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Metastable Ion Studies in the Characterization of Melatonin Isomers†

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The electron ionization mass spectrometric behaviour of melatonin (*N*-acetyl-5-methoxytryptamine) and of a series of its isomers has been studied with the aid of metastable ion experiments. The data show that the different positions of the substituents on the indole ring influence the fragmentation patterns and the relative abundances of the generated ions. © **1998 John Wiley & Sons, Ltd.**

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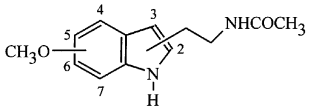
The pineal hormone melatonin (*N*-acetyl-5-methoxytryptamine (MLT), **1**) regulates circadian rhythms in humans and in different animal species,[1] and the entraining of these rhythms by exogenous administration of MLT appears to be beneficial in a number of pathological conditions associated with circadian disorders. The administration of MLT in humans was shown to alleviate jet lag,[2,3] to induce sleep[4–6] and to advance the sleep rhythm of subjects with delayed sleep phase syndrome.[7] A number of studies on elderly people and on depressed patients have also reported a decrease in overnight MLT biosynthesis, thus suggesting a role for MLT in the aging process[8] and in seasonal depression.[9] The importance of claims such as antioxidant[10–12] and immunostimulant[13,14] effects of MLT and its coadjutant role in some antitumour therapies[15–17] have not been fully substantiated as yet. These findings suggest that MLT might have regulatory activities in humans, and the discovery of selective and long-lasting agonists and antagonists for the three receptor subpopulations known to date (Mel_{1a}, Mel_{1b} and Mel_{1c})[18,19] will help clarify these issues. In several instances, mass spectrometry has been used to study the metabolism of MLT.[20,21] We present our results on the behaviour under electron ionization of a series of MLT analogues that were designed to investigate the radical-scavenging properties of compounds strictly related to MLT but not necessarily endowed with binding affinity for all or some of the three receptor subpopulations (Mel_{1a}, Mel_{1b}, Mel_{1c}). The structures of the compounds (**1–9**) are reported below. Compounds **2–9** are MLT isomers, and their synthesis and biological evaluation have been described elsewhere (**2** and **3**,[22] **4–7**,[23] **8**,[24] **9**[25]).



Compound	Substituent position	
	—OCH$_3$	—CH$_2$CH$_2$NHCOCH$_3$
1	5	3
2	4	3
3	6	3
4	4	2
5	5	2
6	6	2
7	7	2
8	6	1
9	5	4

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In the present paper we report and discuss the mass spectrometric behaviour of the isomeric compounds **1–9** as obtained under electron ionization (EI) conditions and with the aid of metastable ion[26] studies. These data may be relevant to the study of the behaviour of these compounds under oxidative conditions.

EXPERIMENTAL

All mass spectrometric measurements were obtained using a VG (Altrincham, UK) ZAB2F instrument operating in EI (70 eV, 200 μA) conditions. Metastable ions were studied by means of mass-analysed ion kinetic energy spectrometry (MIKES).[26]

Compounds **2–9** were analytically pure samples synthesized according to the literature.[22–25] Compound **1** was a commercial sample from Sigma.

RESULTS AND DISCUSSION

Compounds **1–9** behave quite similarly upon EI. For all of them the most abundant peaks are due to the odd-electron molecular ion at *m*/*z* 232 and to the fragments at *m*/*z* 173 and 160 (see ions $\mathbf{a}^{+\cdot}$ and $\mathbf{b}^{+}$ of Table 1) originating from the molecular ion through cleavage of the amide substituent (see Scheme 1). Thus the ions $\mathbf{a}^{+\cdot}$ at *m*/*z* 173 are due to the cleavage of the CH_2-N bond with H rearrangement (see Scheme 2). They are responsible for the base peak of the spectrum for all compounds except **8**. The proposed mechanism for compounds **1–3** is reported in Scheme 2. Ions $\mathbf{a}^{+\cdot}$ can rearrange, through a ring enlargement reaction, to structure **a**′ which is favoured by the acquired aromaticity. Ions **a**′ undergo, as proved by metastable ions, a highly favoured methyl loss, leading to the ionic species at *m*/*z* 158 (ions $\mathbf{c}^{+}$). In principle, the methyl lost could originate either from the methoxy group or from the methyl substituent of the pyridine ring present in structure **a**′.

This process is favoured for the low-internal-energy ions **a**′ originating from **1–9**. As can be seen in Table 2, the ion at *m*/*z* 158 is, for all compounds, the most abundant species in the MIKE spectra of ions at *m*/*z* 173. For high-internal-energy regimes, as in the usual EI spectra, this process is more favoured for compounds **4–6** and **9**. For compounds **4–6**, in the structure **a**′ the methyl group would be in position 2 with respect to the pyridine nitrogen. This

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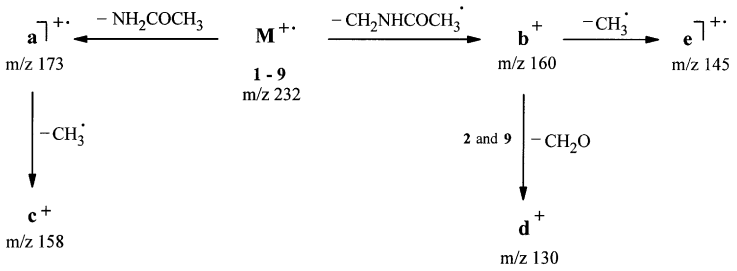
Table 1. 70 eV EI mass spectra of compounds 1–9

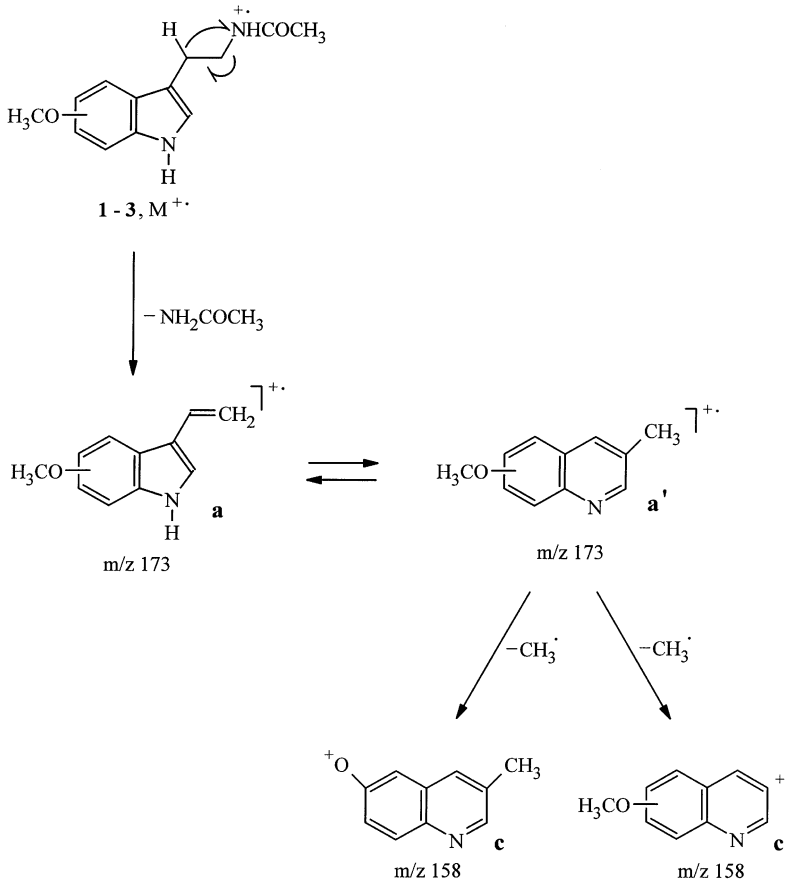
		Relative abundances								
Ionic species	*m/z*	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
$\mathbf{M}^{+\cdot}$	232	52	64	48	45	35	61	37	77	40
$\mathbf{a}^{+\cdot}$	173	100	100	100	100	100	100	100	53	100
$\mathbf{b}^{+}$	160	90	75	95	60	56	96	46	100	59
$\mathbf{c}^{+}$	158	1	9	2	24	13	22	9	1	18
$\mathbf{e}^{+\cdot}$	145	3	–	–	17	–	9	13	–	10
$\mathbf{d}^{+}$	130	2	33	1	11	2	1	8	1	49



Scheme 1.

hypothesis is confirmed by comparison with the behaviour of methyl-substituted pyridines, which show a primary methyl loss only for the isomers bearing the methyl group in positions 2 and 4, while it is completely suppressed in the 3-methyl isomer (see Table 3).

For compound **9**, also showing an abundant ion at *m/z*



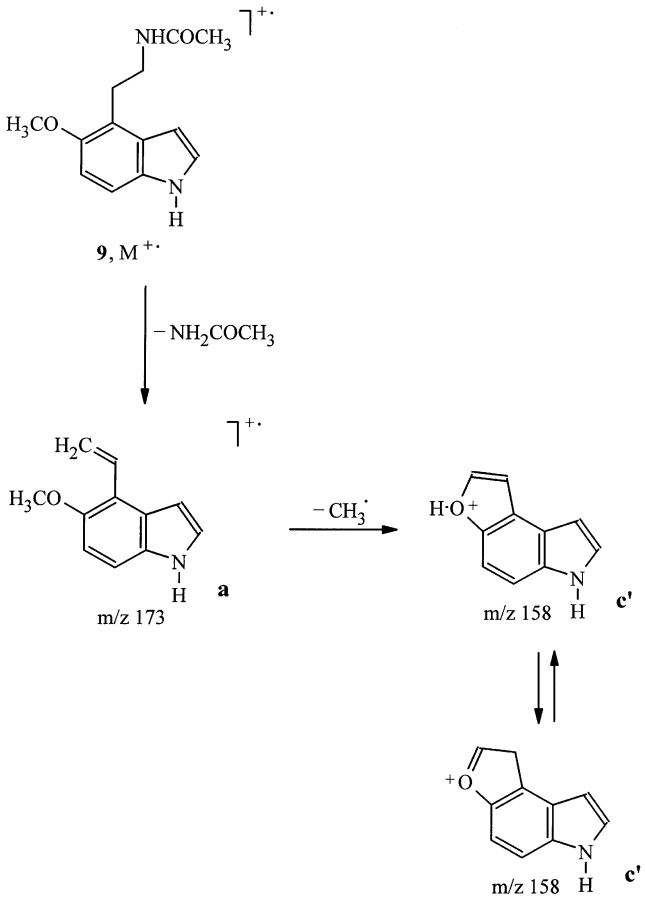
Scheme 2.

Table 2. MIKE spectra of EI-generated ions at *m/z* 173 ($a^{+\cdot}$) of compounds 1–9

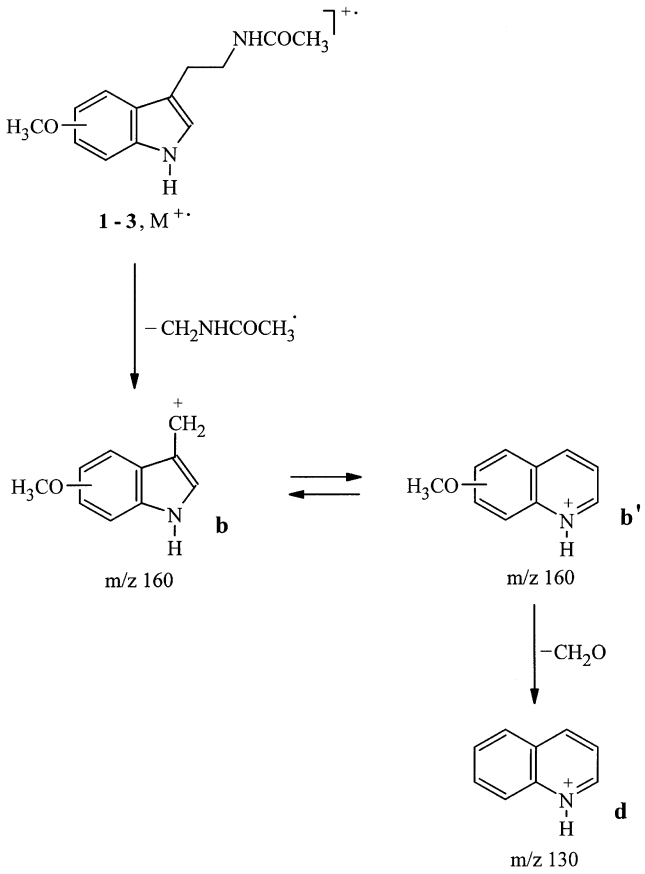
m/z	1	2	3	4	5	6	7	8	9
158	100	100	100	100	100	100	100	100	100
146	–	67	–	5	–	7	4	2	3
144	6	5	4	3	6	5	9	3	5
141	10	4	6	6	6	6	5	4	–
129	16	18	12	9	11	8	15	6	10
115	14	11	9	5	4	7	6	4	4
102	6	5	5	4	3	6	5	4	4
89	3	3	2	2	1	–	2	1	1
76	5	3	3	3	2	–	3	2	2

Table 3. EI-induced methyl loss from methylpyridine isomers[27]

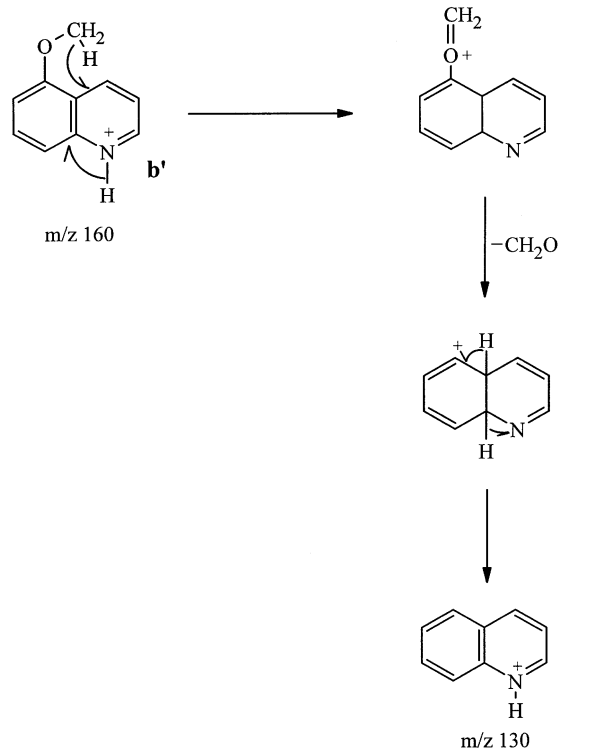
	2-Methylpyridine	3-Methylpyridine	4-Methylpyridine
$M^{+\cdot}$	100	100	100
$[M-CH_3]^+$	20	2	20



Scheme 3.



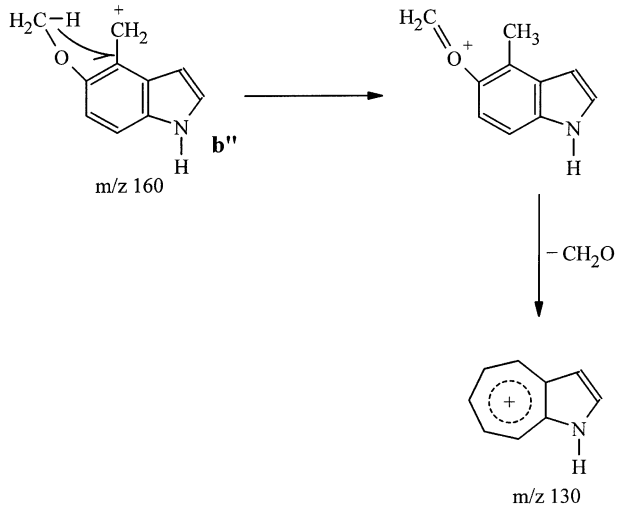
Scheme 4.



Scheme 5.

158, such a mechanism cannot be proposed owing to the different structure of the $\mathbf{a}^{+\cdot}$ ions. In this case the mechanism depicted in Scheme 3 could be considered.

Ions $\mathbf{b}^+$ presumably originate from the cleavage of the CH_2—CH_2 bond of the amide chain, and the protonated methoxy quinoline cation (Scheme 4) can be proposed; in the case of **8** it leads to the base peak of the EI spectrum and this can be justified by the high stability of the resulting



Scheme 6.

Table 4. MIKE spectra of EI-generated $M^{+\cdot}$ ions of compounds 1–9

m/z	1	2	3	4	5	6	7	8	9
189	3	2	4	2	3	2	2	4	2
173	100	100	100	100	100	100	100	100	100
160	20	7	15	9	12	14	6	22	9
147	–	–	–	–	–	–	–	25	–
145	3	1	5	4	3	3	3	2	3
141	2	–	2	3	3	2	2	–	2
130	3	4	2	2	2	2	2	3	5
115	2	1	1	1	1	1	1	2	2

ammonium cation. Ion $\mathbf{b}^+$ shows a further CH_2O loss leading to the protonated quinoline ion ($\mathbf{d}^+$) at *m/z* 130. This last species is of significant abundance in the EI spectra of **2, 4, 7** and **9** (see Table 1).

The CH_2O loss from anisole has been previously described, suggesting that formaldehyde is the neutral fragment lost. Djerassi and co-workers[28], on studying a series of aromatic methoxy and ethoxy compounds under EI, showed that they normally fragment on the C—O bond, leading to the $[M-CH_3]^+$ species, and only in some cases is the CH_2O loss observed. In the present case the methyl loss from the **b** ion is detectable, particularly in the MIKE spectra of all compounds and in the EI spectra of **4, 6, 7** and **9;** the CH_2O loss is favoured only when the molecules are methoxy substituted in position 4 or 7 (compounds **2, 4** and **7**) or when a substituent is present in the position *ortho* to the methoxy group (compound **9**). In the first case the mechanism reported in Scheme 5 can be proposed, starting from the structure **b**′ of Scheme **4**. In the case of **9** the CH_2O loss originates from the structure **b**″, shown in Scheme **6**, and leads to the tropilyum structure.

The MIKE spectra of $M^{+\cdot}$ ions of compounds **1–9** are similar to those observed in the usual EI spectra. However, further decompositions are evidenced, e.g. that due to CH_3CO loss, leading to ions at *m/z* 189 (see Table 4). Compounds **1–7** lead to superimposable MIKE spectra for their $M^{+\cdot}$ ions, with only minor differences in the relative

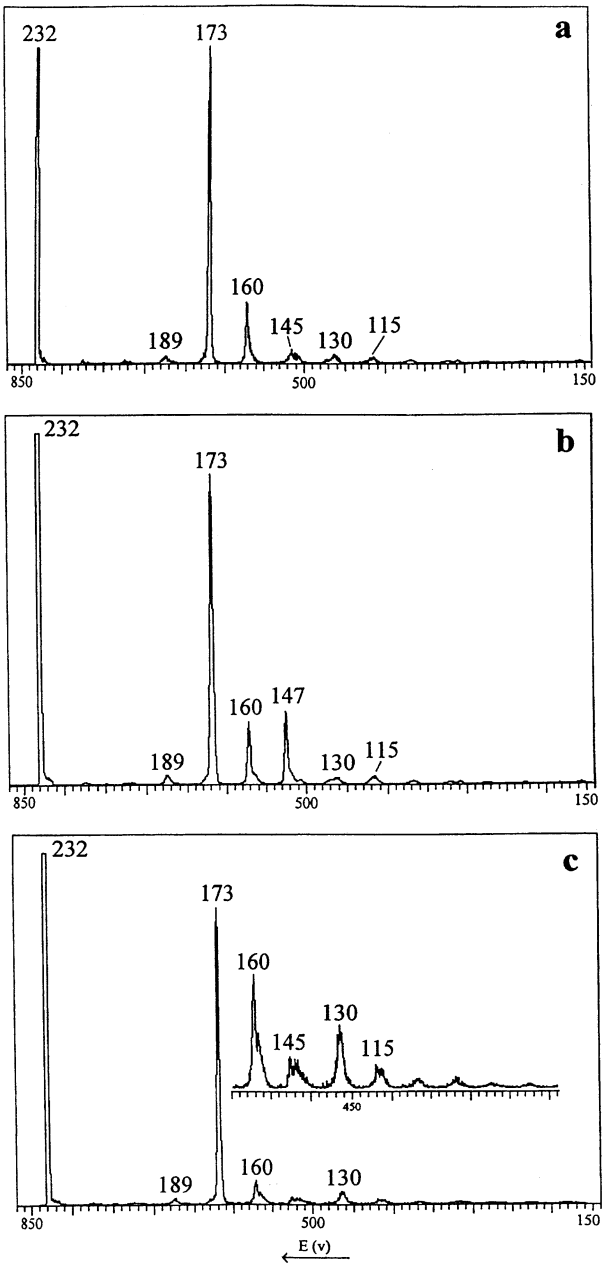
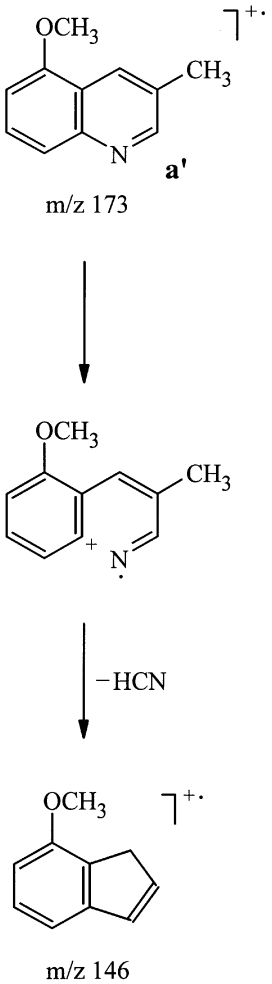


Figure 1. MIKE spectra of EI-generated $M^{+\cdot}$ ions of compounds (a) **1**, (b) **8** and (c) **9**.

abundance of ions at *m/z* 160 (see Fig. 1(a) and Table 4). Some different results are obtained for compound **8**, for which, aside from an abundant ion at *m/z* 160, a further fragment ion at *m/z* 147 is observed, corresponding to the loss of the side chain with H rearrangement and leading to the odd–electron molecular ion of 6-methoxyindole (see Fig. 1(b)). The MIKE spectrum of $M^{+\cdot}$ of compound **9** shows a higher abundance ratio of ions at *m/z* 130 and 160, suggesting the facile loss of formaldehyde from the ion **b**″ of Scheme 6 (see Fig. 1(c)).

The MIKE spectra of the ion **a** at *m/z* 173 obtained for all the compounds are reported in Table 2. Some differences can be seen in the relative abundances of product ions. Compound **2** behaves differently, as it shows a highly favoured HCN loss leading to the ion at *m/z* 146, detectable



Scheme 7.

in the EI mass spectrum at low abundance (2%). This loss could be rationalized by the methoxy group in position 4 activating the cleavage of the C—N bond, as shown in Scheme 7.

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