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# THE DETERMINATION OF RING JUNCTION STEREOCHEMISTRY IN STEROIDS USING MASS-ANALYSED ION KINETIC ENERGY SPECTROMETRY

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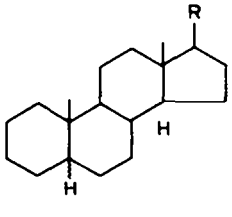
**Abstract**—The unimolecular mass-analysed ion kinetic energy (MIKE) spectra of 9 pairs of hydrocarbon and ketone steroid isomers, differing only in the stereochemistry at the A/B and C/D ring junctions, have been measured and are discussed with a view to unambiguous structural identification. Reproducible differences in the MIKE spectra are observed, which are large enough in certain instances to suggest that MIKE spectrometry may be used for determining the stereochemistry of the A/B and C/D ring junctions in steroidal isomers, even if the second isomer is not available. This fortunate situation is rarely observed in conventional mass spectrometry of stereoisomeric steroids. Furthermore, these differences in the MIKE spectra may be correlated with differences in strain energy between configurational isomers. The sensitivity of MIKE spectrometry to differences in strain energies makes it a potentially powerful stereochemical probe.

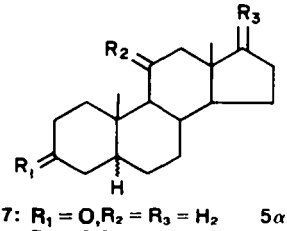
The framentation patterns of steroidal molecular ions, generated by electron impact ionization in the ion source of a mass spectrometer, have been documented for a large number of different functional classes, including isomeric structures. It has been amply demonstrated by these studies that the fragmentation pattern of an ion may be related to the structure of the neutral molecule[1] and hence may be employed in the structural elucidation of unknown compounds. Fragmentation mechanisms of steroids are often complex and with the aid of deuterium labelling studies, particularly by Djerassi *et al.*[2] many of these have been determined.

A large number of investigations have been carried out on the mass spectra of steroidal isomers. In particular, the scope of conventional mass spectrometry in distinguishing between stereoisomers has been studied for isomers differing only in the stereochemistry at the A/B or C/D ring junctions.[1] However, distinction between such isomers is frequently not possible from the normal electron impact mass spectrum, which consists of reaction products formed in the ion source from high energy reactions in a few microseconds or less. The possibility arises, however, that more stereochemical information might be forthcoming from the lower energy reactions occurring in unimolecular mass-analysed ion kinetic energy (MIKE) spectrometry.[3] This is because (i) the spectra are simpler, being only the reaction products of one ion (e.g. the molecular ion) and (ii) the occurrence of unimolecular reactions of metastable ions is very sensitive to changes in the critical energies of those reactions.

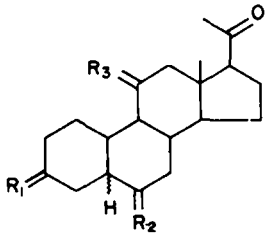
Although the MIKE spectra of a variety of steroidal isomers have been investigated,[4-6] none has been at the epimeric level of stereoisomerism. However, linked scanning of magnetic and electric fields has recently been used to study epimeric t-butyl-dimethylsilyl ethers of androstan-3,17-diols.[7] In this investigation we have measured the unimolecular MIKE spectra of the molecular ions for a series of hydrocarbon (**1–6**) and ketone (**7–18**) steroid stereoisomers (see below). The stereochemistry in each series differs only in the configuration at the A/B or C/D ring junctions.




**1**: R = H 5α, 14α
**2**: R = H 5β, 14α
**3**: R = H 5α, 14β
**4**: R = H 5β, 14β
**5**: R = $C_2H_5$ 5α, 14α
**6**: R = $C_2H_5$ 5β, 14α




**7**: $R_1$ = O, $R_2$ = $R_3$ = $H_2$ 5α
**8**: $R_1$ = O, $R_2$ = $R_3$ = $H_2$ 5β
**9**: $R_1$ = $R_2$ = $H_2$, $R_3$ = O 5α
**10**: $R_1$ = $R_2$ = $H_2$, $R_3$ = O 5β
**11**: $R_1$ = $R_3$ = O, $R_2$ = $H_2$ 5α
**12**: $R_1$ = $R_3$ = O, $R_2$ = $H_2$ 5β
**13**: $R_1$ = $R_2$ = $R_3$ = O 5α
**14**: $R_1$ = $R_2$ = $R_3$ = O 5β




**15**: $R_1$ = $R_2$ = O, $R_3$ = $H_2$ 5α
**16**: $R_1$ = $R_2$ = O, $R_3$ = $H_2$ 5β
**17**: $R_1$ = $R_3$ = O, $R_2$ = $H_2$ 5α
**18**: $R_1$ = $R_3$ = O, $R_2$ = $H_2$ 5β

## RESULTS AND DISCUSSION

### *Androstanes* (1–4)

The full mass spectra of 5α, 14α- and 5β,14α androstane, (1 and 2), have been obtained and the fragmentation patterns determined with the aid of deuterium labelling.[8,9] Although there are differences in the relative abundances observed for certain ions in the mass spectra of these two isomers, these differences are sufficiently minor that an individual mass spectrum cannot identify one isomer without a comparison with the spectrum of the other isomer. Accordingly, the MIKE spectra of the molecular ions of the four androstane isomers (1–4) have been measured (Fig. 1) with a view to identifying the stereochemical differences at the A/B and C/D ring junctions. The MIKE spectra were taken without collision gas in the field-free region between the magnetic and electric sectors and therefore represent the low energy unimolecular reactions of the molecular ions.

The major elimination from the molecular ions of the androstane isomers, observed in the MIKE and normal mass spectra, is of an angular methyl group. In the ion-source, where high energy decompositions predominate, the 18-methyl group is eliminated preferentially to the 19-methyl group in a ratio of 3:2, as evidenced by the mass spectrum of 18-$CD_3$-5α, 14α-androstane[8] (see Table 1). It would thus be anticipated that the metastable peak in the MIKE spectra, resulting from low energy decompositions, for loss of methyl from the androstane molecular ions, should be composite in nature since the rate constant/energy curves for the two methyl elimination reactions should be similar. The relative critical energies of these two competing reactions are of great importance in determining the MIKE spectra in that they affect the composite nature of the metastable peak for methyl loss from the molecular ion among the configurational isomers.

When either the A/B or C/D ring junction stereochemistry is changed from a *trans*-configuration (5α) to a *cis*-configuration (5β), it appears that a change in the critical energy for the reaction involving methyl loss at that particular ring junction occurs. For the A/B ring junction it is proposed that this critical energy is less for the 5β- (*cis*-A/B ring junction) compared with the 5α-isomer (*trans*-A/B ring junction). The situation is reversed for the C/D ring junction where the critical energy for the loss of the 18-methyl group is less for the trans-(14α) compared with the *cis*-(14β) isomer. The rationale for this is as follows: (i) The 5β-isomers are less stable than the corresponding 5α-isomers.[17] Consequently, more strain energy is relieved at the A/B ring junction when the 5β-isomer dissociates by loss of the 19-methyl group, leading to a lower critical energy for the reaction. (ii) The reverse appears to be the case for a stereochemical change at the C/D ring junction where it is the 14β-isomer that is the more stable.[17] (iii) Critical energies ($E_a$) may be roughly estimated from the difference between appearance energy (AE) and ionization energy (IE), $E_a \simeq (AE - IE)$, (see Table 2).[19] This parameter is significantly smaller for the 5β- as compared with the 5α-androstanes and changes only marginally when the configuration at the 14-position is altered.

It can of course be argued that changes in the mode of ring junction will also affect the critical energies of the minor reactions (all involving ring cleavage). This point is discussed further below but it should be emphasized that the data show that the effect of changing the configuration appears to be greatest on the methyl elimination, the reaction of smallest critical energy.

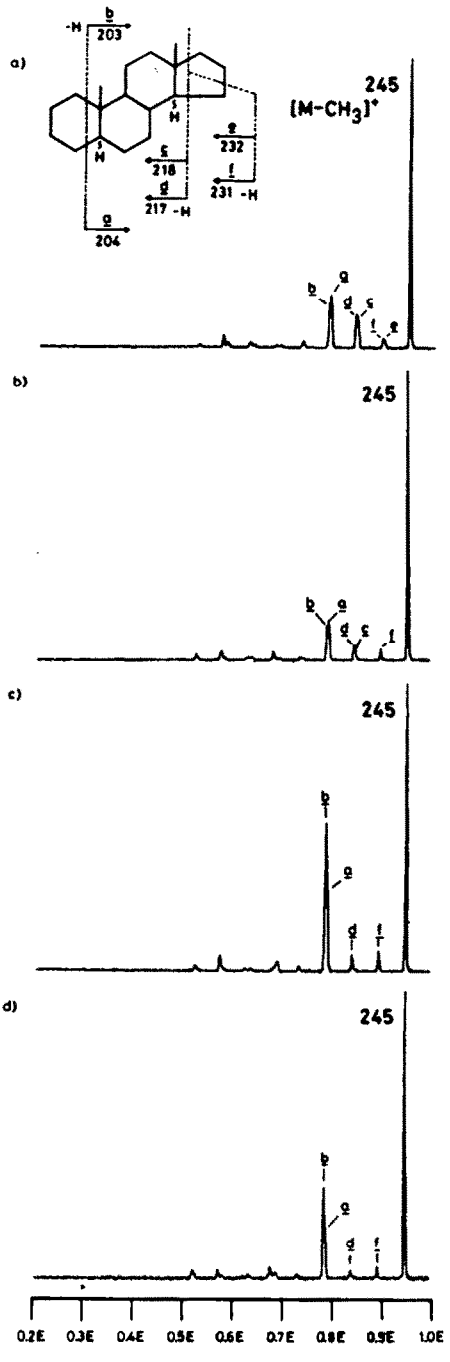



Fig. 1. The unimolecular MIKE spectra of $M^{+\cdot}$ (*m/z* 260) of (a) 5α,. 14α-androstane (1); (b) 5β, 14α-androstane (2); (c) 5α, 14β-androstane (3); and (d) 5β, 14β-androstane (4).

These hypotheses may be employed to rationalize differences in the MIKE spectra of the four androstane isomers (Fig. 1). On changing the configuration of the A/B ring junction from *trans* to *cis* (see Fig. 1b *cf* Fig. 1a and Fig. 1d *cf* Fig. 1c), the relative abundance of the metastable peak due to methyl loss increases owing to decreased critical energy for the loss of the 19-methyl group. However, when the configuration at the C/D ring junction is changed from *trans* to *cis* (see Fig. 1a *cf* Fig.

Table 1. Angular methyl loss ratios for various steroidal stereoisomers

| Compound | 19-$CH_3$/18-$CH_3$ Elimination Ratio | Reference |
|---|---|---|
| 5α, 14α-Androstane (1) | 2:3 | 8 |
| 5α, 14α-Pregnane (5) | 5:1 | 10 |
| 5α-Androstan-3-one (7) | 1:1 | 11 |
| 5α-Androstan-17-one (9) | 3:1 | 12 |
| 5α-Androstan-1-one (19) | 1:1 | 13 |
| 5α-Androstan-2-one (20) | 1:1 | 14 |
| 5α-Androstan-4-one (21) | 4:1 | 15 |
| 5β-Androstane-4-one (22) | 6:1 | 15 |
| 5α-Androstane-6-one (23) | 7:1 | 15 |
| 5α-Pregnan-20-one (24) | 1:0 | 16 |
| 5α-D-Homoandrostan-17a-one (25) | 1:1 | 14 |
| 5α,13α-D-Homoandrostan-17a-one (26) | 3:1 | 14 |

Table 2. Relative intensities[a] of metastable ions and some energy parameters for reactions in stereoisomeric steroid hydrocarbons

| Compound | A/D Cleavage Ratio[b] | $m^*\{[M-CH_3]^+\}/[M]^{+\cdot}$ (x10$^3$) | relative[d] | Relative $\Delta G^\circ$ (kcal/mole)[e] | $E_a$ (eV)[f] |
|---|---|---|---|---|---|
| 5α, 14α-Androstane (1) | 1.6 | 10.1 | 8.0 | 1.77 ± 0.45 | 0.97 ± 0.06 |
| 5β, 14α-Androstane (2) | 2.7 | 20.6 | 16.4 | 2.66 ± 0.22 | 0.67 ± 0.03 |
| 5α, 14β-Androstane (3) | 8.8 | 1.3 | 1.0 | 0.00 | 1.00 ± 0.05 |
| 5β, 14β-Androstane (4) | 10.8 | 5.5 | 4.4 | 1.48 ± 0.35 | 0.68 ± 0.08 |
| 5α, 14α-Pregnane (5) | c | 6.3 | 1.0 | - | 1.01 ± 0.04 |
| 5β, 14α-Pregnane (6) | c | 9.4 | 1.5 | - | 0.80 ± 0.06 |

[a] An error of up to ± 5% is anticipated

[b] $m^*\{[203]^+\}/m^*\{[217]^+\}$

[c] Fragmentation occurs almost exclusively from the D-ring, see reference 18.

[d] The relative ratio of $m^*\{[M-CH_3]^+\}/[M]^{+\cdot}$ between epimeric pairs.

[e] See reference 17

[f] $E_a \approx AE$- IE, see reference 19.

1c and Fig. 1b *cf* Fig. 1d), the relative abundance of the metastable peak due to loss of $CH_3$ decreases due to an increased critical energy for the loss of the 18-methyl group. This effect can be seen more clearly by inspection of the ratio of the intensity of the metastable peak for methyl loss to the intensity of the main beam of molecular ions, $m^*\{[M-CH_3]^+\}/[M]^{+\cdot}$. In Table 2 it can be seen how this ratio varies between isomeric pairs (epimers), becoming larger as the configuration at the A/B or C/D ring junction becomes more strained. An excellent correlation exists between this parameter and the experimental relative stabilities [free energy ($\Delta G^\circ$) data] of the androstanes and is summarized in Table 2.[17] Thus, the magnitude of the difference in this ratio between epimers can be used to judge the difference in strain energy.

The relative abundance of the most abundant metastable peak (that for loss of methyl from the molecular ion) therefore provides some information about the stereochemistry at the A/B and C/D ring junctions in the androstane isomers. Additional stereochemical information is also obtained from the smaller peaks in the MIKE spectra, whose relative abundances are dependent on whether they result from cleavages of the A- or D-rings. The peaks at *m/z* 203 and 204 in the MIKE spectra arise *via* A-ring cleavages and those at *m/z* 217 and 218 *via* D-ring cleavages.[8] Extending the arguments used above, it may be postulated that A-ring cleavage reactions would be more prevalent for the 5β-isomers compared with the 5α-isomers owing to greater relief of steric strain and hence lower critical energies involved in the case of the 5β-isomers. Conversely, D-ring cleavage

reactions would be expected to be more prevalent in the 14$\alpha$-isomers compared with the 14$\beta$-isomers. The effect of varying the configuration (and therefore the steric strain energy and critical energy of these cleavage reactions) can be clearly seen if we look at the relative ratios of reactions occurring at the A- and D-rings. Accordingly, an A/D cleavage ratio can be defined and measured from minor peaks in the MIKE spectra of the four androstane isomers (1–4). Earler work [8,9,18] has established the origin of various peaks with the aid of deuterium labelling. Based on those results, the A/D cleavage ratio is defined as the ratio of the intensity of *m/z* 203 and 217 metastable peaks, $m^*\{[203]^+\}/m^*\{[217]^+\}$.

The sensitivity of this parameter to configurational (and therefore strain energy) differences among the androstane isomers becomes apparent upon inspection of the A/D cleavage ratios summarized in Table 2. From these data, two significant points emerge: (i) The A/D cleavage ratio shows the greatest percentage change between the 14$\alpha$- and 14$\beta$- epimeric pairs. The intensity of the D-ring cleavage reactions is greatly reduced in the 14$\beta$-epimers (3) and (4), to such an extent that the configuration of the C/D ring junction can be reliably predicted from the A/D cleavage ratios alone. (ii) The mode of the A/B ring junction is not so easily determined from the A/D cleavage ratios (Table 2). These ratios for the 5$\alpha$- and 5$\beta$-isomers are however reproducible and afford information diagnostically useful in the determination of configuration at C-5. Given the A/D cleavage ratios for two epimeric androstanes, it should be possible to identify each isomer on any instrument.

Careful inspection of the MIKE spectra and parameters derived from them for the androstane isomers indicates that there are two competitive processes involved in both the loss of an angular methyl group as well as in cleavage reactions of the A- and D-rings. Since it has been shown that A- and D-ring elimination reactions occur via similar mechanisms,[8,9,18] these parameters ($m^*\{[M-CH_3]^+\}/[M]^{+\cdot}$ and A/D cleavage ratios) reflect the dependence of two (or more) competitive fragmentation reactions upon steric strain and upon the critical energies of these processes. Thus, an increase in steric strain at one isomeric centre, with a corresponding decrease in critical energy for reactions at that centre relative to another, would be expected to enhance reactions occurring at that centre. The data support this contention. Furthermore, the sensitivity of MIKE spectrometry to strain energies makes it a potentially powerful stereochemical probe. It is demonstrated below that the principles developed above also pertain to other steroidal stereoisomers.

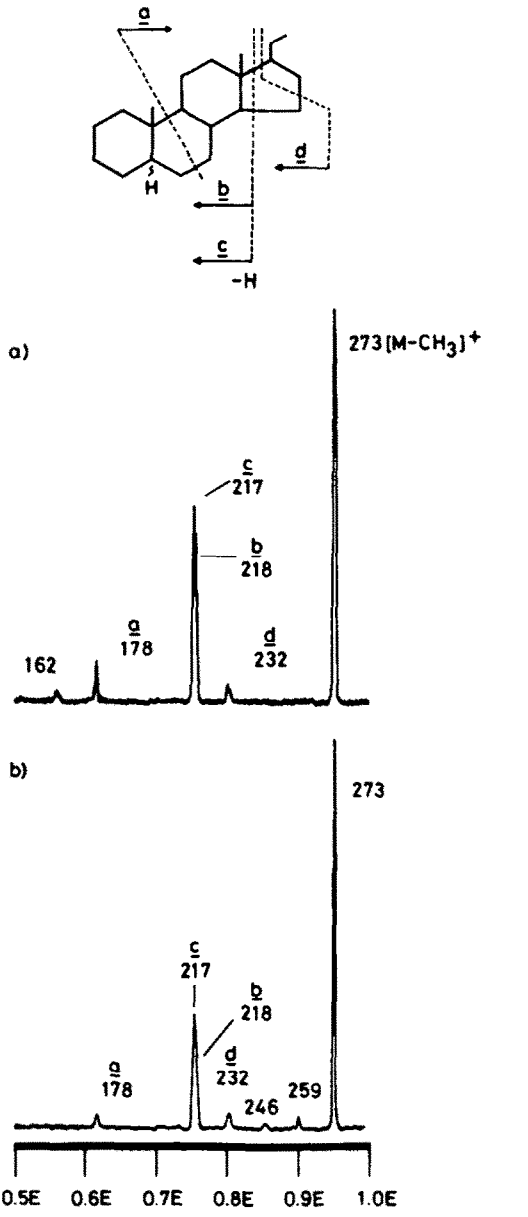



Fig. 2. The unimolecular MIKE specra of $M^{+\cdot}$ (*m/z* 288) of (a) 5$\alpha$, 14$\alpha$-pregnane (5) and 5$\beta$, 14$\alpha$-pregnane (6).

*Pregnanes* (5–6)

The presence of the ethyl side chain at the 17-position in the pregnanes alters the mass spectrometric fragmentation of the steroid skeleton with respect to the androstanes.[10,18] However, the conclusions that can be drawn from the MIKE spectra of the 5$\alpha$- and 5$\beta$-pregnane isomers (5) and (6) (Fig. 2, Table 2) remain broadly the same as those deduced for the androstanes discussed above.

The 17-ethyl group influences the relative tendencies for loss of the 18- or 19-methyl groups. For reactions occurring in the ion source, it has been established for the 5$\alpha$-pregnane isomer that the 19-methyl group is eliminated in 5:1 preference to the 18-methyl group (see Table 1).[10] This situation, which is the reverse of that found for the androstanes, may in part be attributed to a strengthening of the C–13 to C–18 bond in the pregnanes, due to a weakening of the C–13 to C–17 bond. Although labelling results have not as yet been obtained for metastable ions, it is assumed that the strong preference for loss of the 19-methyl group in the pregnanes observed for high energy reactions occurring in the ion source also holds for reactions of metastable ions.

The data in Table 2 illustrate that 5$\beta$-pregnane (6) shows a greater tendency to eliminate methyl, in low energy unimolecular reactions, compared with 5$\alpha$-pregnane (5). This phenomenon is again attributed to a lowering of the critical energy for the loss of the 19-methyl group in the 5$\beta$-isomer (6) due to a greater relief of steric strain, compared with the 5$\alpha$-isomer (5). Arguments based on measurements of A/D cleavage ratios are difficult to venture for the pregnane isomers,

since fragmentations occur almost exclusively from the D-ring.[10]

*Androstan-3-ones* (7–8)

In order to determine the effect of a heteroatom on the MIKE spectra of epimeric steroids, the 5α- and 5β-isomers of some mono-, di and triketo steroids have been investigated.

For the epimeric 5α- and 5β-androstane-3-ones (7) and (8) there is a much greater difference between the two MIKE spectra (Fig. 3 and Table 3) than was demonstrated for the hydrocarbon series above. Since the origins of only major fragmentations from the molecular ion have been elucidated by the appropriate isotopic labelling,[11] a detailed discussion of the MIKE spectra of these two epimers will not be included. However, there are some unifying features that emerge upon inspection of the MIKE spectra (Fig. 3, Table 3): (i) The relative intensity of metastable peak for methyl loss from the molecular ion increases in the MIKE spectrum of the 5β-isomer (8) compared with the 5α-isomer (7), (ii) the intensity of this peak also increases when referred to the intensity of the main beam (see $m^*\{[M-CH_3]^+\}/[M]^{+\cdot}$ ratio, Table 3) as was observed in the hydrocarbon series (see Table 2) and (iii) the majority of metastable reactions appear to occur *via* fragmentation reactions of the A-ring as evidenced by some labelling data.[11] These differences (see Fig. 3 and Table 3) are sufficiently large to permit the distinction between the epimeric 3-ketones (7) and (8), even if only one isomer is available.

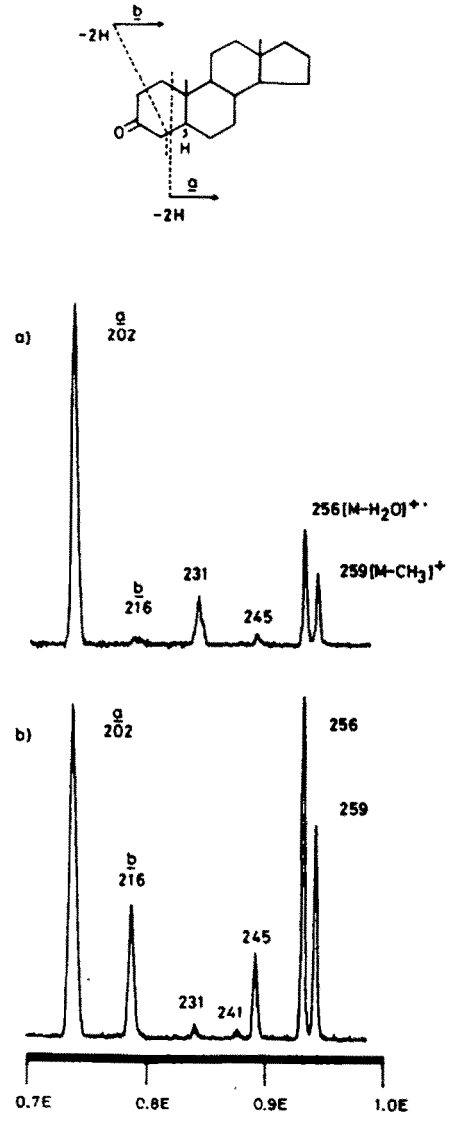



Fig. 3. The partial MIKE spectra of (a) 5α-androstan-3-one (7); and (b) 5β-androstan-3-one (8) showing the principal unimolecular reactions of $M^{+\cdot}$ (*m/z* 274).

The observations are consistent with a lowering of the critical energy for methyl loss from the A/B ring junction in the 5β-isomer (8) as compared to 5α-androstan-3-one (7),[19] due to increased steric strain. Note that the relative ratio of methyl loss between the 3-ketone epimers (7) and (8) is comparable to that observed between hydrocarbon epimers. Charge localization arguments can accommodate the preference for A-ring cleavage reactions in both of the 3-ketone epimers if it is assumed that ionization on the carbonyl group is dominant. The data support this contention.

*Androstan-17-ones* (9–10)

If ionization is preferentially occurring at the carbonyl group in the ketone isomers, then this pair of epimers provides a system for study which illustrates the effect of the proximity of the stereochemical variation (*cis*/*trans* isomerism at the A/B ring junction) to the site of charge localization (the 17-carbonyl group). All of the major fragmentation reactions observed in the MIKE spectra of these epimers (see Fig. 4), with the exception of methyl loss, are believed to arise from the D-ring[12] (consistent with localization of charge on the D-ring carbonyl group). The effect of the position of the carbonyl group with respect to the stereochemical variation, upon the MIKE spectra of epimeric monoketones can clearly be seen by comparison of the MIKE spectra of the 3- and 17-ketone epimers in Figs. 3 and 4. The differences between epimers are significantly greater when the carbonyl group is on the A-ring than on the D-ring, or closer to the stereochemical variation. The relative magnitude of differences in the MIKE spectra among these isomers reflects the greater predominance of reactions occurring near the site of charge localization (the carbonyl group) as well as the effects of the steric strain introduced by the carbonyl group.

The loss of methyl from the molecular ion which exhibits a preference of 3:1 for elimination of the 19-methyl to the 18-methyl in reactions occurring in the ion source (see Table 1)[12] is again higher in abundance for 5β-(10) than for 5α-androstan-17-one (9) (see Table 3). This observation is explicable if it is assumed that methyl loss from the A/B ring junction is promoted by the increased strain imposed in the 5β isomer (10). The relative loss of methyl between the two epimers is again similar to that observed above (see Tables 2 and 3), but the actual amount of methyl lost is slightly less than was observed for the 3-ketone epimers (7) and (8). This difference can be attributed to the presence or absence of the steric strain imposed by the carbonyl group on the A-ring.

*Androstan-3,17-diones* (11–12) *and androstan-3, 11, 17-triones* (13–14)

The general principles derived above to explain the

Table 3. Relative intensities[a] of metastable ions for $[M-CH_3]^+$ formation in stereoisomeric steroid ketones (7–18)

| Compound | $m^*\{[M-CH_3]^+\}/[M]^{+\cdot}$ (x10⁴) | relative[b] | $E_a$(eV)[c] |
|---|---|---|---|
| 5α-Androstan-3-one (7) | 15.3 | 1.0 | 1.05±0.06 |
| 5β-Androstan-3-one (8) | 22.8 | 1.5 | 0.82±0.06 |
| 5α-Androstan-17-one (9) | 4.0 | 1.0 | 0.96±0.05 |
| 5β-Androstan-17-one (10) | 6.1 | 1.5 | 0.83±0.06 |
| 5α-Androstan-3,17-dione (11) | 5.0 | 1.0 | 1.17±0.07 |
| 5β-Androstan-3,17-dione (12) | 31.3 | 6.3 | 1.28±0.07 |
| 5α-Androstan-3,11,17-trione (13) | 2.6 | 1.0 | 1.44±0.08 |
| 5β-Androstan-3,11-17-trione (14) | 35.9 | 13.3 | 0.61±0.07 |
| 5α-Pregnan-3,6,20-trione (15) | 0.7 | 1.0 | - |
| 5β-Pregnan-3,6,20-trione (16) | 43.2 | 61.1 | - |
| 5α-Pregnan-3,11,20-trione (17) | 1.26 | 1.0 | - |
| 5β-Pregnan-3,11,20-trione (18) | 10.6 | 8.4 | - |

[a] An error of up to ± 5% is anticipated.

[b] The relative ratio of $m^*\{[M-CH_3]^+\}/[M]^{+\cdot}$ between epimeric pairs.

[c] $E_a \simeq$ AP-IP, see reference 19.

features in the MIKE spectra of the androstane monoketone isomers persist and can be applied to di-and triketone androstane isomers. The data shown in Figs. 5 and 6, and Table 3 illustrate that the metastable peak for methyl loss from the molecular ions of the epimeric 5α- and 5β-androstan-3, 17-diones, (11) and (12), and 5α- and 5β-androstan-3, 11, 17-triones, (13) and (14), is much more pronounced for the 5β-isomers, (12) and (14). The enhanced loss of methyl from the 5β-isomers is again explained in terms of the increased steric strain at the A/B ring junction, which in turn lowers the critical energy for methyl loss. The greater differences in amount of methyl loss between epimers may be attributed to the greater differences in steric strain between *cis*- and *trans*- A/B ring junction isomers as a result of the presence of the carbonyl groups. The resultant lowering in critical energy for this reaction, relative to others, is evidenced by inspection of the MIKE spectra in Figs. 5 and 6 where elimination of methyl and water are the dominant reactions from the molecular ions.

Additional diagnostically important information about the stereochemistry at the A/B ring junction in androstane-3,17-diones is obtained from the relative intensity of the metastable peak for the loss of the A-ring (*a*, m/z 218, see Fig. 5)[20]. While this peak is practically absent from the MIKE spectrum of the trans-A/B (5α) isomer (11), it can be easily detected in the spectrum of more strained *cis*-A/B (5β) diketone (12) (Fig. 5b). Indeed, the breakdown of ring A could be expected to be more extensive for the 5β-isomer (12) compared to the 5α-isomer (11) due to greater relief of steric strain and hence lower critical energies for the A-ring cleavage reactions in the case of the 5β-isomer (12).

*Pregnan-3,6,20-triones* (15–16) *and pregane-3, 11, 20-triones* (17–18)

The pregnane triketone isomers were investigated to look further at the effect of the position of carbonyl groups upon the MIKE spectra of epimeric steroids and in particular at the effect upon A/B ring junction isomerism. The major peaks in the MIKE spectra of these isomers are summarized in Table 4. The dominant reactions of the molecular ions in all cases were the elimination of the methyl radical or of water. Since the fragmentation mechanisms for these compounds have been documented only in general terms[20], a detailed discussion of minor peaks in the MIKE spectra will not be included at this time.

Inspection of the MIKE spectral data in Tables 3,4 particularly the $m^*\{[M-CH_3]^+\}/[M]^{+\cdot}$ data, reveals striking differences between the epimeric pairs. There is again a strong preference for methyl loss in the more strained 5β-isomers, (16) and (18). The relative difference in the amount of methyl loss between the 5α- and 5β-pregnan-3, 11, 20-trione epimers, (17) and (18), is of the magnitude observed for the androstane ketones discussed earlier (see Table 3). However, the magnitude of the relative difference in the amount of methyl loss between the 5α- and 5β-pregnane-3, 6,20-trione epimers, (15) and (16), is exceptionally large. The magnitude of this difference can be easily rationalized employing principles developed earlier. The presence of carbonyl groups on both the A- and B- rings would be expected to result in a substantial amount of rigidity in the A- and B-rings of the steroidal skeleton. This rigidity, or stiffness, would enhance the difference in steric strain between *cis*/*trans* A/B ring junction isomers in the 5α- and 5β-pregnane-3,6,20-trione epimers, (15) and (16), as compared to the 5α- and 5β-pregnan-3,11,20-trione epimers, (17) and (18), where there is no carbonyl group on the B-ring. Therefore, the difference in steric strain, and thus the critical energy for loss of methyl, should be much greater between the (15) and (16) epimers than for the (17) and (18) epimers. This is in fact what the $m^*\{[M-CH_3]^+\}/[M]^{+\cdot}$ data in Table 3 show. It is the most

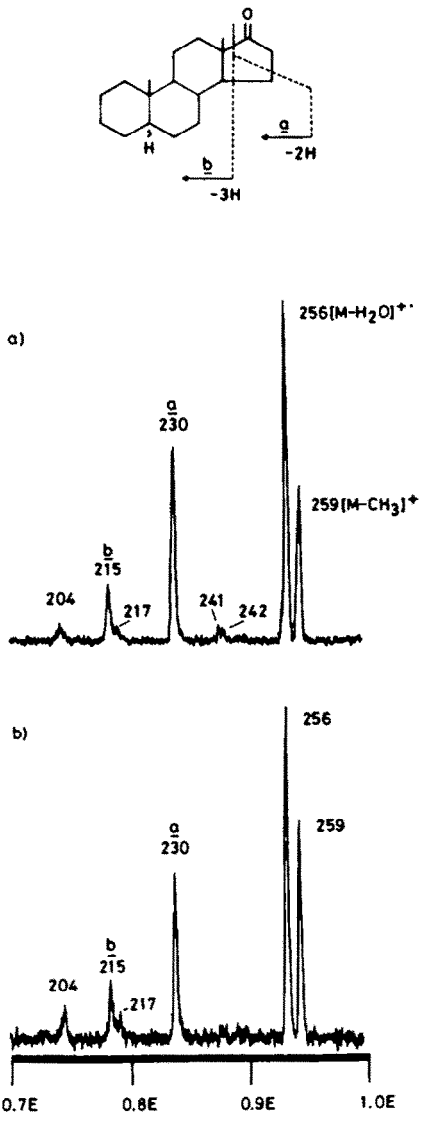



Fig. 4. The partial MIKE spectra of (a) 5α-androstan-17-one (**9**); and (b) 5β-androstan-17-one (**10**) showing the principal unimolecular reactions of $M^+$ (*m/z* 274).

striking example among the steroidal isomers studied of the effect of steric strain upon the critical energy for the methyl elimination reaction.

## CONCLUSIONS

This study reveals that the unimolecular MIKE spectra of several series of epimeric hydrocarbon and ketone steroids, differing only in the mode of the A/B or C/D ring junctions, are different (in many instances distinctly so). The differences in these spectra between epimeric steroids can be rationalized in terms of the influence of steric strain upon the critical energies of competing reactions among isomers. Inspection of the A/D cleavage ratios for the hydrocarbon isomers and the $m^*\{[M-CH_3]^+\}/[M]^{+\cdot}$ ratios for all of the steroids investigated, reveals that as steric strain increases between two epimeric steroids, or within a series of isomers (i.e. the androstane ketones), there is an apparent decrease in the critical energy for reactions arising from the region of

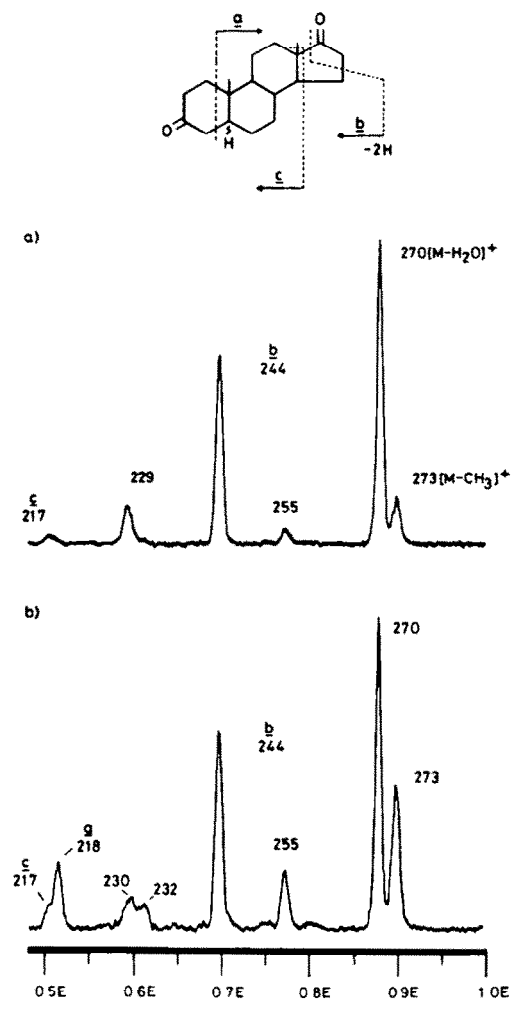



Fig. 5. The partial MIKE spectra of (a) 5α-androstan-3, 17-dione (**11**); and (b) 5β-androstan-3,17-dione (**12**) showing the principal unimolecular reactions of $M^{+\cdot}$ (*m/z* 288).

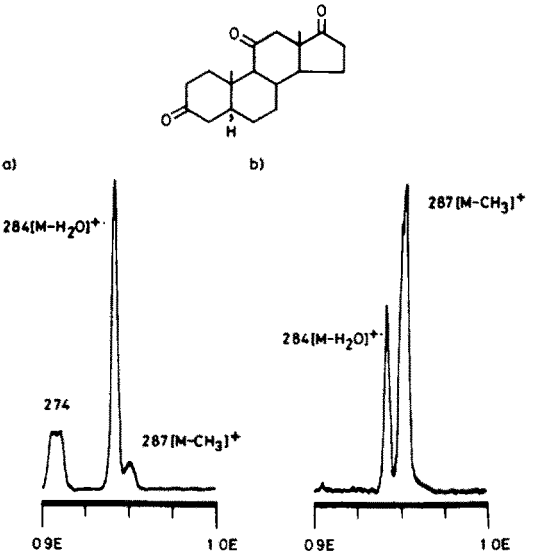



Fig. 6. The partial MIKE spectra of (a) 5α-androstan-3, 11, 17-trione (**13**); and (b) 5β-androstan-3, 11, 17-trione (**14**) showing the principal unimolecular reactions of $M^+$ (*m/z* 302).

Table 4. Relative intensities[a] of the principal metastable peaks in the MIKE spectra of the preganane triketone stereoisomers

| Compound | Relative intensities (% Base) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 315 $[M-H_2O]^{+\cdot}$ | 312 $[M-CH_3]^+$ | 297 | 294 | 287 $[M-Ac]^+$ | 286 | 272 $[M-Ac-CH_3]^{+\cdot}$ | 259 | 246 | 245 | 206 |
| 5α-Pregnan-3,6,20-trione (15) | 20 | 100 | 7 | - | - | 17 | 9 | 8 | - | 9 | - |
| 5β-Pregnan-3,6,20-trione (16) | 44 | 100 | 19 | - | - | 9 | 17 | 16 | - | 17 | - |
| 5α-Pregnan-3,11,20-trione (17) | 8 | 100 | - | 6 | 18 | - | 14 | 8 | 12 | 3 | 7 |
| 5β-Pregnan-3,11,20-trione (18) | 45 | 100 | - | 4 | 4 | - | 2 | - | 2 | - | 6 |

[a] An error of up to ± 5% is anticipated.

the stereochemical variation (i.e. the A/B or C/D ring junctions) resulting in an enhancement of reactions arising from that centre relative to the other reactions of the isomer. This contention is clearly supported by, for example, the data for loss of an angular methyl group from the molecular ions where it was observed that methyl loss is always greater in the more strained 5β- or 14α- isomers as compared with the 5α- or 14β-isomers, respectively.

This work clearly demonstrates the sensitivity of MIKE spectrometry to steric strain, making it a potentially powerful stereochemical probe. Furthermore, through the use of metastable ion abundance data, based on model compound studies, it should be possible to make configurational assignments in a steroidal isomer, even in the absence of the second isomer. This is an extremely fortunate situation which is rarely observed in the conventional mass spectrometry of steroid stereoisomers.

## EXPERIMENTAL

All spectra were measured on a VG-Micromass ZAB-2F reversed geometry mass spectrometer.[21] Source conditions were: accelerating voltage: 5 kV; electron energy: 70 eV; trap current: 100–200 mA; source temperature: 100–200°. Samples were introduced *via* a direct insertion probe. Special care was taken to ensure constant sample evaporation rates during acquisition of data. The reproducibility of the MIKE spectra was confirmed by repeated measurements on each of the steroids on different days. The compounds (1), (2) and (5)–(18) were available commercially from Makor Chemicals Ltd, Jerusalem (Israel), Steraloids, Inc, Wilton, N. H. (U.S.A.) and Sigma Chemical Company, St. Louis, M. O. (U.S.A.), respectively, and their purities were checked mass spectrometrically. Compounds (3) and (4)[22,23] were kindly supplied by Prof. Y. Mazur (The Weizmann Institute of Sciences, Rehovot, Israel) to whom the authors express their gratitude.

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