Cis/trans Stereochemical Effects in the Negative Chemical Ionization/OH^- Mass Spectra of Strained-Ring Azabicycloalkanes Using MIKE and CA/MIKE Spectrometry

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Stereospecific decomposition reactions of isomeric (*cis* and *trans*) deprotonated molecules from azabicycloalkane derivatives as azetidinols generated under negative chemical ionization (NCI)/OH^- have been examined using mass-analysed ion kinetic energy (MIKE) and collisional activation (CA)/MIKE spectra. These measurements together with the ones obtained on specifically labelled compounds enabled us to determine the origin of the stereochemical effects. The results indicate that the hydroxylic proton constitutes the preferential (≈90%) site for the deprotonation process. Subsequent fragmentations of the deprotonated species observed in the second field-free region of a reversed geometry instrument are affected by the stereochemistry of the hydroxylic group. The isomer with the hydroxyl group in the *cis* position relative to the hydrogen at the ring junction mainly loses H_2O, while the *trans* isomer eliminates $CH_3^{\cdot}$, both processes occurring with high specificity. Labelling studies indicate that two major pathways exist for the elimination of H_2O from the *cis* isomer and the loss of $CH_3^{\cdot}$ from the *trans* isomer. The course of the reaction is determined by the ability of the stereoisomers to transfer a proton during the first decomposition step. When the size of the lactam ring is increased from a five-membered ring to a six- or seven-membered ring, these stereochemical effects tend to become less pronounced.

INTRODUCTION

Significant stereochemical effects have been reported previously[1–5] during the decomposition of stereoisomeric depronated molecules, $[M-H]^-$, formed under NCI/OH^- conditions. In particular, the stereospecific loss of H_2 from *trans*-1,4 (or 1,3)-cyclohexane diol has been explained as being due to the anchimeric assistance of the alkoxy group in the $[M-H]^-$, anion,[3] where hydride ion transfer is sterically possible only for the *trans* isomer. An analogous reaction has been observed in 17-substituted steroids having OH groups in the 14- and 17-positions during the elimination of a hydrocarbon molecule.[4] Recently, another stereospecific hydride transfer reaction was reported for the ketal reduction of hydroxyketal stereoisomers characterized by a rigid conformation.[5] The deprotonation process occurs from the most acidic site which leads to the most stable $[M-H]^-$ ion. As noted previously, the decomposition of $[M-H]^-$ can proceed via hydride transfer (for primary and secondary alcohols). However, a proton transfer can be involved during skeletal isomerization of the $[M-H]^-$ ion, as this study will demonstrate.

In the present report, stereospecific decomposition reactions observed for *cis*- and *trans*-azetidinol epimers (the *cis* isomer is the isomer in which R (or the junction hydrogen) is *cis* with the OH group) (Fig. 1) are studied using metastable ion MIKE and CA/MIKE spectra of mass-analysed isomeric deprotonated molecules generated under NCI/OH^- conditions. The differences in the fragmentation routes can be explained by differences in the ability to transfer intramolecularly a proton to the alkoxide site.

EXPERIMENTAL

Compounds

The stereoisomeric azetidinols were prepared by photocyclization of appropriate phenacyl lactams as

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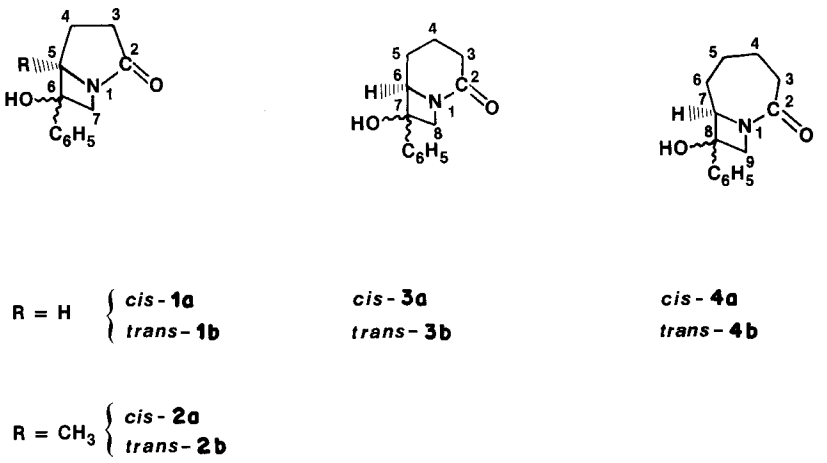


R = H { *cis*-**1a** / *trans*-**1b** | *cis*-**3a** / *trans*-**3b** | *cis*-**4a** / *trans*-**4b**

R = CH_3 { *cis*-**2a** / *trans*-**2b**

Figure 1. Azetidinols used in this study.

described elsewhere.[6] High-pressure liquid chromatography (HPLC) (Waters instrument using a C-18 reversed phase column) was used for the fractionation of the more abundant *cis* from the *trans* isomer. Proton NMR (250 MHz) was used to ascertain the stereochemistry. Specifically deuterium-labelled azetidinols were obtained using the same synthetic scheme[6] from labelled precursors (deuterated acetophenone or/and pyrrolidone).

5-d_1 azetidinol *cis* (1a-d_1) and *trans* (1b-d_1) have been prepared by photocyclization of the phenacyl lactam obtained from phenacyl bromide and 5,5-d_2-2-pyrrolidone. The later was obtained by $LiAlD_4$ reduction of succinimide according to the DJERASSI's procedure.[7]

3,3-d_2 azetidinols *cis* (1a-d_2) and *trans* (1b-d_2) were formed by photocyclization of the phenacyl lactam prepared from phenocyl bromide and 3,3-d_2-2-pyrrolidone (2-pyrrolidone was refluxed 5 days in D_2O containing sodium methoxide and distilled (this procedure was repeated three times).

3,3,7,7-d_4 azetidinols *cis* (1a-d_4) and *trans* (1b-d_4) were obtained by photocyclization of the phenacyl lactam prepared from 3,3-d_2-2-pyrrolidone and 2,2-d_2 bromoacetophenone. The later was obtained by bromination (Amberlyst A-26, Br_3^- form the resin had to be exchanged in D_2O and dried before use, CH_2Cl_2) of 2,2,2-d_3-acetophenone (prepared by H/D exchange with D_2O/K_2CO_3).

Mass spectrometry

Mass spectra, MIKE spectra and CA/MIKE spectra were recorded on a double focusing ZAB-2F mass spectrometer (VG Analytical, Altrincham, UK). The negative ion reagent plasma was prepared using N_2O/CH_4 (1:10). An electron energy of 100 eV and an emission current of 200 μA were used. The ion source temperature was kept at 180 °C. All experiments were carried out with an accelerating voltage of 8 kV. The samples were introduced by the direct insertion probe. Scan rates of 2 s decade^{-1} for recording the mass spectra and 33 meV s^{-1} for the MIKE experiments were used. Helium was used as collision gas at a pressure giving a 50% attenuation of the main beam. The readings of the ion gauge located at the throat of the diffusion pump below the collision cell were 8×10^{-8} Torr for CA experiments and 10^{-8} Torr for metastable ion studies. The neutral–neutral H/D exchange reaction was performed using ND_3 (replacing CH_4) and OD^- which was produced by the following sequence:

$$N_2O + e \rightarrow N_2 + O^{-\cdot}$$
$$O^{-\cdot} + ND_3 \rightarrow OD^- + ND_2^{\cdot}$$

RESULTS AND DISCUSSION

The structures of the various isomeric azetidinols considered here are given in Fig. 1. Their NCI/OH^- mass spectra exhibit an abundant signal for the $[M-H]^-$ ion (base peak). Very little fragmentation (<1% relative abundance) within the ion source is observed. Thus under these conditions, the distinction between *cis* and *trans* isomers is not possible. This is in contrast to the previously reported NCI/OH^- spectra of bifunctional stereoisomeric diols[3,4] and hydroxyketals[5] where stereochemical effects are readily observed in their respective CI/OH^- mass spectra. However, effective differentiation of isomers becomes possible when deprotonated azetidinol ions with a longer lifetime are sampled and either their spontaneous decompositions (MIKE) or collisionally activated dissociations (CA/MIKE) are studied.

Deprotonation site

In order to assess the location of the deprotonation site during the ionization step, the NCI/OD^- spectra of the azetidinols were recorded using a mixture of N_2O and ND_3 as described. It has been established

Table 1. Isotopic distributions[a] in the NCI/OD⁻ mass spectra of stereoisomeric azetidinols (using N_2O/ND_3) of deprotonated molecular ions

Azetidinols	$[M_{d_2} - H]^-$	$[M_d - H]^-$ or $[M_{d_2} - D]^-$	$[M_d - D]^-$
cis-**1a**	—	6	94
trans-**1b**	2	10	88
cis-**2a**	1	8	91
trans-**2b**	4	10	86
cis-**3a**	2	20	78
trans-**3b**	3	25	72
cis-**4a**	4	22	74
trans-**4b**	5	18	77

[a]Normalization of the molecular ions to 100% after correction for natural isotopic contribution.

earlier for hydroxyketones[3,4] that under these conditions the H/D neutral–neutral exchange reaction is restricted to the hydroxyl position. Also, no H/D exchange by ion–molecule reaction[9,10] is possible. Therefore, it is reasonable to assume that the exchange reaction generates an azetidinol, M_d, monolabelled at the hydroxyl site.

Analysis of the results summarized in Table 1 indicates that, among the most likely two active protons for the deprotonation, the hydroxylic proton prevails (>75%; Eqn (1)) over that in the position α to the carbonyl group (Eqn (2)).

$$M_d + OD^- \rightarrow [M_d - D]^- + D_2O \text{ (major pathway)} \quad (1)$$

$$M_d + OD^- \rightarrow [M_d - H]^- + HDO \text{ (minor pathway)} \quad (2)$$

Furthermore, the higher degree of hydroxylic deprotonation observed for strained *cis* isomers (**1a** and **2a**) decreases when the size of the lactam ring increases. Similar exchange ratios have been reported for hydroxyketones.[3,4]

Metastable and collisionally activated dissociations of deprotonated strained azetidinols 1a and 1b

The specific decompositions of stereoisomeric anions (*m/z* 202) **1a** and **1b** have been further studied by recording MIKE and CA/MIKE spectra of $[M - H]^-$ (Fig. 2). The most interesting observation is the existence of a $[M - H - H_2O]^-$ peak in the *cis* isomer (**1a**) spectra and its absence in the spectra of the *trans* isomer (**1b**) which instead loses a radical methyl from $[M - H]^-$. Each pathway is stereospecific.

In order to examine these reactions further, the CA/MIKE spectra of labelled compounds, e.g. 5-d_1 (**1a**-d_1, **1b**-d_1), 3,3-d_2 (**1a**-d_2, **1b**-d_2) and 3,3,7,7,-d_4 azetidinols (**1a**-d_4, **1b**-d_4) have been recorded and the results are displayed in Table 2. The discussion will be focused on the decompositions of the alkoxy forms rather than of the enolate ions. Indeed, the comparison of the MIKE and CA/MIKE spectra of $[M_d - D]^-$ (formed under NCI/OD⁻, Eqn (1)) and of $[M - H]^-$ reproduced in Fig. 2 formed under NCI/OH⁻ conditions show an identical structure for both $[M_d - D]^-$ and $[M - H]^-$ anions and therefore only the alkoxy form must be considered.

H_2O loss from deprotonated *cis*-azetidinol 1a (Table 2a). The elimination of H_2O from $[M - H]^-$ of *cis*-azetidinol **1a** is induced by proton transfer to the alkoxy group involving primarily (95%) the ring-junction hydrogen as depicted in Scheme 1 (ion *a*). In the second step, the cyclobutane methylene proton in position 7 or, to a lesser extent, the methylene proton in position 3 (α to the carbonyl group) is transferred to the hydroxyl group followed by water loss to generate *m/z* 184. The former mechanism accounts for about 80% or more of the reaction as determined

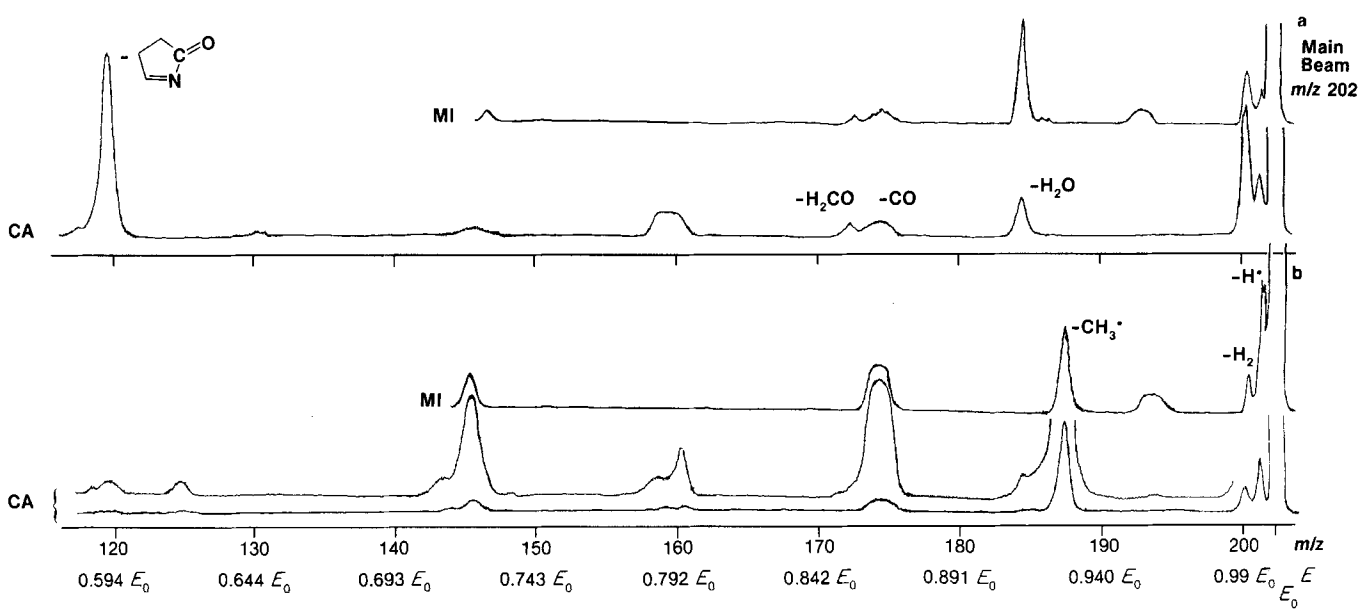


Figure 2. Metastable ion (MI) and CA/MIKE spectra of $[M - H]^-$ from azetidinols: (a) *cis* isomer **1a**, (b) *trans* isomer **1b**.

Table 2. Partial CA/MIKE spectra[a] of labelled *cis*- and *trans*-azetidinols representing the isotopic distributions in the signals corresponding to the loss of H_2O or $CH_3^{\cdot}$ from $[M—H]^-$

(a) *cis*-Azetidinols	$[M-H-H_2O]^-$	$[M-H-HDO]^-$	$[M-H-D_2O]^-$
5-d_1(**1a** – d_1)	5	95	—
3,3-d_2(**1a** – d_2)	81	19	—
3,3,7,7-d_4(**1a** – d_2)	6	92	4

(b) *trans*-Azetidinols	$[M-H-CH_3]^{-\cdot}$	$[M-H-CH_2D]^{-\cdot}$	$[M-H-CHD_2]^{-\cdot}$	$[M-H-CD_3]^{-\cdot}$
5-d_1(**1b**-d_1)	15	85	—	—
3,3,-d_2(**1b**-d_2)	82	18	—	—
3,3,7,7-d_4(**1b**-d_4)	—	5	78	17

[a]The relative abundance are normalized to 100% for each species (water or methyl radical).

from the MIKE spectra of the labelled compounds. The minor pathway, in which a proton α to the carbonyl group is involved, constitutes less than 20% of the water elimination reaction.

$CH_3^{\cdot}$ loss from deprotonated *trans*-azetidinol 1b (Table 2b). The specific methyl loss involves the cyclobutane methylene carbon atom and occurs after cleavage and opening of the C_4 ring (first step in Scheme 2, ion *b*) followed by a proton transfer from both acidic positions (3 and 5). The labelled deprotonated molecules $[M_{d_2}-H]^-$ (*m/z* 204 for **1b**-d_2 and $[M_{d_4}-H]^-$ (*m/z* 206 for **1b**-d_4) decompose in the 2nd FFR to produce $[M_{d_2}-H-CH_3]^{-\cdot}$ (*m/z* 189, 85%) and $[M_{d_4}-H-CHD_2]^{-\cdot}$ (*m/z* 189, 78%). In the latter case, 17% of $CD_3^{\cdot}$ loss is also observed.

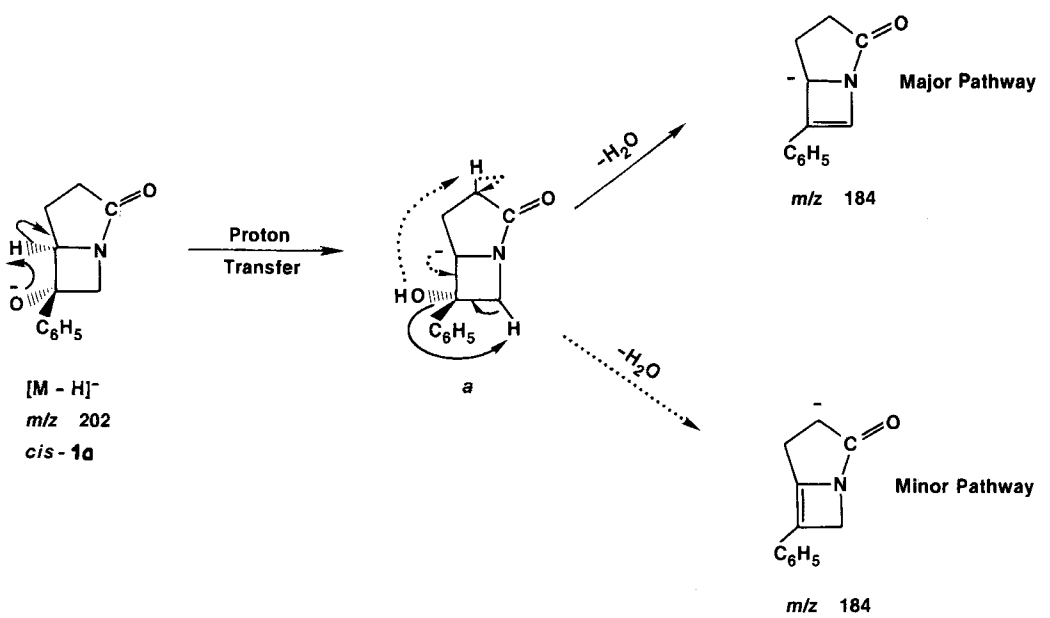
Thus, the cyclobutane methylene group is involved in the radical methyl elimination. As for the third hydrogen, it originates nearly exclusively from the proton at the ring junction (major pathway ≃85%) but also, as these labelling experiments suggest, from an α-carbonyl hydrogen in position 3 (minor pathway ≃15%).

Stereochemical driving force. The stereochemical effect observed for the *cis*- and *trans*-$[M-H]^-$ anions (**1a**, **1b**) can be rationalized in terms of the ease of transfer of the proton at the ring junction to the alkoxy group during the first step (Scheme 1). The steric requirements are such that this first step is possible for the *cis* isomer (**1a**) but not for the *trans* isomer (**1b**). The proximity of the junction ring to the alkoxy group (for *cis*) is evident from X-ray crystallographic data.[11]

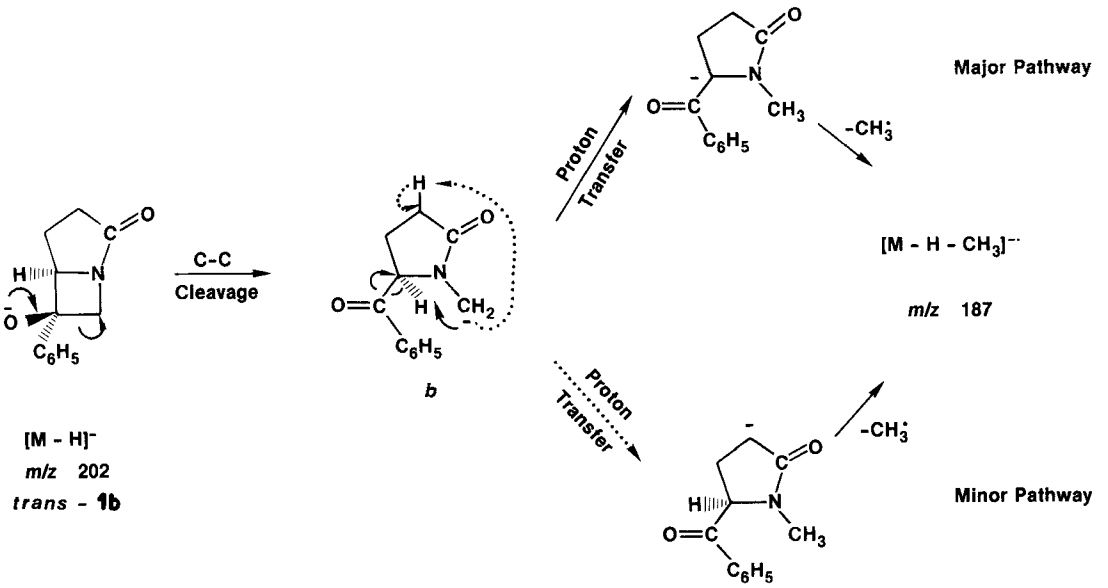
This unusual proton transfer relieves the ring strain by creating a carbanion at the ring junction (ion *a*, Scheme 1). Consequently, the flexibility of this bicyclic system is enhanced and the isomerized *a* form is stabilized.

An alternative pathway, as shown in Scheme 3, leading to the isomerized ion *c*, is rejected since H/D exchange between the hydroxy group and the enolate site is not observed. Indeed, such a enolate structure would induce a large H/D exchange. As mentioned earlier for monocyclic hydroxyketones,[3,4] the enolate formation (minor contribution) is accompanied by an H transfer from the hydroxy group.

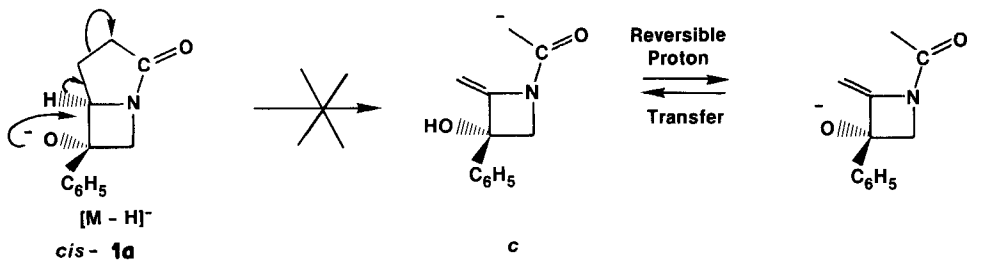
Furthermore, the fundamental role played by the H-transfer reaction at the ring junction, as a decisive step in the stereochemical orientation of the



Scheme 1



Scheme 2



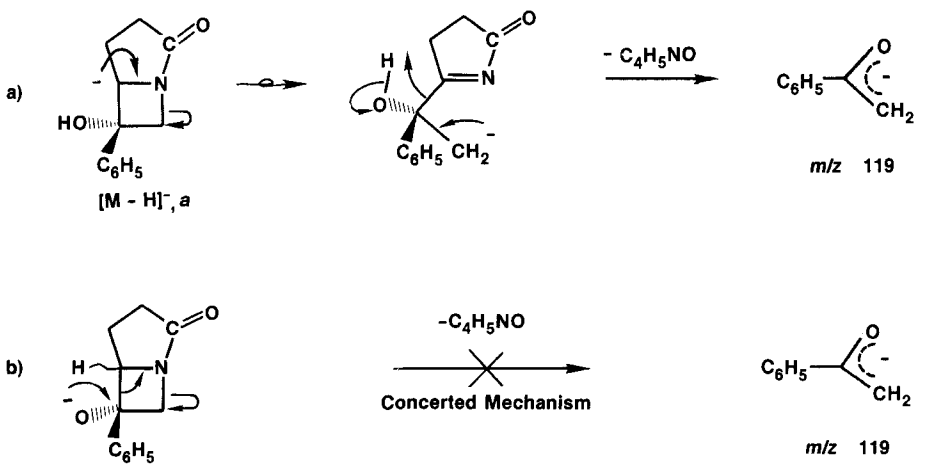
Scheme 3

decomposition, finds some additional support from the consideration of low-energy ions in the CA/MIKE spectra of $[M-H]^-$ (**1a**, **1b**). The loss of the lactam ring (C_4H_5NO) from $[M-H]^-$ appears to be very specific to the *cis* isomer (Scheme 4(a)). The carbanion (*a*) is likely to induce the cleavage of the N—C(7) bond which is followed by the hydroxylic proton migration during the formation of the acetophenone enolate (*m/z* 119).

The concerted retro-cycloaddition as an alternative process cannot explain the stereochemical effects on the formation of the ion *m/z* 119 (Scheme 4(b)).

It is difficult to give evidence for the proposed mechanism since the double proton transfer only involves the hydrogen at the C(5) position. However, this hypothetical process is consistent with the CA/MIKE spectra of labelled *cis*-azetidinols which show that:

(i) the peak at *m/z* 119 is not shifted (≃95%) for the 5-d_1 (**1a**-d_1) and 3,3-d_2 (**1a**-d_2) labelled *cis*



Scheme 4

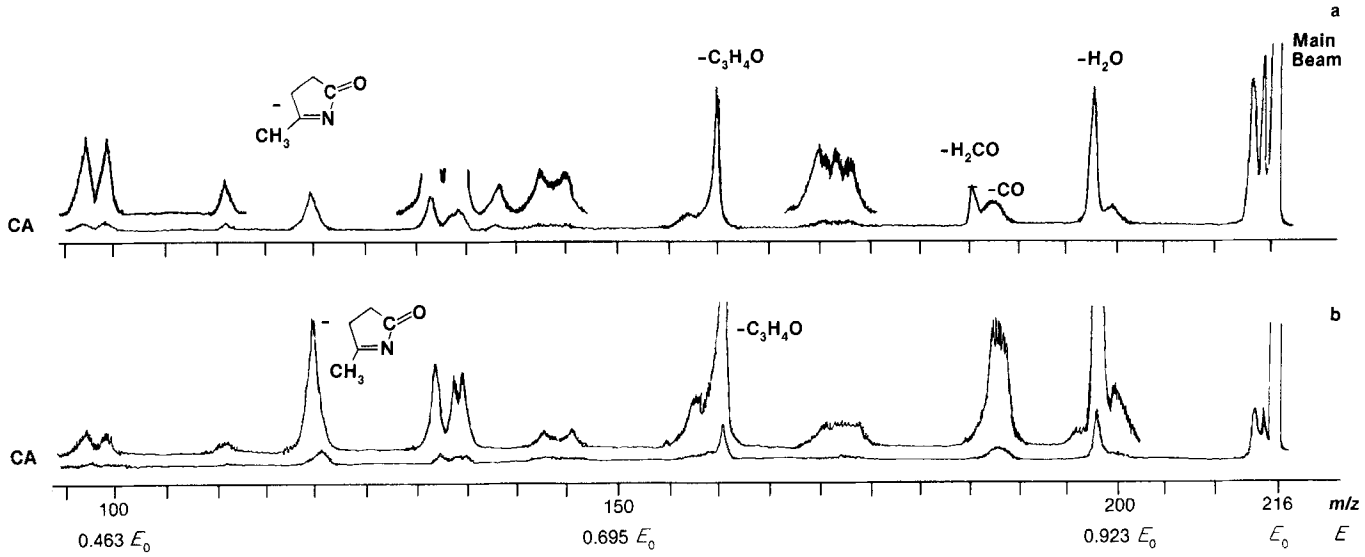


Figure 3. CA/MIKE spectra of $[M-H]^-$ from 6-methylazetidinols: (a) *cis* isomer **2a**, (b) *trans* isomer **2b**.

epimers, while

(ii) this signal is shifted to m/z 121 (≈88%) in the case of 3,3,7,7,-d_4-*cis*-azetidinol **1a**-d_4.

As far as *trans*-$[M-H]^-$ (**1b**) is concerned, the first proton transfer is not possible. Instead, a direct cleavage of the cyclobutane ring generates a carbanion (Scheme 2, ion *b*) which can lose $CH_3^{\cdot}$ after proton transfer to the carbanion from neighbouring acidic positions. However, such a loss from an even-electron ion is not straightforward.

The important role of ring strain on the proton migration is confirmed by other experimental results. Indeed, the derivatives **3a/3b** and **4a/4b** with larger lactam rings, where the ring strain is smaller, do not present any characteristic and/or specific metastable ion or collisionally activated fragmentation as discussed previously for the pair of epimers **1a/1b**. This absence of strong stereochemical effects indicates that the rings are insufficiently strained to induce a migration of the H atom at the ring junction which, according to our hypothesis, would relieve the tension of the ring.

The case of the methyl derivatives 2a/2b. The substitution of the H atom at the ring junction by a methyl group in **1a/1b** leads to the suppression of the aforementioned stereochemical effect in the CA/MIKE spectra (Fig. 3) of the isomeric pair **2a/2b**. Thus, the formation of a carbanion intermediate is hindered since the migration of CH_3^+ to the alkoxy group in *cis*-$[M-H]^-$ does not occur. Loss of the lactam ring does not occur with the same stereospecificity as observed for the **1a/1b** epimers. These results demonstrate the importance of the hydrogen at the ring junction in inducing stereospecific decompositions of strained-ring compounds as **1a** and **1b**.

Finally, although less important, another stereospecific fragmentation is observed in the MIKE spectra of azetidinols **1a/1b** and **2a/2b**. The loss of CH_2O, which is characteristic of the azetidinols presenting groups OH/H (or OH/CH_3) in a *cis* configuration, is not present in the MIKE spectra of the *trans*-epimeric azetidinols (Fig. 3).

CONCLUSION

Contrary to aliphatic or cyclic alkoxides known to generate short-lived $[M-H]^-$ species, the negative chemical ionization of azetidinols produces stable deprotonated molecules. Stereospecific decompositions are, nevertheless, observed for strained azetidinols within the 2nd FFR. The underlying stereochemical effects depend on the ability to transfer a proton onto the alkoxide group within the $[M-H]^-$ ion in the first step.

Only the *cis* isomer eliminates specifically a water molecule by this mechanism. This type of proton transfer is rather unusual for alkoxides since normally their decompositions involve a hydride transfer. However, such exceptional behaviour is believed to be triggered by the ring strain in the bicyclic structure of compound **1a**.

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