A Mass Spectrometric Investigation on some 4,7-Dioxo-4,5,6,7-tetrahydroindole Derivatives

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The mass spectrometric behaviour of a series of 2-aryl substituted 4,7-dioxo-4,5,6,7-tetrahydroindoles has been studied in different ionization conditions (Electron Ionization and Fast Atom Bombardment), with the aid of the metastable ion studies. In electron ionization conditions all the compounds exhibit a highly favoured, primary H_2 loss giving rise to the corresponding indole-4,7-diones; in the usual spectra no evidence for the molecular ions in the enolic form was found, while the $OH^{\bullet}$ loss observed in the MIKE (mass analyzed ion kinetic energy) spectra of molecular ions suggests that species at low internal energy content isomerize to the corresponding tautomeric enolic form. FAB mass spectra show easy formation of an unusual $[M + 2H]^{+\bullet}$ species, together with abundant $[M + H]^+$ and $M^{+\bullet}$ cations.

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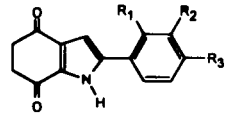
Introduction.

The 4,7-dioxoindole derivatives exhibit considerable pharmacological interest, related to the antibacterial and antitumor properties of antibiotics of the mitomicyn group [1,2], in which the central skeleton is the 4,7-dioxoindole structure [3-6]. Some derivatives differently substituted in positions 2, 3, 5 and 6, sometimes showed considerable antibacterial activity [7].

In particular the 2-phenyl derivative, if it did not show any antibacterial activity against *Staphylococcus aureus* (I 67), *Escherichia coli* (Pd 2) and *Candida albicans* (Ba 61) until the 100 μg/ml concentration was reached, it gave a 23% of inhibition against mouse Ehrlich ascites tumor when administered intraperitoneally at daily dosage of 5 mg/kg [8].

In previous work devoted to the demethylation of 4,7-dimethoxyindole, it was found that these compounds, on heating with anhydrous aluminium chloride in benzene solution, give rise to the 4,7-dioxo-4,5,6,7-tetrahydroindoles [9]. For 2-aryl substituted compounds both 4,7-dioxo-4,5,6,7-tetrahydro- and 4,7-dihydroxyindole derivatives are obtained and such behaviour was investigated in detail by Malesani *et al.* [10]. It was proposed that greater electron density is present at the C-5 and C-6 positions of the indole nucleus, with a consequent migration into these positions of the hydrogen atoms from the hydroxyl groups present in the C-4 and C-7 positions, thus stabilizing the 4,7-dioxo form. The presence of an electron-withdrawing group at the C-2 position reduces the electron density at the C-5 and C-6 positions, therefore making the dihydroxy structure more stable.

This paper pertains to the mass spectrometric behaviour fo some indole-4,7-dione derivatives, compounds **1-6**, as observed under different ionization conditions, *i.e.* elec-



1: $R_1=R_2=R_3=H$
2: $R_1=R_3=H$ $R_2=OH$
3: $R_1=R_2=H$ $R_3=OH$
4: $R_1=OH$ $R_2=R_3=H$
5: $R_1=R_2=H$ $R_3=Cl$
6: $R_1=R_3=OH$ $R_2=H$

Table 1

70 eV EI Mass Spectra of Compounds **1-6**

	1	**2**	**3**	**4**	**5**	**6**
$M^{+\bullet}$	225(4) [a]	241 (63)	241(31)	241(30)	259(52)	257(14)
$[M-H]^+$	224(16)	240(24)	240(24)	240(84)	258(14)	256(36)
$[M-2H]^{+\bullet}$	223(100)	239(100)	239(100)	239(100)	257(100)	255(27)
$[(M-2H)-OH]^+$	–	–	–	222(30)	–	–
$[(M-2H)-HCN]^{+\bullet}$	196(8)	–	–	–	–	–
$[(M-2H)-CO]^{+\bullet}$	195(34)	211(42)	211(40)	211(18)	229(29)	227(45)
$[M-C_3H_4O]^{+\bullet}$	169(12)	185(53)	185(31)	185(20)	203(29)	201(16)
$[M-C_4H_4O_2]^{+\bullet}$	141(11)	157(24)	157(19)	157(20)	175(8)	173(15)
$[M-C_6H_5O_2]^+$	–	132(2)	132(3)	132(3)	151(2)	148(100)
$[M-C_6H_5O_2N]^{+\bullet}$	102(20)	118(42)	118(55)	118(22)	136(31)	134(14)

[a] ( ) Relative abundances corrected from the isotopic contributuion.

tron impact (EI), fast atom bombardment (FAB) and by means of metastable ion studies.

All these compounds so analysed by mass spectrometry, exhibited no appreciable inhibition toward the growth of *Staphylococcus aureus* (J 67), *Streptococcus pyrogenes* (C 203), *Streptococcus sp. betahemolyticus* (BTCC 9994), *Escherichia coli* (CCM 180) and *Candida albicans* (T 69). They also were found to be inactive against the transplantable Ehrlich ascite tumour in mice [9].

EXPERIMENTAL

Compounds **1-6** were synthesized and purified according to literature [8,9,10]. All mass spectrometric measurements were performed by a VG ZAB 2F instrument [11]. The electron impact spectra were obtained with an electron energy of 70 eV (200 μA) and with a source temperature of 180°; the samples were introduced *via* a direct inlet system. Fast atom bombardment [12] spectra were obtained bombarding glycerol, for compounds **2-6**, or nitrobenzyl alcohol, for compound **1**, solutions with 8 keV Xenon atoms. Metastable ion studies were performed by mass analyzed ion kinetic energy (MIKE) spectroscopy [13].

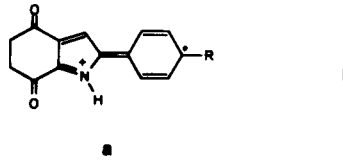
Results and Discussion.

The 70 eV EI mass spectra of compounds **1-6** are reported in Table 1.

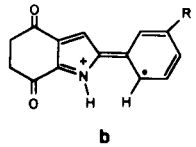
By means of metastable studies and accurate mass measurements the common fragmentation pattern shown in Scheme 1 has been obtained. Compounds **2-5** exhibit particularly abundant molecular ion, suggesting that the presence of one electron-withdrawing group on the phenyl in position 2 increases the stability of the molecular species.

In the condensed phase it was emphasized that the presence of an electron-withdrawing group in the C(2) position reflects on the reduction of the electron density in C(5) and C(6) position, leading to a stabililzation of the dihydroxy tautomeric structure [10].

In the present case the stability of the odd electron molecular ions of compounds **2-5** can be explained in terms of the stability of their distonic form, as the structures **a, b** and **c** shown below for *para, meta* and *ortho* isomers respectively, indicate.



a



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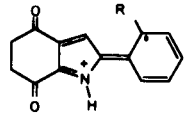


c

No evidence is present in the EI mass spectra on the existence of the molecular ion in the enolic form, being absent either OH· or H_2O primary losses. For all the compounds examined, primary losses of H· and 2H· are present. The former is particularly favoured for compound **4**, *i.e.* the *ortho*-hydroxyphenyl derivative. It is reasonable to assume that, for compounds **2-4** and **6**, it originates from the phenolic hydroxyl group, but its presence also for compounds **1** and **5** indicates that other possible acidic hydrogens are present in the molecule.

The $[M-2H]^{+\cdot}$ ions give rise, for compounds **1-5**, to the base peak of the spectra, indicating their high stability. This process can be envisioned as leading to the corresponding 4,7-indoledione derivatives. It could originate through two different mechanisms *i.e.* by a sequential loss of two H· radicals or by a concerted loss of an hydrogen

Table 2

Kinetic Energy Release Values ($T_{1/2}$, meV) Related to Primary H· and H_2 Losses in Compounds **1-6**

Compounds	$T_{1/2}$ $M^{+\cdot} \rightarrow [M-H]^+$	$T_{1/2}$ $M^{+\cdot} \rightarrow [M-H_2]^{+\cdot}$
1	155	1822
2	165	2018
3	161	2039
4	155	1990
5	178	2055
6	152	1560

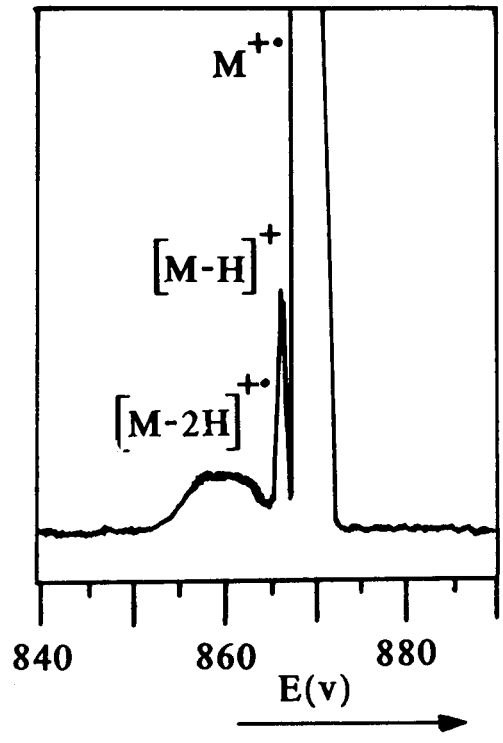


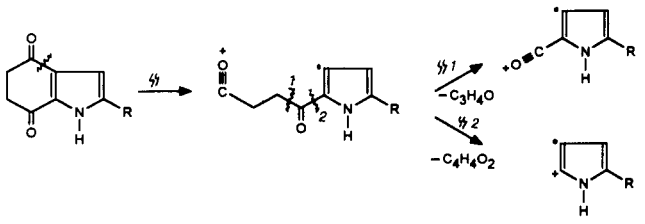
Figure 1. Partial MIKE spectum of $M^{+\cdot}$ of compound **1**.

molecule. Metastable ion data were important in clarifying this point. In Table 2 the kinetic energy release data related to the primary H· and 2H· losses are reported. For the latter process a high kinetic energy release (from 1560 to 2055 meV) is always observed (see, for example, Figure 1), strongly suggesting the occurrence of a concerted mechanism for the H_2 loss, leading to particularly stable product ions. Interestingly the relative abundance of $[M-2H]^{+\cdot}$ results are strongly reduced in the MIKE spectra, thus indicating that it must be a fast process, kinetically favoured by precursors with high internal energy. $[M-H_2]^{+\cdot}$ ions are precursors of further decomposition processes. Thus, while the CO loss from $[M-H_2]^{+\cdot}$ is observed for all the compounds examined, in agreement with the dione structure of $[M-H_2]^{+\cdot}$ ions, the loss of OH· is present only for compound **4** and the loss of HCN is characteristic for compound **1**.

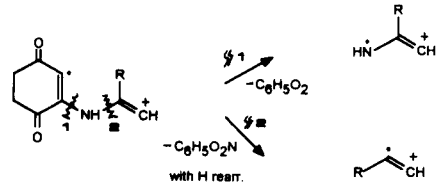
The other primary decomposition pathways are related to ring cleavages (see ruptures 1, 2, 3 and 4 of (Scheme 1). Cleavages 1 and 2 can easily originate from molecular species in "open" structure and distonic form, originating by the easy cleavage in the α-position to the carbonyl group, according to the pathways reported in Scheme 2.

Cleavages 3 and 4 could analogously originate by a rupture first of the pyrrole ring, as shown in Scheme 3, and further cleavages in the α-position to the nitrogen atom giving rise to the abundant fragments reported in Table 1.

Scheme 2



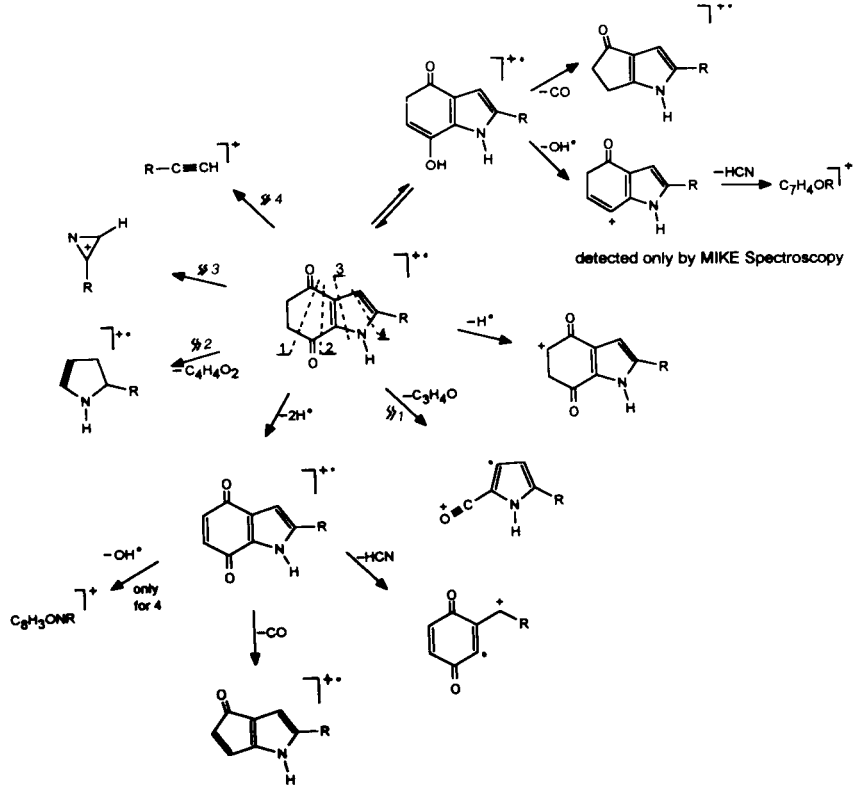
Scheme 3



It is to emphasize that in the MIKE spectra the most abundant species originate from a process completely absent in the normal EI spectra, consisting in primary OH· loss, giving evidence for the enolic structure of the molecular ion of low internal energy.

The results described above can be explained by considering the activation of different decomposition channels with respect to the internal energy of the decomposing species. Thus, while the molecular species generated by electron ionization and decomposing into the source (*i.e.*

Scheme 1

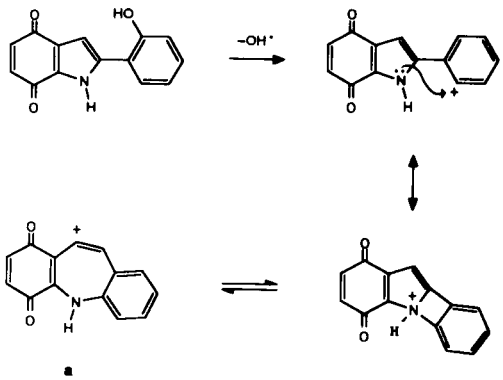


in a time window of 10^{-6}-10^{-7}s), undergo a series of decomposition pathways all requiring high activation energies (see Scheme 1), the lower internal energy ions studied by MIKE spectroscopy decompose only through reaction pathways requiring low activation energy. Among these, the OH· radical loss is good evidence for the occurrence of keto-enol tautomerism, which in the EI mass spectra is not revealed, being under these conditions kinetically unfavoured and concurrent with decomposition processes more favoured for higher internal energies.

From the analytical point of view, a problem could arise in the differentiation of the isomeric compounds **2-4**.

Compound **4** shows different behaviour in the EI spectrum. Leaving aside the difference in abundance of $[M-H]^+$ ions, explicable in terms of higher acidity of the phenolic moiety, for such a compound a specific hydroxyl loss from the $[M-H_2]^{+\cdot}$ cation is observed. Such behaviour could be explained by the interaction between the cation generated by hydroxyl loss with the nitrogen atom of the pyrrole ring leading, through a further skeletal rearrangement, to the highly stable tricyclic structure **a** shown in Scheme 4.

Scheme 4



Compounds **2** and **3** lead to practically identical EI spectra, with only minor differences in the relative abundances of ionic species originating from cleavage 1 of Scheme 1. The MIKE spectra of the related $M^{+\cdot}$ show evident differences in the abundances of the peaks related to the most favoured processes (see Figure 2), *i.d.* those due to CO and C_3H_4O losses. The abundant ratios of the related fragment ion are 5 and 2 for compounds **2** and **3** respectively. The easier loss of C_3H_4O for compound **3** can be explained by the different, all highly stable, mesomeric structures (see Scheme 5).

Scheme 5

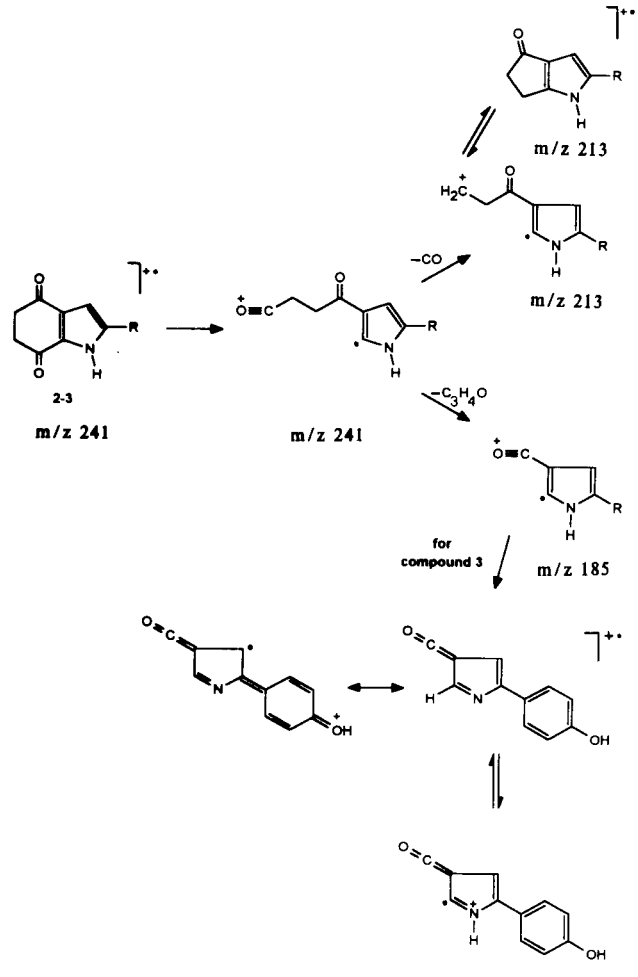


Table 3

Positive Ion FAB Mass Spectra of Compounds **1-6**

	1	**2**	**3**	**4**	**5**	**6**
$[M+2H]^{+\cdot}$	227(22) [a]	243(48)	243(8)	243(20)	–	259(20)
$[M+H]^+$	226(100)	242(90)	242(14)	242(35)	258(4)	258(16)
$M^{+\cdot}$	225(90)	241(100)	241(100)	241(100)	259(20)	257(15)
$[M-H]^+$	224(46)	240(20)	240(2)	240(15)	258(4)	256(6)
$[M-2H]^{+\cdot}$	223(23)	239(18)	239(8)	239(16)	257(20)	255(8)
$[(M-2H)-H_2O]^{+\cdot}$	–	–	–	–	–	241(100)
$[M-H_2O]^{+\cdot}$	–	223(30)	223(9)	223(16)	241(100)	241(65)
$[(M-2H)-CO]^{+\cdot}$	195(7)	211(45)	211(20)	211(31)	–	227(20)

[a] ( ) Relative abundances corrected from the isotopic contribution.

Table 4

Negative Ion FAB Spectra of Compounds **1-6**

	1	**2**	**3**	**4**	**5**	**6**
$M^{-\cdot}$	-	241 (36) [a]	241 (35)	241 (42)	259 (26)	257 (55)
$[M-H]^-$	224(100)	240(100)	240(100)	240(100)	258(100)	256(100)
$M-2H]^{-\cdot}$	223(80)	239(32)	239(41)	239(39)	257(20)	255(45)

[a] ( ) Relative abundances corrected from the isotopic contribution.

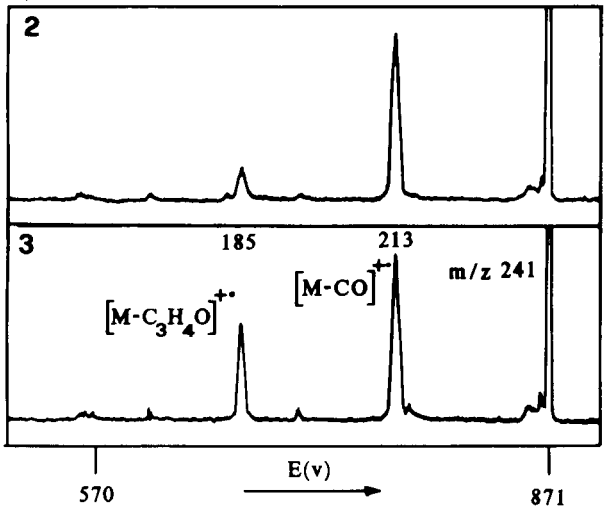


Figure 2. MIKE spectum of $M^{+\cdot}$ for compounds **2** and **3**.

The compounds examined ionized under FAB conditions lead to quite striking differences, in particularly related to the molecular ion clusters (see Table 3).

As it is known, in positive ion FAB the most favoured ionization process generally consists in a protonation reaction occurring in the selvedge region close to the solution [14]. In the present case the $[M+H]^+$ formation is observed for compounds **1-4** and **6** but it is accompanied by the formation of $[M+2H]^{+\cdot}$ ions, particularly abundant for compounds **2-4** and **6**. This unusual formation of $[M+2H]^{+\cdot}$ species can be explained with the formation of the 4,7-dihydroxy derivative and it must be explained by a sequential addition of H^+ and $H^\cdot$.

It is worth noting the presence, for all the compounds examined, of abundant odd electron molecular species $M^{+\cdot}$ which, for compounds **2-4**, is responsible for the base peak of the spectrum.

In contrast to what was observed under EI conditions, the ions due to losses to $H^\cdot$ and H_2 are in this case of lower abundance, indicating that the energy deposition achieved by FAB is lower than that obtained by EI. This is further confirmed by the lack of some decomposition pathways observed under the latter conditions; only the CO loss from $[M-2H]^{+\cdot}$ ions is observed, except for compound **5**. A new decomposition pathway is observed in FAB for compounds **2-6** consisting of the metastable supported loss of H_2O from $M^{+\cdot}$ ions, leading for compound **5** to the base peak of the spectrum, and giving evidence that in the moderate ionization conditions typical of FAB, the keto-enolic tautomerism is present.

Compound **6** shows a peculiar behaviour consisting of the loss of H_2O from the $[M-2H]^{+\cdot}$ ion, responsible for the base peak of the spectrum at m/z 241.

The negative ion FAB mass spectra results reported in Table 4 particularly simple: the $[M-H]^-$ anion is the base peak for all the compounds examined and the only other two ions are due to $M^{-\cdot}$ species, but not for **1**, and $[M-2H]^{-\cdot}$.

Conclusions.

The EI mass spectrometric behaviour of compounds **1-6** mainly consists in primary losses of $H^\cdot$ and H_2, the latter being confirmed by metastable ion studies and kinetic energy release measurements; ring cleavages are also present.

There is no evidence in the EI mass spectra for the presence of tautomers in the enol form. In contrast the most favoured $OH^\cdot$ loss, present in the MIKE spectra of $M^{+\cdot}$ for all the compounds, is a substantial evidence that molecular ions with low internal energy are content to rearrange to the enol form. This dependence of enol formation at low internal energy values is further confirmed by the data obtained under FAB conditions. In fact, while the unusual presence of $[M+2H]^{+\cdot}$ is observed for compounds **1-4** and **6**, the MIKE spectra of $M^{+\cdot}$ ions, still in quite high abundance, show the present loss of H_2O.

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