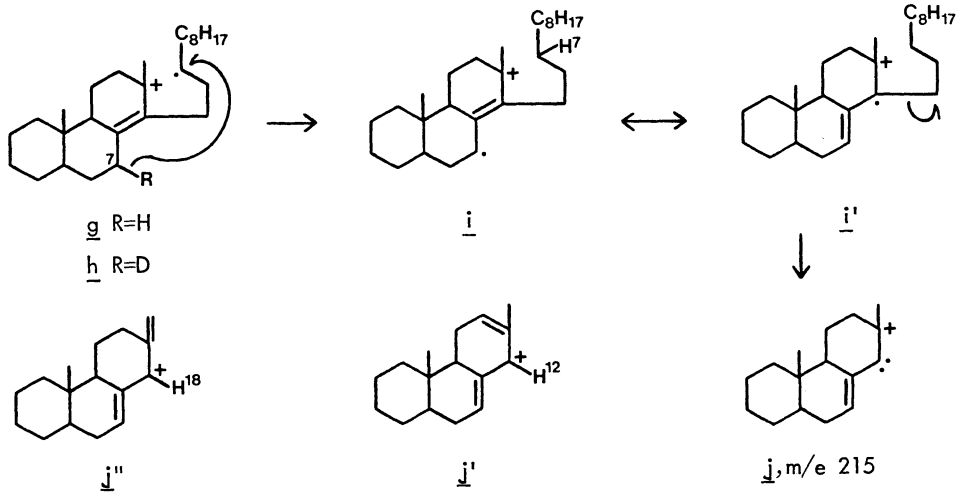
12

11

14

13

15a R=R'=H
b R=R'=D
c R=D;R'=H



Additional evidence for double bond migration (10a → 15a) is provided by the data in Table 1, which lists the origin of the $M^+ - CH_3$ peak in various steroid hydrocarbons. In pregnane and other C-17 substituted hydrocarbons, interaction between the C-17 side chain and the 18-methyl group increases the strain inherent in the C/D trans ring junction (16). This factor and the generation of a secondary vs. a primary radical at C-17 are probably responsible for the increased ring D fission in pregnane as compared to androstane (17). Rupture of the 13-17 linkage effectively blocks the cleavage of a second bond to C-13, which would explain why in pregnane only 20% of $M^+ - CH_3$ is associated with the loss of C-18, whereas in androstane (with its greatly reduced ring D fission) 63% is lost from that site. The Δ^7 double bond presumably occupies a "neutral" site in the steroid skeleton without favoring unduly expulsion of a given methyl group (cf. androstane vs. Δ^7-androstene in Table 1), whereas the $\Delta^{8(14)}$ double bond should promote loss of the 18-methyl group because of allylic activation, which is precisely what happens (cf. pregnane vs. $\Delta^{8(14)}$-cholestene in Table 1). The data in Table 1 show that the bulk of the $M^+ - CH_3$ peak in both Δ^7- and $\Delta^{8(14)}$-cholestenes (76 and 78% respectively) arises from loss of the 18-methyl group, thus supporting the concept that isomerization of the Δ^7- to the $\Delta^{8(14)}$-cholestene occurs in the mass spectrometer after electron impact but prior to fragmentation. The importance of allylic activation in the methyl elimination is further documented (Table 1) by the observation that introduction of a $\Delta^{9(11)}$ bond completely reverses the situation in favor of predominant (80%) 19-methyl expulsion.

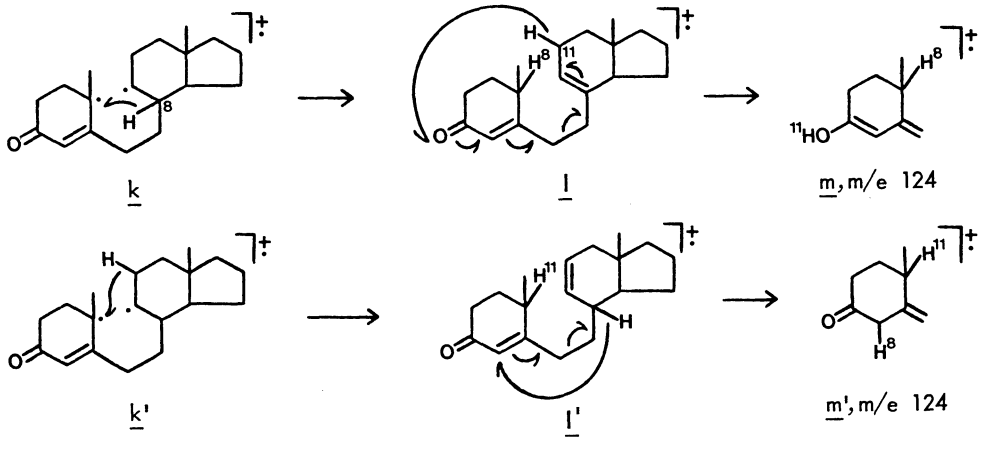
TABLE 1. Relative percent loss of C-18 and C-19 in the $M^+ - CH_3$ fragment of various steroid hydrocarbons

Steroid	C-19 loss	C-18 loss
Androstane	37	63
Δ^7-Androstene	37	63
Pregnane	80-83	17-20
Δ^7-Pregnene	32	68
Δ^7-Cholestene	24	76
$\Delta^{8(14)}$-Cholestene	22	78
$\Delta^{7,9(11)}$-Cholestadiene	80	20

Both angular methyl groups were labelled in androstane and pregnane, C-18 was labelled in Δ^7-pregnene, and C-19 was labelled in the remaining steroids.

FRAGMENTATION OF α,β-UNSATURATED 3-KETOSTEROIDS

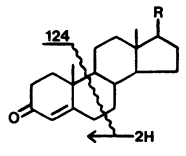
The mass spectrometric behavior of α,β-unsaturated 3-keto steroids, notably Δ^4-3-keto steroids, has stimulated interest during the earliest days of modern organic mass spectrometry (2) because of the almost ubiquitous presence of the Δ^4-3-keto moiety among biologically active steroid hormones. Our early labelling studies (18) seemed to have settled the question of the mechanism of the most diagnostic peak (m/e 124) involving the fission of ring B by identifying C-8 and C-11 as the origins for the double hydrogen transfer which accompanies this process. With this information in hand, the reaction can be visualized as proceeding in one of two ways, depending upon whether the C-8 (k → ℓ) or the C-11 (k' → ℓ') hydrogen transfer occurs first with the second hydrogen migration invariably proceeding from an allylically activated position. This second hydrogen migration is depicted from C-11 in terms of transfer to oxygen (ℓ → m), whereas we assume migration of the C-8 hydrogen to C-4 (ℓ' → m') because of appropriate interatomic distance factors as determined with Dreiding models.



Zaretskii has summarized additional data indicating that this cleavage is less intense (19) in 19-nor-Δ^4-3-ketosteroids (Note b) and that it is sensitive to stereochemical changes (20) - the "abnormal" 10-iso steroids showing more intense m/e 124 ions than their "natural" 10β-counterparts. The latter observation can be readily rationalized in the light of the structural and mechanistic representations given above irrespective of whether initial C-8 (k) or C-11 (k') hydrogen transfer occurs. In either event, this process must be concerted with the attachment of the migrating hydrogen to C-10 occurring synchronously with 9-10 bond fission and detachment of the hydrogen from its original locus. Inspection of models clearly demonstrates that this is more favored when the hydrogen can approach C-10 from the β-side and hence should proceed more readily in 10α-Δ^4-3-keto steroids.

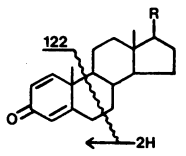
We became interested in re-examining the mass spectral behavior of steroidal α,β-unsaturated ketones when we isolated Δ^4-, $\Delta^{1,4}$- and especially $\Delta^{4,6}$-3-keto steroids from some marine animals and noted that only cursory attention had been paid to the mass spectrometric behavior of such dienones (21). The shift of the above discussed m/e 124 peak of Δ^4-3-keto steroids (16) to m/e 122 in $\Delta^{1,4}$-3-ketones (17) was expected on mechanistic grounds, but this is clearly not the case with the equally diagnostic m/e 136 peak of $\Delta^{4,6}$-3-keto steroids (18). That this latter fragmentation does indeed involve loss of rings C and D as represented graphically in structure 18 is confirmed by its shift to m/e 137 in 7-d-$\Delta^{4,6}$-androstadiene-3,17-dione (19) (ref. 22) and to m/e 122 in 19-nor-6-dehydrotestosterone (20) (ref. 23). Deuterium labelling was clearly indicated in order to uncover the origin of the two migrating hydrogens and since 17 and 18 are usually synthesized from a Δ^4-3-keto precursor (16), we were led automatically to a reinvestigation (24) of the mass spectral fragmentation of the earlier studied (18) Δ^4-3-ketones (16) while delving into that of $\Delta^{1,4}$- (17) and especially of $\Delta^{4,6}$- (18) 3-ketosteroids.

Note b. This may be an erroneous conclusion associated with other substituents, because in our hands both Δ^4-androsten-3-one and its 19-nor analog exhibited Σ_{40}=10% for this ring B fragmentation.



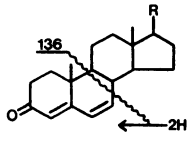
16

Σ_{40} 8% (R=C_8H_{17})



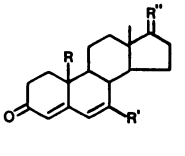
17

Σ_{40} 36% (R=C_8H_{17})



18

Σ_{40} 5% (R=C_8H_{17})

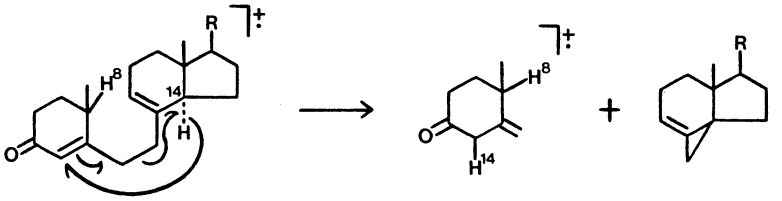


19 R=CH_3;R'=D;R"=O

20 R=R'=H;R"=OH

While the synthetic work (24) is not yet completed the results to date (Table 2) shed new light on this important ring B fragmentation. The first conclusion that can be reached is that all three unsaturated ketone types (16-18) behave similarly in terms of predominant transfer of the C-8 hydrogen atom, but differ significantly in the source of the second migrating hydrogen. The simplest situation exists in $\Delta^{1,4}$-dien-3-ones (17), which is precisely the series in which this ring B fragmentation is of overwhelming significance (Σ_{40} 36%), since the second hydrogen originates almost exclusively from C-11. We conclude that the two alternative paths expressed in terms of k → ℓ → m or k' → ℓ' → m' (with the additional Δ^1 double bond) adequately describe this fragmentation, the mass 122 ion then simply being a tautomer of ionized o-xylene. The great abundance of this ion is surely associated with the doubly allylic activation of C-10 in the first step (k or k').

Δ^4-3-Keto steroids (16) display a somewhat more complicated behavior as can be judged from the data collected in Table 2. Only half of the second hydrogen atom originates from C-11 - consistent with the k → m (or k' → m') sequence - while the remainder comes from several positions including C-14 (e.g. ℓ'' → m''). In $\Delta^{4,6}$-3-keto steroids (18) the situation is still more complicated (see Table 2) and this is also reflected by the metastable defocussing data (24), which show that m/e 122 and 124 arise exclusively from the molecular ions of 17 and 16 respectively, while in the $\Delta^{4,6}$-dienones (18) only 67% of m/e 136 is derived from the molecular ion, the other two progenitors (14.4% and 18.4% respectively) being m/e 269 (M^+ - side chain) and m/e 247 ($C_{18}H_{31}^+$ encompassing rings C and D). Since the common denominator in all three series (16-18) is the predominant or virtually exclusive migration of one hydrogen atom from C-8, we assume that this is the principal initiating process (see k → ℓ) in all three fragmentations of the respective molecular ions and that the more subtle differences are then associated with the transfer of the second hydrogen atom. A detailed discussion of these differences and of the hydrogen migrations involved in some of the other significant fragmentations of such steroid α,β-unsaturated ketones are beyond the scope of this presentation and will be covered in the detailed paper dealing with the labelling work (24).



l" m"

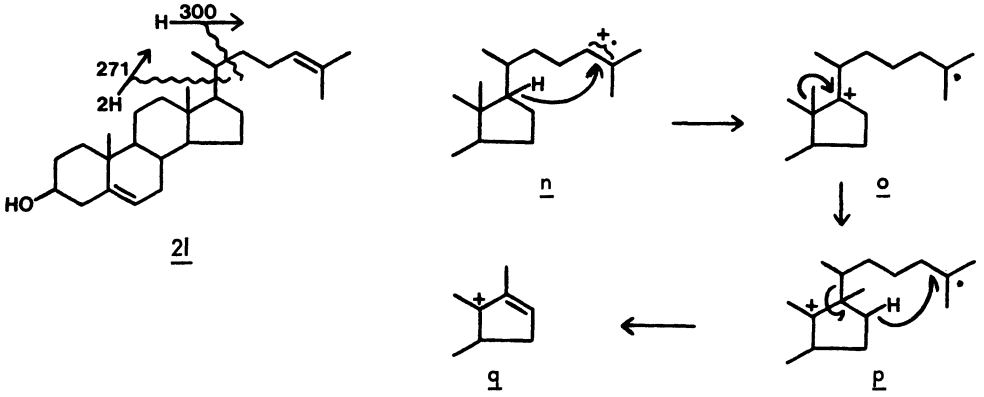
TABLE 2. Deuterium transfers (%) in ring B cleavage of labelled ketones (24)

Steroid	7-d_2	8β-d_1	9α-d_1	11-d_2	12-d_2	14α-d_1
Δ^4-Cholesten-3-one (16)	2	76	9	48	0	20
$\Delta^{1,4}$-Cholestadien-3-one (17)		94		92		12
$\Delta^{4,6}$-Cholestadien-3-one (18)	0	76	1	15	12	22

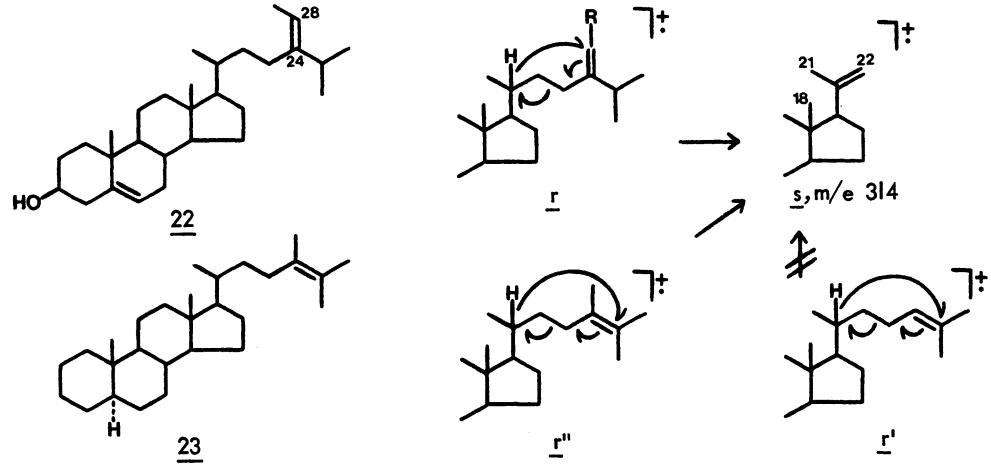
STEROLS WITH UNSATURATED SIDE CHAINS

The most interesting structural feature of marine sterols is the nature of the side chain and especially the fact that the latter is usually unsaturated (25). It is not surprising, therefore, that those mass spectral features which are peculiar to these centers of unsaturation have proved particularly useful in structure elucidation work, but with the exception of one isotope labelling study (26) all other conclusions are based on circumstantial evidence or surmise (e.g. refs. 27 - 29). The purpose of this brief discussion is to point out that there exist major gaps in our knowledge of the mechanism of some of these fragmentations and at the same time to illustrate the utility of the presently available mass spectral information in structure work.

Desmosterol (21) is a pertinent example, since it is an important intermediate in sterol biosynthesis and illustrates one of the most diagnostic mass spectral peaks (m/e 271) of certain sterols with unsaturated side chains. Its mass spectrum as reported usually in the literature (e.g. ref. 29) is reproduced in Fig. 3a (30) - the most prominent feature being the peak at m/e 271 associated with the loss of the side chain together with two hydrogen atoms. Labelling studies (26) have shown that one hydrogen originates exclusively from C-17 (n), while the second one comes from C-12,14 and 16. The proposed mechanism (26) is depicted below (n → o → p → q) solely for the C-16 transfer, since allylic carbonium ions similar to q would be generated from C-12 or C-14.



Fucosterol (22) is one of the most abundant marine sterols and its mass spectrum (Fig. 3c) is typical of $\Delta^{24(28)}$-unsaturated sterols with a base peak at m/e 314. Several workers (27-29) have suggested that this ion arises by a McLafferty rearrangement (r → s), but no deuterium labelling has been performed to prove that the C-20 hydrogen is actually involved. While similar McLafferty rearrangement (r' → s) should be equally feasible in desmosterol (21), such an m/e 314 peak is virtually absent (Fig. 3a). It does, however, reappear (26) as the base peak (r'' → s) in tetrasubstituted 24-methyl-Δ^{24}-steroids (23) and since no obvious explanation can be offered for this anomaly, it will be desirable to confirm the actual operation of such McLafferty rearrangements by deuterium labelling of 22 and 23 at position 20.



In preparation for such work, we have carried out a detailed high resolution and metastable defocussing study (31) on desmosterol and fucosterol (see Table 3). The most interesting result is that while in desmosterol (21) the molecular ion is generally the most important precursor of all fragment peaks above m/e 229 (Fig. 3a), the situation is completely altered in fucosterol (22) in that the mass 314 ion (Fig. 3c) becomes the most significant progenitor. If the ion of mass 314 really is represented by structure s then this raises a number of questions, which would require extensive isotopic labelling: (1) Loss of the methyl group (m/e 314 → 299) could involve expulsion of the C-18 angular methyl group or of the C-21 methyl function or hydrogen rearrangement to C-22 followed by methyl loss. (2) Part of the substantial m/e 281 peak arises by loss of the elements of CH_5O and thus involves concerted water and methyl loss which may differ from the two stepwise processes. (3) The formal loss of the side chain together with two hydrogens (m/e 271) clearly must arise by a different mechanism from that (n → q) established (26) for desmosterol (21). Answers to these questions are primarily of intrinsic mechanistic interest; however the empirical utilization of these characteristic fragmentations is still quite useful for structure elucidation work as will be demonstrated below.

TABLE 3. High resolution and metastable defocussing data of desmosterol (21) and fucosterol (22)

Important Fragment Peaks	Desmosterol(21)(M^+ 384) Rel.Int.(%)	Major Progenitors	Fucosterol(22)(M^+ 412) Rel.Int.(%)	Major Progenitors
395, $C_{28}{}^{13}CH_{46}$			1	
394, $C_{29}H_{46}$			4	412(100)
369, $C_{26}H_{41}O$ (21)	34	384(100)	0.2	
351, $C_{26}H_{39}$ (21)	17	384(21),369(79)	0.2	
329	0.2		0.2	
315, $C_{21}{}^{13}CH_{34}O$ (22)	0.7		25	
314, $C_{22}H_{34}O$	2	384(85),329(9)	100	412(100)
300, $C_{21}H_{32}O$ (or) $C_{20}{}^{13}CH_{31}O$	34	384(100)	8	
299, $C_{21}H_{31}O$	29	384(93)	22	412(26),314(71)
297, $C_{21}H_{29}O$ (21) $C_{21}{}^{13}CH_{32}$ (22)	1.5		5	
296, $C_{22}H_{32}$ (22)	0.7		16	394(9),314(91)
282, $C_{21}H_{30}$ (or) $C_{20}{}^{13}CH_{29}$	3	300(96)	5	
281, $C_{21}H_{29}$	5		20	314(40),299(35) 296(25)
274, $C_{20}H_{34}$ (or) $C_{19}{}^{13}CH_{33}$	2		0.6	
273, $C_{20}H_{33}$ (40%) $C_{17}{}^{13}C_2H_{27}O$ (60%)	12	384 or 385(66) 274(?)(22)	3	413(12),395(14) 315(18),274(56)

TABLE 3 (cont.)

272,	$C_{19}H_{28}O$ (or) $C_{18}{}^{13}CH_{27}O$	31	384 or 385(92)	7	413(16),314(56), 273(28)
271,	$C_{19}H_{27}O$	100	384(91)	15	412(18),314(71)
258,	$C_{18}H_{26}O$ 21(76%)22(100%) $C_{19}H_{30}$ 21(24%)	5		3	
255,	$C_{19}H_{27}$	9	366(8),273(83)	5	314(16),296(30), 273(46)
253,	$C_{19}H_{25}$	15	271(96)	4	
247,	$C_{18}H_{31}$ (21) $C_{16}{}^{13}CH_{26}O$ (22)	1		1	
246,	$C_{17}H_{26}O$ 21(21%)22(100%) $C_{18}H_{30}$ 21(79%)	3		3	
245,	$C_{18}H_{29}$ (21) $C_{17}H_{25}O$ (22)	5	384(21),368(14), 260(8),247(?)(42)	3	
231,	$C_{16}H_{23}O$		384(44),369(9) 246(32)	7	413 or 412(23), 314(32),246(34)
229,	$C_{16}H_{21}O$(50%) $C_{17}H_{25}$(50%)	6	384(28),258(9), 274(33)	20	314(69),247(15)
228,	$C_{17}H_{24}$	5		5	
213,	$C_{16}H_{21}$	16	231(64),228(18)	11	297(8),231(60), 228(18)
211,	$C_{16}H_{19}$	5		9	314 or 315(8), 296(39),229(36)

In Figure 3 are reproduced two separate mass spectra of desmosterol (21) measured on the same instrument (Varian-MAT 711 double focussing mass spectrometer) to illustrate a potentially serious instrumental problem in mass spectrometry discovered by A. Wegmann of this laboratory (30). A conventional way of measuring mass spectra of rare, solid natural products, such as certain marine sterols isolated in trace quantities, is to introduce a very small amount of substrate into the ion source via a solid probe inlet system. The probe is then heated until the sample starts to vaporize just sufficiently so that the base peak of the mass spectrum shows a full response on the UV recorder. This procedure has the advantage of using the least amount of substance and the mildest conditions. Higher probe temperatures yield "saturated" spectra (i.e. unnecessary loss of material by vaporizing too much material), possible thermal decomposition and excess contamination of the mass spectrometer source. Periodically, the solid probe needs to be cleaned and electropolishing has been recommended (32) as an efficient and cheap method. Such a procedure has also been employed by us and the sensitivity usually increases greatly after such electropolishing. However, Wegmann (30) noticed that in the process "active sites" are apparently produced on the probe surface that can result in major and unexpected differences in fragmentation modes. The problem is illustrated in terms of the $M^+ - H_2O$ peak (m/e 366) of desmosterol (21), but similar observations were also made (30) with fucosterol (22) and cholesterol. A priori one would expect less dehydration at the lower temperature (80°, Fig. 3b) than at 140° (Fig. 3a), but exactly the opposite is observed - the $M^+ - H_2O$ peak (m/e 366) as well as the peak at m/e 253 corresponding to the loss of water from m/e 271 (see Table 3) increasing to a

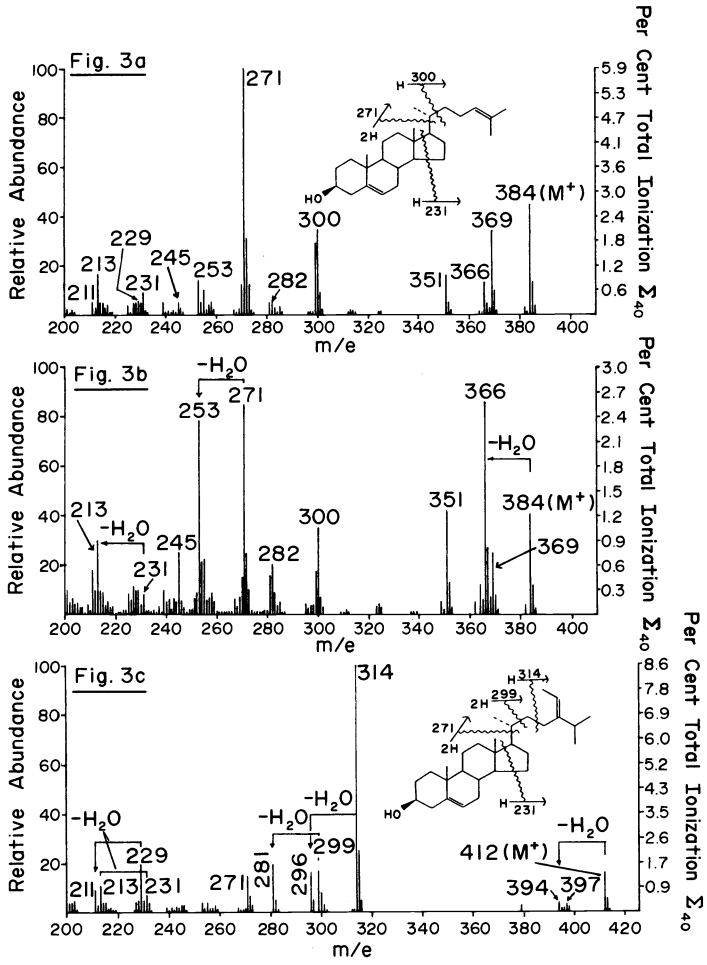


Fig. 3 Mass spectra (70 eV), Varian-MAT 711 mass spectrometer, solid probe inlet system of (a) desmosterol (21) at 140°; (b) desmosterol at 80°; (c) fucosterol (22) at 140° (identical spectrum obtained with MAT 711 or AEI MS-9 mass spectrometer)

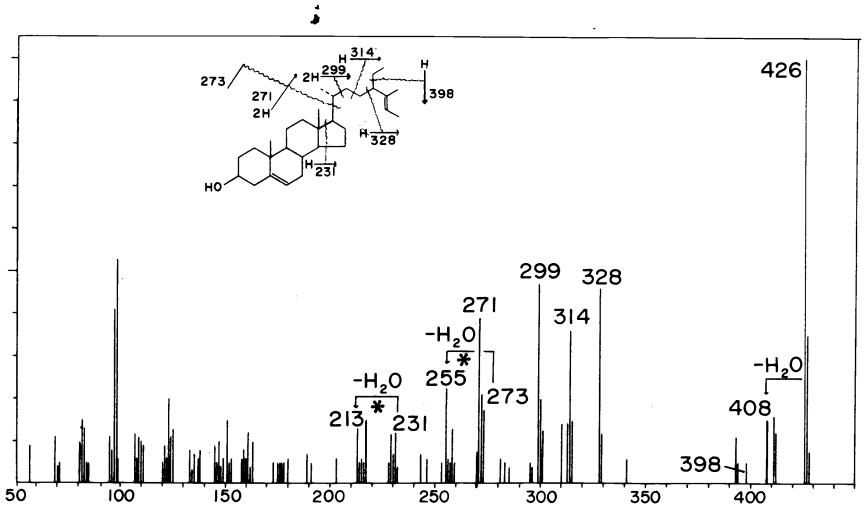
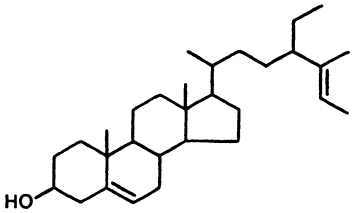


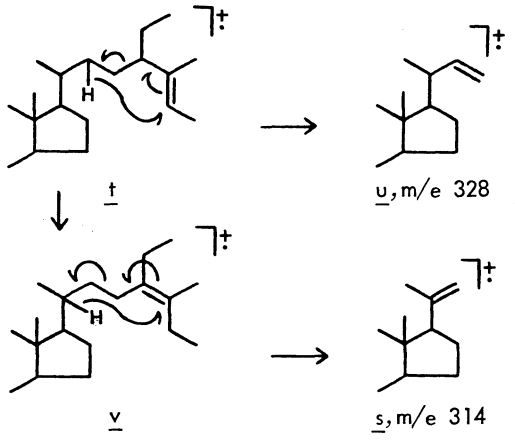
Fig. 4 Mass spectrum (15 eV) of stoliferasterol (24)

dramatic extent under these mild conditions. This unexpected effect can be nearly eliminated by adding a "source filler" such as perfluorokerosene or methyl stearate to the sterol sample prior to probe insertion. Wegmann's tentative conclusion (30) is that when low temperatures with minimal sterol samples are used, fragmentation at the "active sites" can manifest itself. At higher temperatures, these sites are flooded by the large excess of sterol produced by sudden massive evaporation and such "flooding" of active sites can also be accomplished at the low temperature by using a large amount of a volatile component such as perfluorokerosene or methyl stearate. These observations are clearly of importance when comparing the mass spectra of rare natural products with those of reference spectra in the literature and it will be interesting to observe how wide spread this phenomenon is among compounds other than sterols. It would be reasonable to predict that this would also be found among the indole alkaloids - a class of compounds where mass spectrometry has played a very significant role.

The semi-empirical use to which the presently available information on mass spectral fragmentation of steroids with unsaturated side chains can be put is illustrated by the new marine sterol, stoliferasterol (24), which has recently been isolated (33) from the Australian sponge Jaspis stolifera. Its structure is based so far solely on two physical measurements, NMR and mass spectrometry, and is of considerable biogenetic interest since it represents the first example of biosynthetic alkylation at both C-27 and C-28. Its mass spectrum is depicted in Fig. 4 and exhibits most of the diagnostic peaks of sterols with unsaturated side chains as indicated graphically in structure 24. Of particular significance is the peak at m/e 328, which does not have its counterpart in the mass spectra of other marine sterols (e.g. fucosterol - see Table 3), but which can be rationalized readily via the McLafferty rearrangement t → u. In order to explain the simultaneous presence of an m/e 314 peak - typical of a $\Delta^{24(28)}$ double bond (cf. r → s) which is absent in stoliferasterol (24) - we propose the occurrence of an electron impact-induced migration of the Δ^{25}-double bond to the tetrasubstituted Δ^{24}-position (v), which would then undergo the well-documented (26) fragmentation (cf. r'' → s) to m/e 314 (s). Precedent for this double bond migration is provided by the earlier discussed isomerization in the mass spectrometer of Δ^{7} - (10a) to $\Delta^{8(14)}$ - (15a) unsaturated steroids.



24



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