Tetrahedron. Vol. 31, pp. 2391 to 2400. Pergamon Press 1975. Printed in Great Britain

# MASS SPECTROMETRY IN STRUCTURAL AND STEREOCHEMICAL PROBLEMS—CCXLIV[1]

## THE INFLUENCE OF SUBSTITUENTS AND STEREOCHEMISTRY ON THE MASS SPECTRAL FRAGMENTATION OF PROGESTERONE

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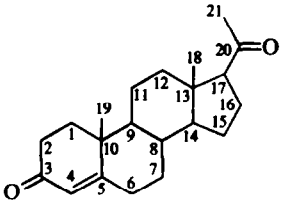
(*Received in UK 24 March 1975; Accepted for publication 17 April 1975*)

**Abstract**—The complete high resolution mass spectra of progesterone ($\Delta^4$-pregnene-3,20-dione) and twenty-nine stereoisomers and alkyl substituted analogs have been analyzed with the aid of the recently developed computer program INTSUM. Progesterone analogs with "normal" configuration at the six chiral skeletal carbon atoms give rise to abundant ions corresponding to cleavage of the 1–2 and 3–4 bonds (ketene elimination), to cleavage of the 6–7 and 9–10 bonds (ring B cleavage), and to cleavage of the 13–17 and 15–16 bonds (partial ring D cleavage); these reactions are frequently followed by elimination of alkyl radicals. Alkyl groups at C-6 and C-10 exert a pronounced influence on the formation and fragmentation of the [M-ketene] ions. Reversal of configuration at C-10 increases the importance of ring B cleavage, whereas reversal at C-17 favors the partial cleavage of ring D. The fragmentation of 17-alkylprogesterones differs significantly from the general pattern, with acetyl loss (cleavage of the 17–20 bond) and partial ring D cleavage as the predominating reactions. Loss of ring D by cleavage of the 13–17 and 14–15 bonds is not an important reaction of progesterones. Direct interaction of the two ketonic functions was not observed.

The mass spectra of numerous classes of compounds incorporating the steroid skeleton have been examined within the last decade,[3] and the major fragmentation reactions of most common steroids are by now well known. However, in order to utilize this information for the determination of structures of previously unknown steroids it is often necessary to understand not only the basic decomposition pathways of a particular class of compounds, but also to know the effects of substituents, and of the configuration at the point of substitution, as well as the influence of "abnormal" skeletal stereochemistry on the major decomposition reactions. For this reason we have undertaken an investigation of the complete high resolution mass spectra of progesterone ($\Delta^4$-pregnene-3,20-dione, **1**), of a number of alkylsubstituted progesterones, and of various stereoisomers of these compounds (see Table 1).

Progesterones were chosen for this study for a number of reasons; the mass spectral fragmentation reactions of this biologically important group of steroids have not previously been subject to extensive study, though the low resolution mass spectra of progesterone itself and a number of alkyl substituted analogs have been reported[4-8] and the genesis of some of the major peaks briefly discussed.[7,8] The influence of the configuration at C-17 has been discussed by Genard *et al.*[8] and by Zaretskii and collaborators;[6] the latter group has also briefly reported on the mass spectrum of 9β,10α-progesterone.[5] Furthermore, a study of progesterones allows us to assess the extent of the interaction, if any, between two functional groups situated sufficiently far from each other on the steroid skeleton to make direct, through-space interaction unlikely. This is possible since previous studies have established in detail the electron impact induced fragmentation reactions of steroids possessing either an α,β-unsaturated keto group in ring A[9] or a 20-keto group.[10] The impetus to undertake this study at this time has been the recent advent of computer programs (such as INTSUM[11])



**1**

Table 1. Alkylprogesterones examined

| | | |
|---|---|---|
| 1. Progesterone | 11. 16β-Methylprogesterone | 21. 6α-Methyl-17α-ethylprogesterone |
| 2. 19-Norprogesterone | 12. 18-Methylprogesterone | 22. 17α-Progesterone[a] |
| 3. 1α-Methyl-19-norprogesterone | 13. 19-Methylprogesterone | 23. 6-Methyl-17α-progesterone |
| 4. 1β-Methyl-19-norprogesterone | 14. 2,2-Dimethylprogesterone | 24. 16β-Methyl-17α-progesterone |
| 5. 18-Methyl-19-norprogesterone | 15. 6α,16α-Dimethylprogesterone | 25. 10α-Progesterone |
| 6. 6α-Methylprogesterone | 16. 6β,16α-Dimethylprogesterone | 26. 6α-Methyl-10α-progesterone |
| 7. 6β-Methylprogesterone | 17. 6α-Methyl-16α-isopropylprogesterone | 27. 9β,10α-Progesterone[b] |
| 8. 7α-Methylprogesterone | 18. 17α-Methylprogesterone | 28. 6α-Methyl-9β,10α-progesterone |
| 9. 14α-Methylprogesterone | 19. 17α-Ethylprogesterone | 29. 8α,10α-Progesterone |
| 10. 16α-Methylprogesterone | 20. 6α,17α-Dimethylprogesterone | 30. 9α-Methyl-19-norprogesterone |

[a] Sometimes referred to as 17-isoprogesterone.
[b] Sometimes referred to as *retro*-progesterone.

capable of interpretation and summary of large amounts of mass spectral information. It is well known that mass spectra of isomers are often very much alike, and it was felt that an automated initial treatment would be of considerable value in the detection and analysis of systematic differences between the potentially very similar spectra of the many isomeric compounds examined in this study.†

## EXPERIMENTAL

Complete high resolution mass spectra of all compounds included in the present study were recorded by Ms. A. Wegmann using a Varian-MAT 711 mass spectrometer at 70 eV with the direct insertion probe. Low resolution mass spectra were recorded by Mr. R. G. Ross on an AEI MS-9 mass spectrometer; metastable defocusing measurements were performed on both instruments. Samples of compounds **1–4** were available from previous investigations in this laboratory while compounds **5–7**, **10–17**, and **24** were placed at our disposal for this study by Dr. John Edwards of Syntex Research, Palo Alto, California. Compounds **22** and **23** were prepared by Serini-Logemann reactions from the corresponding 17α-hydroxy-20-acetoxy compounds as described by Rubin and Blossey.[12] Progesterone-$d_9$ was prepared from progesterone by exchange twice with $MeOD/D_2O/Na_2CO_3$ for 24 hr; the isotopic purity was 4% $d_7$, 41% $d_8$, and 55% $d_9$ (low voltage mass spectrometry). The deuterium atoms in the $d_9$ species occupy positions 2, 2, 4, 6, 6, 17, 21, 21, 21; the $d_8$ species appears to have one deuterium less at C-6, judged from the mass and NMR spectra. The exchange reaction gave rise to some 17α-progesterone, which was separated by fractional crystallization.

The remaining compounds were kindly donated by Prof. G. R. Pettit (Arizona State University) (**9**), Dr. J. C. Babcock (The Upjohn Company) (**8**), Dr. M. J. Weiss (American Cyanamid) (**18**, **19**, **21**), Dr. M. Uskokovic (Hoffmann–La Roche) (**25**, **27**), Dr. K. Heusler (Ciba–Geigy) (**26**), Dr. R. Deghenghi (Ayerst) (**20**), Dr. J. Schlatmann (Philips–Duphar) (**27–29**), and Dr. R. V. Coombs (Sandoz–Wander) (**30**).

### *Data analysis*

Examination of a relatively large number of complete high-resolution spectra of complex molecules lacking strongly fragmentation directing groups is complicated by the sheer amount of information present in each spectrum. In the present case a typical mass spectrum contains peaks of significant intensity (i.e. >1% rel. int.) corresponding to between 150 and 200 different elemental compositions. In order to extract a maximum of information while limiting the number of specific data to a manageable order of magnitude we have utilized a computer program, INTSUM, available from previous investigations.[11] INTSUM operates in three stages; first, it generates an exhaustive list of possible fragmentations of the molecular skeleton (here, the progesterone molecule); second, it analyzes each individual spectrum to match the elemental composition of the ions formed to the list of possible fragmentations (the list is amended, if necessary, to take substituents into account); third, the program produces a summary of the mass spectral evidence, listing for each possible fragmentation those compounds whose mass spectra display peaks corresponding in elemental composition to that fragmentation, together with the peak intensities. To limit the actual computing time, as well as the size of the final output, the program was prevented from considering fragmentations that involve cleavage of two or more bonds to the same carbon atom (not counting bonds to hydrogen), and from considering fragmentations that consist of more than two physically separate processes or involve migration of more than two hydrogen atoms (excepting reciprocal transfer reactions) to or from the fragment expelled. Also, peaks of less than 0·4% of the total ion current (~4% of base peak) were not considered.

The exclusion of processes involving cleavage of two bonds of the same C atom from the data analysis renders the program unable to consider and correlate fragmentations that actually proceed in this manner. To examine if such reactions actually contribute to the formation of ions that give rise to intense peaks in the spectra two measures were taken, namely test runs on a limited number of spectra with this constraint removed, and metastable defocusing of intense peaks in several spectra. As a result one general process was found in which two bonds to the same carbon atom are broken, viz. elimination of $C_3H_7$ from $[M-C_2H_2O]$ (see below).

Even with these constraints applied to the reduction and summary of the mass spectral information only those processes (with a few exceptions) that give rise to peaks in the high mass range could be unambiguously determined from the results of the computer analysis. The human fares only little better, since the ambiguity lies in the multiple origins of the ions that give rise to a specific peak; most of the ions of low mass could in principle be formed by several different processes, a situation common to nearly all complex molecules that lack strongly fragmentation directing moieties. Metastable defocusing experiments performed on various hydrocarbon ions formed from progesterone have confirmed that these usually have multiple origins, e.g. the $C_{11}H_{15}$ ions (*m/e* 147 in Table 2) arise by decomposition of ions of seven different *m/e* ratios.[13] It is therefore difficult to establish the origin(s) of even reasonably abundant ions in the low mass range, in the absence of complete series of deuterium labeled analogs,‡ and our analysis of the mass spectra of progesterones is thus limited largely to oxygen-containing ions and to high-mass ions.

## RESULTS AND DISCUSSION

A preliminary investigation of the progesterone mass spectra (Tables 2 and 3) reveals that many of the decomposition reactions observed are quite dependent upon the configuration of certain C atoms, and that the presence of substituents at certain loci—most prominently C-17—changes the course of reactions dramatically. It is, however, possible to determine the general fragmentation pattern of progesterones by studying the spectra of those compounds that have the same configuration as progesterone at all six chiral carbon atoms—excepting 17-substituted progesterones. These general features are discussed below, followed by a discussion of the effects of changes of skeletal stereochemistry and of introduction of 17-substituents.

The molecular ions give rise to abundant peaks in the spectra of all 30 progesterones examined, except in the case of 17α-progesterones (**22–24**, *cf.* Ref. 6). Differences in the abundance of $M^{+\cdot}$ are observed between stereoisomeric pairs such as 6α- and 6β- (**6**,**7**) and 16α- and and 16β-methylprogesterones (**10**, **11**), where the 6β and 16α isomers give rise to the more intense molecular ion peaks; these differences are not of diagnostic significance, since the 6α,16α- and 6β,16α-dimethyl compounds (**15** and **16**) do not show similar variations.

*Single bond cleavages.* Elimination of an angular Me group is frequently encountered in steroid mass spectrometry.[3,14] In progesterone (**1**) loss of the allylic methyl (C-19) would be expected to predominate over loss of the other angular Me (C-18), but examination of the spectra of the 18- and 19-Me substituted progesterones, **12** and **13**, shows that the issue is more complicated. In **12**, the Et (C-18) and Me (C-19) groups are eliminated with equal facility, whereas in **13** the peak corresponding to loss of the Et group (C-19) is eight times as intense as that

†Among the compounds examined, nine have the elemental composition $C_{21}H_{30}O_2$, thirteen $C_{22}H_{32}O_2$, and five $C_{23}H_{34}O_2$.

‡The probable occurrence of non-specific and reciprocal hydrogen transfer reactions as part of many processes leading to low mass ions would conceivably obscure the modes of formation even in a study of an extensively labeled series of compounds.

Table 2. Partial[a] mass spectra of alkylprogesterones examined[b]

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $M^{+\cdot}$ | 6·4 (314) | 2·8 (300) | 3·7 (314) | 4·4 (314) | 9·3 (314) | 5·9 (328) | 10·8 (328) | 6·5 (328) | 4·6 (328) | 11·5 (328) | 6·5 (328) | 6·3 (328) | 5·3 (328) | 7·2 (342) | 6·5 (342) | 7·6 (342) | 4·1 (370) |
| M-alkyl | 0·6 (299) | 0·3 (285) | 0·4 (299) | — (299) | 0·5 (285) | 0·8 (313) | 1·7 (313) | 0·7 (313) | 1·0 (313) | 2·1 (313) | 0·8 (313) | 0·5 (313) | 0·2 (313) | — (327) | 1·6 (327) | 2·6 (327) | 0·9 (355) |
| | | | | | | | 0·4 (299) | | | | | 0·5 (299) | 1·2 (299) | | | | 7·4 (327) |
| M-$CH_3CO$ | 0·5 (271) | 0·8 (257) | 0·6 (271) | 0·7 (271) | 0·6 (271) | — (285) | — (285) | — (285) | — (285) | 0·8 (285) | 0·5 (285) | — (285) | — (285) | 0·9 (299) | 0·7 (299) | 0·8 (299) | — (327) |
| a (M-$C_2H_2O$) | 4·4 (272) | 0·8 (258) | 4·3 (272) | 2·7 (272) | 0·3 (272) | 2·6 (286) | 4·2 (286) | 3·8 (286) | 1·3 (286) | 4·6 (286) | 3·2 (286) | 2·2 (286) | 3·1 (286) | 3·8 (272)* | 2·6 (300) | 2·2 (300) | 0·8 (328) |
| b (M-$C_2H_2O$-$C_3H_7$) | 1·6 (229)* | 0·4 (229)[f] | 1·9 (229)* | 1·6 (229)* | 0·3 (243)[f] | 0·9 (243)* | 2·5 (243)* | 1·1 (243)* | 0·5 (243)* | 1·2 (243) | 0·7 (243) | 1·0 (243)* | 1·4 (229)** | — (229) | 0·6 (257) | 1·2 (257) | 0·3 (285) |
| c (M-$C_2H_2O$-$CH_3CO$) | 1·8 (229) | 0·4 (215) | 0·8 (229) | 0·9 (229) | 0·3 (229) | 1·9 (243) | 0·5 (243) | 0·9 (243) | 0·3 (243) | 1·7 (243) | 1·7 (243) | 0·5 (243) | 1·0 (243) | 1·4 (229) | 1·5 (257) | 0·3 (257) | 1·0 (285) |
| f[c] | 2·0 (191) | 0·8 (191) | 1·2 (191) | 2·3 (191) | 0·8 (205) | 0·9 (191) | 0·6 (191) | 1·7 (205) | 1·2 (205) | 1·7 (205) | 2·1 (205) | 1·8 (205) | 0·9 (191) | — (191) | 0·9 (205) | 0·4 (205) | 0·6 (233) |
| g[c] | 0·9 (190) | 0·3 (190) | 0·4 (190) | 0·7 (190) | 0·3 (204) | — (190) | — (190) | 1·1 (204) | 0·4 (204) | 0·8 (204) | 0·9 (204) | 0·8 (204) | 0·4 (190) | — (190) | — (204) | — (204) | — (232) |
| d[c] | 9·0 (124) | 4·2 (110) | 3·9 (124) | 8·7 (124) | 4·7 (110) | 10·2 (138) | 4·2 (138) | 6·3 (124) | 7·8 (124) | 8·0 (124) | 6·8 (124) | 4·4 (124) | 3·9 (138) | 2·2 (152) | 11·3 (138) | 4·3 (138) | 6·3 (138) |
| e[c] | 1·3 (123) | 0·8 (109) | 0·6 (123) | 0·6 (123) | 0·9 (109) | 3·0 (137) | 3·5 (137) | 1·6 (123) | 1·5 (123) | 1·2 (123) | 1·3 (123) | 1·3 (123) | 0·9 (137) | 0·6 (151) | 3·1 (137) | 3·4 (137) | 3·3 (137) |
| d-alkyl | 1·0 (109) | — (95) | 0·5 (109) | 0·6 (109) | — (95) | 0·9 (123) | 1·9 (123) | 0·5 (109) | 0·6 (109) | 0·9 (109) | 1·0 (109) | 0·9 (109) | 0·5 (123) | 0·8 (137) | 0·9 (123) | 1·9 (123) | 0·9 (123) |
| | | | | | | 0·7 (109) | 0·7 (109) | | | | | | 0·6 (109) | | 0·8 (109) | 0·7 (109) | 0·7 (109) |
| h | 1·3 (244) | 1·8 (230) | 0·7 (244) | 2·5 (244) | 1·1 (244) | 0·9 (258) | 1·1 (258) | — (258) | — (258) | 2·5 (244) | 1·8 (244) | 0·8 (258) | 0·7 (258) | 3·8 (272)* | 2·1 (258) | 2·3 (258) | 3·1 (258) |
| h-alkyl | 1·6 (229)* | 1·8 (215)* | 1·9 (229)* | 1·6 (229)* | 2·5 (229)* | 0·9 (243)* | 2·5 (243)* | 1·1 (243)* | 0·5 (243)* | 0·7 (229)* | 0·7 (229)* | 1·0 (243)* | 0·4 (243)* | — (257)* | 0·3 (243)* | 0·9 (243)* | 1·5 (243)* |
| | | | | | 0·4 (215) | | | | | | | 0·3 (229) | 1·4 (229)* | | | | |
| i | 1·0 (71) | 2·5 (71) | 1·6 (71) | 1·4 (71) | 1·8 (71) | 0·9 (71) | 0·9 (71) | 1·4 (71) | 0·5 (71) | 2·2 (85) | 2·0 (85) | 1·4 (71) | 1·1 (71) | 1·0 (71) | 2·1 (85) | 2·8 (85) | 1·0 (113) |
| k | 1·6 (229)* | 1·8 (215)* | 1·9 (229)* | 1·6 (229)* | 2·5 (229)* | 0·9 (243)* | 2·5 (243)* | 1·1 (243)* | 0·5 (243)* | 0·7 (229)* | 0·7 (229)* | 1·0 (243)* | 0·4 (243)* | — (257)* | 0·3 (243)* | 0·9 (243)* | 1·5 (243)* |
| Other oxygen-containing ions[d] | 0·9 (122) | 0·9 (217) | 0·5 (272) | 1·4 (137) | 3·4 (231) | 1·0 (136) | 1·3 (259) | 0·6 (245) | 1·5 (137) | 0·6 (231) | 0·9 (122) | 1·8 (245) | 0·9 (257) | 11·1 (286) | 1·1 (136) | 0·9 (273) | 1·7 (312) |
| | | | ($C_{18}H_{24}O_2$) | | | | ($C_{17}H_{23}O_2$) | 1·3 (85) | 1·3 (122) | | | | | ($C_{19}H_{26}O_2$) | | ($C_{18}H_{25}O_2$) | |
| | 0·9 (85) | 2·6 (84) | 1·6 (84) | 1·4 (84) | 4·1 (148) | | 1·2 (136) | 1·3 (84) | | | | | 1·0 (136) | | | | 0·5 (245) |
| | | | | | 1·2 (147) | | | | | 0·8 (98) | | 0·9 (85) | 1·8 (110) | 1·4 (271) | 0·8 (98) | 0·6 (245) | 0·9 (136) |
| | | | | | 1·0 (85) | | | | | | | 3·9 (84) | 1·0 (85) | ($C_{18}H_{23}O_2$) | | 1·1 (136) | 1·0 (111) |
| | | | | | 2·4 (84) | | | | | | | | | | | 1·1 (98) | |
| Other hydrocarbon ions[e] | 1·6 (147) | 1·9 (105) | 1·6 (107) | 1·7 (105) | 1·6 (119) | 1·5 (105) | 1·9 (105) | 1·5 (161) | 2·6 (149) | 2·1 (107) | 2·0 (107) | 1·7 (105) | 1·5 (147) | 2·2 (107) | 1·6 (109) | 1·5 (109) | 1·5 (119) |
| | 1·6 (133) | | 1·7 (105) | | 1·6 (105) | | | 1·5 (133) | 1·5 (105) | 1·8 (105) | 1·9 (105) | | 1·5 (133) | 1·9 (105) | 2·0 (107) | 1·8 (107) | 1·8 (107) |
| | 1·5 (107) | | | | | | | 1·7 (105) | | | | | 1·8 (105) | | 1·8 (105) | 2·1 (105) | 2·0 (105) |
| | 1·6 (105) | | | | | | | | | | | | | | | | |

[a] Ions of more than 0·5% of $\Sigma_{40}$ above $m/e$ 70 included if discussed in text; other ions as given in footnotes *d* and *e*. Presented in terms of percent total ionization ($\Sigma_{40}$) followed by nominal mass in parenthesis. An asterisk denotes that ions of the same elemental composition are also formed in other reactions.

[b] 17-Alkyl substituted progesterones and configurational isomers are listed in Table 3.

[c] See Scheme 3.

[d] Incorporating one oxygen atom unless otherwise noted; ions of $\Sigma_{40} > 0·9\%$ above $m/e$ 70 included.

[e] Ions of $\Sigma_{40} > 1·5\%$ with masses above $m/e$ 100 included.

[f] M-$C_2H_2O$-$C_2H_5$.

[g] M-$C_2H_2O$-$C_4H_9$.

Table 3. Partial[a] mass spectra of alkylprogesterones examined[b]

| | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $M^+$ | 4·0 (328) | 3·1 (342) | 4·7 (342) | 2·7 (356) | 9·1 (314) | 8·1 (328) | 0·6 (328) | 4·4 (314) | 5·3 (328) | 2·6 (314) | 1·8 (328) | 5·0 (314) | 3·1 (314) |
| M-alkyl | 0·3 (313) | — (327) | 0·4 (327) | — (341) | 0·4 (299) | 0·8 (313) | — (313) | 0·5 (299) | 0·7 (313) | 0·3 (299) | 0·3 (313) | 1·0 (299) | 0·3 (299) |
| M-$CH_3CO$ | 2·1 (285) | 3·0 (299) | 1·8 (299) | 2·7 (313) | — (271) | — (285) | — (285) | 0·5 (271) | 0·3 (285) | 0·3 (271) | — (285) | 0·3 (271) | 0·5 (271) |
| a (M-$C_2H_2O$) | 0·5 (286) | 0·5 (300) | 0·5 (300) | — (314) | 1·1 (272) | 0·4 (286) | — (286) | 1·9 (272) | 1·2 (286) | 0·7 (272) | 0·5 (286) | 0·6 (272) | 0·4 (272) |
| b (M-$C_2H_2O$-$C_3H_7$) | — (243) | — (257) | — (257) | — (271) | 2·0 (229)* | 2·0 (243)* | — (243) | 1·3 (229)* | 1·3 (243)* | 0·5 (229)* | 0·4 (243)* | 0·7 (229)* | — (229) |
| c (M-$C_2H_2O$-$CH_3CO$) | — (243) | — (257) | — (257) | — (271) | 0·3 (229) | — (243) | — (243) | 1·5 (229) | 0·3 (243) | 0·8 (229) | 0·5 (243) | 0·5 (229) | — (229) |
| f[c] | 0·3 (205) | — (219) | 0·3 (205) | — (219) | 0·8 (191) | 0·7 (191) | — (205) | 1·2 (191) | 0·8 (191) | 2·3 (191) | 2·6 (191) | 2·0 (191) | 4·4 (205) |
| g[c] | — (204) | — (218) | — (204) | — (218) | — (190) | 0·4 (190) | — (204) | 1·0 (190) | 0·3 (190) | 0·5 (190) | — (190) | 0·4 (190) | 1·4 (204) |
| d[c] | 1·6 (124) | 1·1 (124) | 3·0 (138) | 2·2 (138) | 3·8 (124) | 3·8 (138) | 2·6 (124) | 16·1 (124) | 17·5 (138) | 16·4 (124) | 14·5 (138) | 11·4 (124) | 19·2 (110) |
| e[c] | 1·4 (123) | 1·2 (123) | 4·8 (137) | 4·2 (137) | 1·1 (123) | 3·1 (137) | 1·1 (123) | 1·9 (123) | 4·2 (137) | 1·2 (123) | 5·2 (137) | 1·4 (123) | 0·5 (109) |
| d-alkyl | 0·9 (109) | 0·8 (109) | 0·9 (123) | 0·7 (123) | 1·0 (109) | 0·9 (123) | 1·3 (109) | 1·5 (109) | 1·3 (123) | 1·3 (109) | 2·0 (123) | 1·0 (109) | — (95) |
| | | | 0·6 (109) | 0·7 (109) | | 0·6 (109) | | | 0·8 (109) | | 0·8 (109) | | |
| h | 9·1 (244) | 9·9 (244) | 11·0 (258) | 11·7 (258) | 14·5 (244) | 20·2 (258) | 19·1 (244) | 0·4 (244) | 0·6 (258) | — (244) | — (258) | 0·5 (244) | 0·3 (244) |
| h-alkyl | 2·5 (229)* | 2·8 (229)* | 2·3 (243)* | 1·8 (243)* | 2·0 (229)* | 2·0 (243)* | 1·2 (229)* | 1·3 (229)* | 1·3 (243)* | 0·5 (229)* | 0·4 (243)* | 0·7 (229)* | — (229) |
| i | 2·6 (85) | 2·5 (99) | 2·4 (85) | 2·4 (99) | 2·9 (71) | 2·6 (71) | 8·5 (85) | 1·3 (71) | 0·9 (71) | 0·9 (71) | 1·2 (71) | 1·4 (71) | 0·6 (71) |
| k | 2·5 (229)* | 2·8 (229)* | 2·3 (243)* | 1·8 (243)* | 2·0 (229)* | 2·0 (243)* | 1·2 (229)* | 1·3 (229)* | 1·3 (243)* | 0·5 (229)* | 0·4 (243)* | 0·7 (229)* | — (229) |
| Other oxygen-containing ions[d] | 0·9 (270) | 1·1 (149) | 0·8 (284) | 0·9 (163) | 1·1 (122) | 1·5 (136) | 0·6 (258) ($C_{17}H_{22}O_2$) | 1·0 (85) | 1·4 (136) | 1·9 (137) | 1·9 (136) | 1·1 (137) | 1·0 (136) |
| | 1·0 (98) | 1·1 (97) | 0·9 (163) | 0·9 (136) | 0·9 (85) | 0·9 (85) | 0·8 (246) | | 1·0 (85) | 1·7 (122) | 1·6 (85) | 1·0 (136) | 3·5 (123) |
| | 0·9 (97) | | 0·9 (136) | 0·9 (135) | | | | | | 1·3 (85) | | 1·0 (85) | 1·1 (85) |
| | | | 0·9 (135) | | | | | | | | | | |
| Other hydrocarbon ions[e] | 1·9 (121) | 1·1 (281) | 2·2 (121) | 2·2 (121) | 1·7 (147) | 1·7 (121) | 2·0 (121) | 1·8 (147) | 1·5 (147) | 1·7 (173) | 2·6 (147) | 2·0 (147) | 1·7 (161) |
| | 1·5 (107) | 1·5 (121) | 1·5 (109) | 1·8 (105) | 1·5 (121) | 1·5 (105) | 1·7 (105) | 1·8 (133) | 1·5 (133) | 3·1 (147) | 1·9 (133) | 1·7 (133) | 1·6 (107) |
| | 1·9 (105) | 2·2 (105) | 1·7 (105) | | | | | 2·1 (107) | 1·8 (107) | 2·5 (133) | 2·1 (109) | 1·9 (107) | 1·7 (105) |
| | | | | | | | | 2·3 (105) | 1·9 (105) | 2·0 (105) | 2·0 (105) | 1·9 (105) | |

[a] Ions of more than 0·5% of $\Sigma_{40}$ above *m/e* 70 included if discussed in text; other ions as given in footnotes *d* and *e*. Presented in terms of percent total ionization ($\Sigma_{40}$) followed by nominal mass in parenthesis. An asterisk (*) denotes that ions of the same elemental composition are also formed in other reactions.

[b] Progesterones with "normal" configuration are listed in Table 2.

[c] See Scheme 3.

[d] Incorporating one oxygen atom unless otherwise noted; ions of $\Sigma_{40} > 0·9\%$ above *m/e* 70 included.

[e] Ions of $\Sigma_{40} > 1·5\%$ with masses above *m/e* 100 included.

corresponding to Me loss (C-18). This shows that the mass of the eliminated fragment and the inherent preference for cleavage of the 10–19 bond both play a role. This is especially interesting since Me loss in 20-keto pregnanes occurs exclusively[10] by loss of C-19, even though this is not allylically activated in these compounds. The reason for this may well be that progesterones in contrast to many other steroids do not readily produce *seco*-molecular ions by cleavage of the 13–17 bond (see below), which would render a subsequent fission of the 17–18 bond unfavorable. α-Cleavage at the C-20 CO group, which would also give rise to an [M–$CH_3$] ion is not important (no significant M–$CD_3$ in $d_9$-labeled **1**).

Loss of alkyl substituents other than C-18/19 from the molecular ions of the Me substituted compounds is dependent upon the position of the additional Me groups. Progesterones with a Me substituent in positions 1, 2, 6α, 7α, 9α, 16β, or 17α do not give rise to significantly increased [M–$CH_3$] peaks relative to **1**, whereas compounds with 6β or 16α alkyl groups show considerably enhanced alkyl loss. Alkyl elimination is, as expected, favored by increased mass of the substituent, and this reaction even gives rise to the base peak in the spectrum of the 16α-isopropyl substituted compound (**17**).

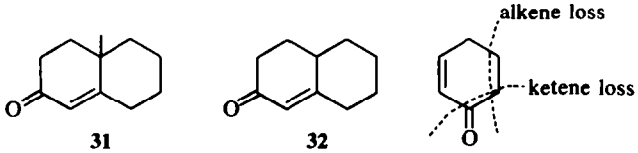
Elimination of the C-17 acetyl side chain with charge retention in the [M–$CH_3CO$] fragment takes place only to a small extent in the decomposition of most of the progesterones examined (Tables 2 and 3), but this process is a major reaction of the 17α-alkylprogesterones (**18–21**, Table 3). The product ion has been shown by defocusing experiments on 17α-ethylprogesterone (**19**) to be among the precursors to a number of other fragment ions, whereas this is only rarely the case for the [M-alkyl] ions of progesterones lacking additional substituents at C-17 (Table 5). It should be noted, however, that $CH_3CO^+$ ions give rise to substantial peaks in all progesterone spectra examined.

*Cleavage reactions associated with the α,β-unsaturated ketonic function.* Elimination of the elements of ketene from the molecular ions of cyclic α,β-unsaturated ketones has been observed in steroid systems[3] as well as in simpler compounds.[15,16] Egger[17] has shown that this process in $\Delta^1$-androsten-3-ones depends on the stereochemistry at the A/B ring junction; our results show that ketene elimination in the progesterone series (ion **a** in Tables 2 and 3) is likewise dependent upon the stereochemistry at the B/C ring junction, since the intensity of the [M-$C_2H_2O$] peak is seen to be significantly decreased in progesterones with reversed configurations at C-8 or C-9 ($\Sigma_{70} = 0{\cdot}6$–$0{\cdot}7$ in **27** and **29**, Table 3) relative to progesterone itself (**1**, $\Sigma_{70} = 4{\cdot}4\%$). Similarly, the [M-ketene] peak in the spectrum of 8α-testosterone is 1/3 as intense as that found in the spectrum of testosterone itself;[18] this may be a general feature of the mass spectra of steroidal B/C *cis* 4-en-3-ones.

The abundance in the progesterone series of the [M-ketene] ions (**a**) is also significantly reduced in the presence of a number of other structural features, such as reversed configuration at C-17 (**22–24**), substituents at C-17 (**18–21**), large alkyl substituent at C-16 (**17**), and in the 19-nor compounds **2**, **5**, and **30**. In the three former cases the reduced intensity is probably related to the concurrent increase in propensity to undergo other reactions (partial ring-D cleavage or acetyl loss in **18–24**, elimination of the substituent in **17**).† Removal of the C-19 angular Me group results in a considerable decrease of the [M-ketene] peak not only in **2**, **5**, and **30** relative to progesterone (**1**), but also in related steroids and octalones (Table 4). This effect parallels earlier observations on the mass spectra of 2-cyclohexenones[15,16] regarding the importance of the presence of substituents at C-4 (which corresponds to C-10 in progesterones and androsterones); likewise, loss of $C_2H_2O$ is significantly more pronounced from ionized **31** than from **32** (Table 4), model compounds perhaps more appropriate than cyclohexenones.

It appears, however, that the mechanisms suggested for ketene loss in the 2-cyclohexenones do not apply in the steroid series, since both cyclization to cyclobutanone intermediates[15] or rearrangement to bicyclic structures[16] (Scheme 1) would involve cleavage of the steroid 1–10 bond. This is regarded as unlikely in view of the subsequent decomposition (see below) of the [M-ketene] ion by loss of a $C_3H_7$ radical incorporating C-1, C-10 and C-19. Also, the mechanisms suggested for the fragmentation of 2-cyclohexenones were designed to accomodate

†The apparent reduction of the relative intensity of the [M-$C_2H_2O$] peak is not simply a consequence of the increased intensity of another peak, since also the intensity ratio [M-$C_2H_2O$]: [M] is similarly decreased in most of these spectra.



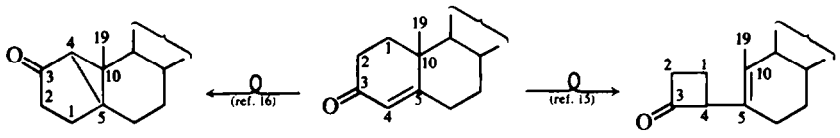

**31** **32** **33**

Table 4. Relative intensity of M and [M-$C_2H_2O$] peaks in the mass spectra of $\Delta^4$-androsten-3-ones and $\Delta^{1(9)}$-octal-2-ones

| | M | [M-$C_2H_2O$] | M: [M-$C_2H_2O$] |
|---|---|---|---|
| $\Delta^4$-Androsten-3,17-dione | 100 | 62 | 1·6 |
| 19-Nor-$\Delta^4$-androsten-3,17-dione | 100 | 7 | 14·3 |
| $\Delta^4$-Androsten-17-ol-3-one | 72 | 51 | 1·4 |
| 19-Nor-$\Delta^4$-androsten-17-ol-3-one | 94 | 18 | 5·2 |
| $\Delta^4$-Androsten-3-one | 47 | 51 | 0·9 |
| 19-Nor-$\Delta^4$-androsten-3-one | 47 | 15 | 3·2 |
| 10-Methyl-$\Delta^{1(9)}$-octal-2-one (**31**) | 85 | 100 | 0·85 |
| $\Delta^{1(9)}$-Octal-2-one (**32**) | 56 | 32 | 1·75 |

the alkene elimination which takes place concurrently in these compounds, but a similar reaction is only exhibited by one of the 30 progesterones (**14**, where, however, it gives rise to the base peak, presumably because of the high degree of substitution at C-2).

alkyl fragment originates from C-8; the other was presumed to come from C-14. Our results show, in support of this, that the [M-$C_2H_2O$] ion formed from 14$\alpha$-methylprogesterone (**9**) eliminates a $C_3H_7$ radical at most to the extent of 1/3 of that occurring in proges-




Scheme 1.

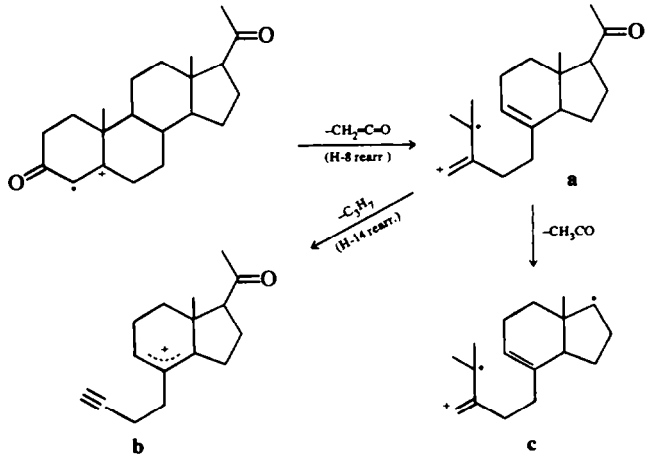
In progesterone (**1**) ketene elimination is followed by loss of 43 a.m.u., producing an ion of mass 229. The corresponding peak is a doublet ($C_{17}H_{25}$ and $C_{16}H_{21}O$) and arises from four distinct reactions: M-($C_2H_2O + C_3H_7$), M-($C_2H_2O + CH_3CO$), M-($C_4H_6O + CH_3$) and M-$C_5H_9O$. The high resolution data permit a distinction to be made between the second reaction and the remaining ones, and consideration of the shifts caused by alkyl substitution at C-16 (**10**, **11**, **15–17**) shows that the latter two reactions both involve elimination of parts of ring D. The identity of the C atoms that make up the $C_3H_7$ fragment expelled from **a** (Scheme 2) in the first reaction is indicated by the loss of $C_4H_9$ in the case of 19-methylprogesterone (**13**), showing that C-19 is eliminated. Further, the 1-methyl-19-norprogesterones **3** and **4** also eliminate $C_3H_7$ from [M-$C_2H_2O$], whereas the 19-norprogesterones **2** and **5** lose Et, indicating that C-1 is also incorporated in the alkyl radical. It appears reasonable, then, to assume that C-10 is also part of the $C_3H_7$ fragment. These observations are corroborated by the partial† shift of *m/e* 229 to *m/e* 236 in the spectrum of progesterone-$d_9$, which shows that in the M-($C_2H_2O + C_3H_7$) reaction no further loss of label occurs after ketene expulsion. Earlier studies on $\Delta^4$-androsten-3-ones[9] have also identified the carbon atoms of the alkyl fragment lost in conjunction with ketene elimination as C-1, C-10 and C-19. These studies also showed that one of the two H atoms transferred to the terone; no further quantitative elaboration is possible even on the basis of the high resolution data since three ions (**b**, **h**-$CH_3$, and **k**, Table 2) have the same elemental composition.

The elimination of ketene and, subsequently, $C_3H_7$ involves the net cleavage of the 1–2, 3–4, 5–10 and 9–10 bonds. It may not be meaningful to attempt to decide the precise sequence of cleavage of these bonds, even though the effect of the presence of a substituent on C-10 suggests that the 9–10 bond is broken early in the process, probably in most cases prior to loss of ketene. The formation of abundant [M-ketene] ions in the decomposition of the 1-methyl-19-norprogesterones, **3** and **4**, suggests that in these cases cleavage of the 1–2 bond may precede cleavage of the 9–10 bond. Hypothetical ion structures without mechanistic implications are shown in Scheme 2.

The M-($C_2H_2O + C_3H_7$) ions (**b**) are, expectedly, of low abundance in those cases where the precursor [M-$C_2H_2O$] ions are not very abundant. More puzzling is the observation that $C_3H_7$ elimination is significantly decreased in the 6$\alpha$-methyl substituted progesterones **6** and **15**, relative to the 6$\beta$ analogs **7** and **16**, and relative to progesterone (**1**) itself (see also below).

Elimination of $CH_3CO$ from the [M-$C_2H_2O$] ion gives rise to the hydrocarbon peak of the *m/e* 229 doublet. The abundance of the product [M-$C_4H_5O_2$] ions, **c**, vary considerably, following to a certain extent the abundance of the precursor, **a**. Alkyl substituents at C-6 have a significant effect also on the abundance of **c**, and hence on its formation and/or fragmentation, as witnessed by the more than threefold reduction in abundance when going from 6$\alpha$- to 6$\beta$-methylprogesterones (**6–7**, and **15–16**). We

†The *m/e* 229 peak associated with the four processes M-($C_2H_2O + C_3H_7$), M-($C_2H_2O + CH_3CO$), M-($C_4H_6O + CH_3$) and M-$C_5H_9O$, is replaced in the spectrum of 1-$d_9$ by a series of peaks from *m/e* 233 to *m/e* 236, corresponding to loss of 2, 5, 4 and 4 deuterium atoms, respectively, in the four reactions.




Scheme 2.

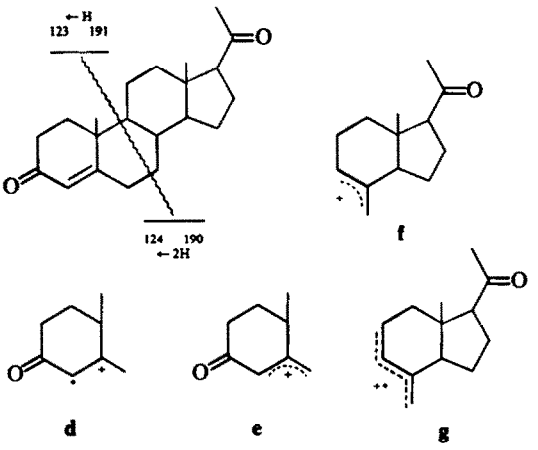
cannot offer any rationale for these observations, except to point out that the abundance of the isobaric [M-$C_4H_5O_2$] and [M-$C_5H_9O$] ions **b** and **c** change in opposite directions and by the same order of magnitude; the operation of the two effects is therefore not immediately evident from low resolution spectra. It is perhaps significant that the relationship between the intensity of the two peaks and the configuration at C-6 is reversed in the 10$\alpha$ series, where 6$\alpha$-methyl-10$\alpha$-progesterone (**26**) yields abundant [M-$C_5H_9O$] ions, but negligible [M-$C_4H_5O_2$] ions.

A very characteristic feature of the mass spectral fragmentation of progesterone and many other steroidal $\Delta^4$-3-ketones is the ring B cleavage (fission of the 6–7 and 9–10 bonds with concomitant transfer of two hydrogen atoms originally bound to C-8 and C-11[9]) producing in progesterone an abundant *m/e* 124 ion, **d** ($C_8H_{12}O$). All the progesterone mass spectra examined in the present study exhibit a reasonably intense peak associated with such a cleavage. Possible mechanistic rationalizations have been given in an earlier communication[9] from this laboratory. The relative abundances of these ions (**d**, Tables 2 and 3) vary considerably with changes in substitution and configuration. Very pronounced effects are found in the spectra of 10$\alpha$-progesterones (**25–29**) and 9$\alpha$-methyl-19-norprogesterone (**30**), where the intensity of **d**, as noted earlier for the 10$\alpha$ compounds,[5] is significantly greater than in the remaining compounds. This is believed to show that the transfer of at least one of the H atoms (that originating on C-11) takes place on the $\beta$-face of the molecule, and that it occurs before the stereochemical integrity of C-10 is lost, possibly concomitant with cleavage of the 9–10 bond. Further support for transfer of the axial $\beta$ hydrogen from C-11 is found in Grostic and Rinehart's observation[7] that the *m/e* 124 peak is much more pronounced in the spectrum of 11$\alpha$-hydroxyprogesterone than in the spectrum of the 11$\beta$ isomer.

Compounds with a 6$\beta$ substituent (**7** and **16**) give rise to **d** ions of significantly lesser abundance than do the corresponding 6$\alpha$ isomers (**6** and **15**, Table 2); this is consistent with a preferred mechanism of hydrogen migration via a transition state where the 6$\beta$-Me and the 8$\beta$-H atom have retained their spatial proximity. Of further mechanistic relevance is the observation that the presence of 6$\alpha$- or 7-alkyl substituents does not significantly enhance the abundance of **d** relative to the corresponding unsubstituted progesterones. This suggests that the 6–7 bond is not broken in the rate-determining step of the formation of these ions and that the stability of the products is not sensitive to the presence of these substituents.

2,2-Dimethylprogesterone (**14**) gives rise to the analog of **d** (here: *m/e* 152) of lesser abundance than any of the other progesterones examined (excepting the 17-alkyl analogs); this is associated with the occurrence of other, more favored reactions of the molecular ion, in particular elimination of $C_4H_8$ (C-1, C-2 and their substituents).

Ion **d** decomposes further via elimination of a methyl group; this process gives rise to reasonably abundant ions when **d** itself is very abundant as in the decomposition of the 10$\alpha$-progesterones (*m/e* 109 in **25**, **27**, **29**, *m/e* 123 in **26**, **28**; Table 3), but the corresponding peaks are also noteworthy in the spectra of many of the remaining compounds. The fact that peaks corresponding to **d**-$CH_3$ and **d**-$C_2H_5$ are both observed in the spectrum of 19-methylprogesterone (**13**) (with the latter slightly predominating), while the 19-norprogesterones **2**, **5** and **30** do not give rise to significant **d**-$CH_3$ peaks shows that C-19 is frequently implicated in this process. The only other compounds that give rise to peaks corresponding to both **d**-$CH_3$ (*m/e* 123) and **d**-$C_2H_5$ (*m/e* 109) are the 6-methylprogesterones (**6**, **7**, **15–17**, **20**, **21**, **23**, **26**, **28**); this shows that C-6 may also be a source of the eliminated alkyl group.




Scheme 3.

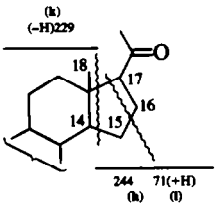
Ion **d** is always accompanied by an ion **e** (Scheme 3) with one less H atom, which is usually considerably less abundant. It is interesting, however, to note that the abundance of **e** is dependent upon the presence of 6-alkyl substituents, but not on their stereochemistry. Typically, **e** (*m/e* 137) formed from 6-alkyl progesterones is about three times as abundant as the **e** ions (*m/e* 123) formed from the corresponding 6-unsubstituted compounds; this indicates that either the stability of these ions or the rate of cleavage of the 6–7 bond is significantly dependent upon the presence of the 6-alkyl group, in marked contrast to the situation existing for **d**. The fact that 7$\alpha$-methylprogesterone (**8**) does not give rise to **e** in greater abundance than does progesterone (**1**) points to increased stability of the product ion rather than lability of the 6–7 bond as the major reason for the effect of 6-alkyl substitution. No labeling data are available to indicate the origin of the hydrogen atom transferred in the formation of **e**.

Progesterones with 17$\alpha$ groups (**18–24**) undergo the ring B cleavage reaction to a lesser extent than the remaining compounds, probably because of their increased tendency to decompose by way of reactions that take place in ring D (see below). Characteristically, the peak corresponding to **d** does not stand out in the low mass range of the spectra of 17$\alpha$-alkyl progesterones; in fact, the intensity of the peak corresponding to **e** in 6,17$\alpha$-disubstituted progesterones (**20**, **21**) is even greater than that corresponding to **d**, in marked contrast to the situation in the rest of the alkylprogesterone spectra examined. A similar lack of prominence of the otherwise characteristic *m/e* 124 peak (**d**) is observed in the spectra of C-17 oxygenated progesterones.[19]

Cleavage of ring B with transfer of one or two H atoms to the ring A portion but with charge retention in the fragment incorporating rings C and D also gives rise to reasonably abundant ions, with the ions (**f**) formed after transfer of one H atom present in greater abundance than those (**g**) formed after transfer of two H atoms. The

abundances of the ions corresponding to **f** are generally less when these are formed from progesterones with 6-alkyl groups than from compounds without. This corresponds well with the results presented above for **e**, (cleavage of the same skeletal bonds but with reversal of charge retention), indicating again that 6-alkyl groups increase the stability of the fragment incorporating ring A. Progesterone isomers with rings B and C joined *cis* (**27–29**) afford an exception to this, since they give rise to intense peaks corresponding to **f** (*m/e* 191) irrespective of alkyl substitution. A 9α-Me group likewise enhances the formation of **f** and **g** (compare **2** and **30** in Tables 2 and 3).

*Cleavage of ring D.* Decomposition reactions resulting in loss of fragments that incorporate parts of ring D usually give rise to prominent peaks in the mass spectra of steroids;[3] two such peaks are observed in the mass spectrum of progesterone, *m/e* 244 (**h**) and *m/e* 229 (**k**).




The reaction leading to **h**, cleavage of the 13–17 and 15–16 bonds (*partial* ring D cleavage), is as expected favored by 16-alkyl groups (**10, 11, 15–17**). Less predictable is the observation that the presence of a 14α-methyl group (**9**) causes this peak (expected at *m/e* 258) virtually to disappear from the spectrum; this probably demonstrates that the 14α hydrogen in progesterone migrates (to C-15?) during this fragmentation; such an assumption is not unreasonable since cleavage of the 13–17 and 15–16 bonds would otherwise lead to a product ion with charge or radical at a primary position (C-15). It is also possible that even more deep-seated skeletal reorganization accompanies the formation of **h**, since the abundance of this ion is significantly decreased also in the 10α-progesterones (**25–29**) and when 7α- or 9α-methyl groups (**8** and **30**) are present.

Introduction of 17α-alkyl substituents (**18–21**) and of reversal of configuration at C-17 (**22–24**) causes a dramatic increase in the abundance of **h** (Tables 2 and 3). The reason for this could be an increased lability of the 13–17 bond in **18–21**, as a consequence of the increased degree of substitution, even though ring D cleavage (ion **k**, see below) is not similarly enhanced in these compounds. The increased abundance of **h** in the 17α compounds (**22–24**) relative to the corresponding 17β isomers, which has also been observed by Zaretskii[6] and Genard,[8] is in striking contrast to the results obtained previously[10] for 17α- and 17β-pregnan-20-ones, where only minor differences were observed between the spectra of the two epimers. This shows, in agreement with the results presented above for methyl loss, that formation of a *seco* molecular ion by cleavage of the 13–17 bond is not generally an important initial reaction in the fragmentation of progesterones, since the configurational difference between the 17α and 17β isomers would be destroyed in this process.

Defocusing measurements (Table 5) have shown that **h** (*m/e* 244) formed from 17α-ethylprogesterone (**19**) is among the precursors to ions **d** and **e** (*m/e* 124 and 123), in contrast to the situation in progesterone (**1**). This may either reflect that the structure of the ions formed in the *partial* ring D cleavage (**h**) is dependent upon whether or not C-17 is substituted, or that this process is only observed when the precursor (**h**) is very abundant.

In all cases where it is formed in appreciable abundance, ion **h** fragments further by elimination of an alkyl radical, usually methyl, which may be expelled from various sites on the steroid skeleton. The two angular Me groups are not the primary sources of the eliminated fragment, for $CH_3$ elimination from **h** is an important reaction of the 19-norprogesterones (**2–5**), while the 18-methyl analogs (**5** and **12**) eliminate Me in preference to Et; the peak corresponding formally to loss of Et from **h** in the spectrum of 19-methylprogesterone (**13**) cannot be distinguished from that corresponding to **c**. More rigorous labeling, isotopic as well as with substituents, is needed to resolve this problem more completely.

Cleavage of the 13–17 and 15–16 bonds also takes place with charge retention in the small fragment, accompanied by hydrogen migration, to produce an ion **l** (*m/e* 71 in progesterone). The formation of this ion is also favored by the presence of 16- and 17-alkyl groups (**10, 11, 15–17,**

Table 5. Metastable ions decomposing in the first field free region

| Cpd. | Daughter ion | | Parent ion(s)[a] |
|---|---|---|---|
| **1** | 272 | (**a**) | 314 (M) |
| | 244 | (**h**) | 272 (**a**), 314 (M) |
| | 229 | (**b, c, k, h**-$CH_3$) | 244 (**h**), 272 (**a**), 314 (M) |
| | 191 | (**f**) | 314 (M) |
| | 190 | (**g**) | 314 (M)[b] |
| | 124 | (**d**) | 314 (M)[b] |
| **11** | 243 | (**b, c**) | 286 (**a**)[c] |
| | 244 | (**h**) | 328 (M) |
| **12** | 245 | (**l**) | 328 (M) |
| | 243 | (**b, c, k, h**-$CH_3$) | 258 (**h**), 286 (**a**), 328 (M)[d] |
| | 204 | (**g**) | 328 (M)[b] |
| **13** | 258 | (**h**) | 328 (M) |
| | 257 | | 286 (**a**) |
| | 243 | (**c, k, h**-$CH_3$) | 286 (**a**)[e] |
| | 229 | (**b, h**-$C_2H_5$) | 258 (**h**), 286 (**a**)[f] |
| **15** | 258 | (**h**) | 342 (M) |
| | 257 | (**b, c**) | 300 (**a**)[g] |
| **16** | 273 | | 342 (M) |
| | 258 | (**h**) | 342 (M)[h] |
| | 257 | (**b, c**) | 300 (**a**), 327 (M-$CH_3$), 342 (M)[i] |
| | 245 | (**l**) | 342 (M) |
| | 243 | (**k, h**-$CH_3$) | 258 (**h**), 300 (**a**), 327 (M-$CH_3$), 342 (M)[j] |
| **19** | 229 | (**h**-$CH_3$) | 244 (**h**), 299 (M-$CH_3CO$), 342 (M)[k] |
| | 124 | (**d**) | 244 (**h**), 342 (M)[l] |
| | 123 | (**e**) | 244 (**h**), 299 (M-$CH_3CO$)[l] |

[a] Only parent-daughter relationships shown by *intense* metastable peaks included.

[b] Also small metastable for formation from **a**.

[c] Also small metastable for one-step formation from M.

[d] Metastable peak for formation from **h** much less intense than the two other metastables.

[e] Very small metastable peaks show formation also from **h** and M.

[f] Metastable peak for formation from **h** much less intense than that for formation from **a**. Very small metastable corresponding to one-step formation from M also present.

[g] Also small metastable peaks for formation from M and [M-$CH_3$].

[h] Also small metastable peak for formation from [M-$CH_3$].

[i] Metastable peak for formation from **a** much more intense than the two other.

[j] Intensity relationship: 258 > 342 > 300 ~ 327.

[k] Intensity relationship: 244 > 342 > 299.

[l] Six other very small metastable peaks.

18–21) (see also Ref. 20) and by reversed configuration at C-17 (22–24). The decomposition of pregnan-20-ones has been shown[10] to give rise to a similar ion; the H atom transferred was suggested to come from C-14. This appears reasonable when considering the reduced intensity of the peak corresponding to i in the spectrum of 14α-methylprogesterone (9).

Loss of ring D by cleavage of the 13–17 and 14–15 bonds is the most characteristic fragmentation of steroidal hydrocarbons bearing a side chain at C-17,[3,21] and also gives rise to very intense peaks in pregnan-20-ones.[10] In the latter case the process takes place nearly exclusively with concomitant transfer of a H atom to the eliminated fragment, as is also observed in the progesterone series. The corresponding ions, **k**, (*m/e* 229 in progesterone) are, however, of quite low abundance. Furthermore, other reactions give rise to ions of the same elemental composition,† and it appears that ring D cleavage (formation of **k**) is not a diagnostically significant reaction of progesterones.

This deviation from the "usual" fragmentation of steroids is caused by (indirect) interaction of the two ketonic functions. The previous investigation of pregnan-20-ones[10] established that the H atom transferred to the fragment subsequently expelled in the process leading to **k** originates to a substantial degree from C-8. However, in progesterone and other steroidal $\Delta^4$-en-3-ones this H atom is also implicated in the ring B cleavage reaction (see above), and it appears that transfer of the C-8 hydrogen to ring A is more favorable than transfer to ring D, causing the ring D cleavage to diminish in relative importance.

Another peak is frequently observed two mass units above the peak corresponding to **k**. This peak, **l**, is quite intense in the spectra of 18-methylprogesterones (**5**, **12**) and apparently corresponds to loss of ring D with (net) transfer of one hydrogen atom to the charge retaining moiety. It is interesting to note that whenever **l** is reasonably abundant there are also abundant ions corresponding in elemental composition to ring D cleavage with charge retention in the low mass fragment (*m/e* 84 in **5** and **12**).

*Metastable defocusing experiments.* Many of the processes discussed above are accompanied in the low resolution mass spectra by metastable peaks. In order to examine further the origin(s) of the more abundant ions we have carried out a number of experiments by the metastable defocusing technique. The results, which are summarized in Table 5, show that many fragment ions have more than one precursor. It is generally assumed[22] that it is possible to obtain qualitative but not quantitative information about fragmentations taking place in the ion source from the observation of metastable peaks, and hence it is not possible to relate the relative intensities of metastable peaks to the relative importance of the corresponding processes in the ion source. Therefore the data presented in Table 5 do not allow an assessment to be made of, e.g. the relative contributions of the decompositions of the parent ions of mass 244, 272 and 314 to the formation of *m/e* 229 in the mass spectrum of progesterone (**1**), but rather they serve to substantiate the parent-daughter relationships described above.

In summary, the present paper illustrates two important points which are not only relevant to the mass spectrometry of steroids but also of other organic compounds. First, the INTSUM program can be of great help to the chemist in handling large numbers of high resolution data, especially when dealing with a series of closely related molecules. This help does not only refer to the interpretation of mass spectrometric fragmentation processes but also to the fact that attention is frequently called to reactions which merit additional experimental work, be it by isotopic labeling or other methods. Second, contrary to the generally held assumption about the relative insensitivity of mass spectrometry to stereochemistry, the present study shows that even relatively minor stereochemical changes can at times have an important impact on mass spectrometric fragmentation processes. However, in order to detect these or even more importantly to employ such information for eventual assignment of stereochemistry it is usually highly desirable to have both isomers at one's disposal.

†It has not been possible to distinguish between ions formed by elimination of Me from **h** and ions formed in one-step loss of ring D (**k**), since no progesterone analogs with substituents at C-15 were available.

*Acknowledgements*—The patient instruction and assistance of Mr. W. C. White and Dr. D. H. Smith with the computer programs is gratefully acknowledged. We thank the National Institutes of Health, U.S. Public Health Service (Grants No. AM-04257 and RR-612-05), for financial assistance, and Statens Naturvidenskabelige Forskningsraad for a supporting grant (to S.H.).

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