HETEROCYCLES, Vol. 75, No. 9, 2008, pp. 2213 - 2223. © The Japan Institute of Heterocyclic Chemistry
Received, 27th February, 2008, Accepted, 21st April, 2008, Published online, 24th April, 2008. COM-08-11374

THE BEHAVIOUR OF $M^{+\cdot}$ AND $[M+H]^+$ IONS OF SOME OXOISOAPORPHINES AND QUINOLINONE ANALOGS

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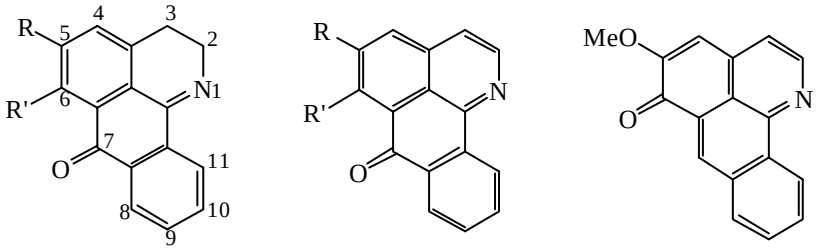
Abstract – The mass spectrometric behaviour of some oxoisoaporphines and quinolinone analogs has been studied by both electrospray and electron ionization methods. By the former approach, information can be obtained on the decomposition pattern of the compounds under investigation in acidic condition, while by the latter the behaviour related to both cationic and radical character of molecular ion can be put in evidence. The collisional spectra of the protonated molecules indicate that protonation has taken place on both oxygen and nitrogen atoms. This can be justified by the fact that even if the most basic site present in the molecule is surely the N atom, in mass spectrometry conditions the protonation reactions are not governed by thermodynamics only, but kinetic effects can also play a fundamental role. Some exception to the even electron rule have been evidenced, and can be well justified by the high stability of the odd electron fragment ion. In electron ionization conditions fragmentation patterns well related to the original structures are present, allowing the characterization of isomeric compounds by the presence of specific fragmentation routes.

INTRODUCTION

A small group of compounds with the *7h*-dibenzo[*de,h*]quinoline skeleton, known as 1-azabenzanthrones, were synthesized three decades ago as intermediates for the formation of dyes,[1] and due to their possible photo- and electrochemical properties.[2] In addition, the synthesis of *7h*-dibenzo[*de,h*]quinolin-7-one derivatives via n-phenethylphthalimides was reported due to their possible antiviral activity,[3] together with the synthesis of some 2,3-dihydro derivatives by cyclization of 3-(b-dialkoxyarylethylamino)phthalides.[4] Since the 1980's, a small group of alkaloids possessing the

7h-dibenzo[*de,h*]quinoline skeleton and bearing different substitution patterns have been isolated from *Menispermum dauricum DC*. (Menispermaceae) and called as oxoisoaporphines.[5] Some of them have exhibited cytotoxic activities against a small panel of cancer cell lines.[6] The reactivity of this type of alkaloids in reductive conditions afforded them unusual oxoisoaporphine and annelated quinoline derivatives.[7] Also, when these compounds were photoreduced *via* a stepwise mechanism of electron proton electron transfer, affording an n-hydrogen oxoisoaporphine anion, give a butadienyl derivative formed with the usage of TEA as electron donor in anaerobic conditions.[8]

While the mass spectrometric behaviour of substituted quinolines and reduced quinolines has been extensively studied,[9] to our knowledge any mass spectrometric investigation on oxoisoaporphines and the quinolinone analogs has not been reported in literature. For this reason we undertook the study on the behaviour of compounds **1** – **8** (Figure 1) in two different ionizing conditions, electrospray and electron ionization and we compared the results related to protonated molecules and odd electron molecular ions.



1: R=R'=H
2: R=OMe, R'=H
3: R=OMe, R'=OH

4: R=R'=H
5: R=OMe, R'=H
6: R=OMe, R'=OH
7: R=OH, R'=H

8

Figure 1

RESULTS AND DISCUSSION

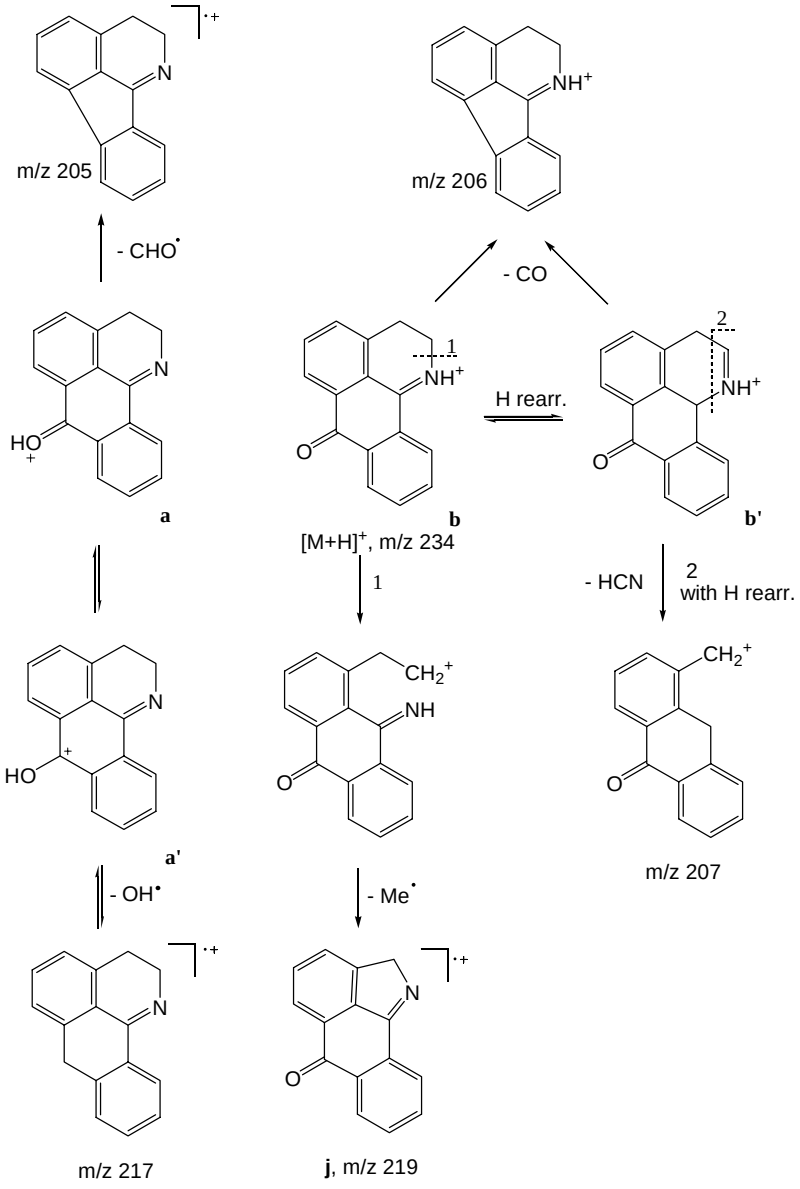
In order to describe the general behaviour of compounds **1 - 8** (see Figure 1) in ionic form, two different sets of experiments were performed: the first in positive ion electrospray conditions, so to produce protonated molecules of **1 - 8** to be characterized by collisional experiments, the second by electron ionization MS, so to produce odd electron cations with high internal energy content. What is expected is to gain in the former case information on the decomposition pattern of **1 - 8** in acidic conditions, in the latter on the behaviour related to both the cationic and radical character of molecular ions. Compounds **1 - 8** give rise in electrospray conditions to the production of abundant $[M+H]^+$ ions. Interestingly compounds **1 - 3**, the reduced molecules, show also the presence of fragment ions, which for **4 - 8** are completely absent. These fragments are the same generated by collisional experiments performed on $[M+H]^+$ inside the ion trap. They reasonably originate by "in source" fragmentation processes and, considering the low voltage (15 V) applied to the source (lower than that usually employed for in source fragmentation studies, typically in the range 25-100 V, they indicate a relatively high lability of $[M+H]^+$

of **1 - 3**. The collisional conditions in an ion trap privilege the decomposition processes exhibiting the lowest activation energy, due to the step-by-step internal energy acquisition typical of this approach.[10] Consequently the decomposition routes induced by collisions represent those energetically most favoured. Furthermore it must be underlined that the typical collisionally induced fragmentation pathway of even electron species as $[M+H]^+$ ions, generally implies losses of neutral molecules and not radical species, in accordance with the Karni's and Mandelbaum's even electron rule.[11] The loss of radicals must be justified by the formation of highly stable product ions. Following these considerations, the behaviour of $[M+H]^+$ of **1** can be justified by the fragmentation pattern reported in Scheme 1 (the related spectrum is reported in Table 1).

Table 1 – Collisional spectra of ESI-generated $[M+H]^+$ species of compounds **1 - 8**

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Ionic species	m/z(rel.ab.)	m/z(rel.ab.)	m/z(rel.ab.)	m/z(rel.ab.)	m/z(rel.ab.)	m/z(rel.ab.)	m/z(rel.ab.)	m/z(rel.ab.)
$[M+H]^+$	234	264	280	232	262	278	248	262
- Me·	219(65)	249(100)	265(100)	-	247(37)	263(13)	-	-
- OH·	217(41)	-	-	-	-	-	-	-
- H_2O	-	-	262(81)	-	-	260(100)	-	-
- HCN	207(100)	237(21)	-	-	-	-	-	-
- CO	206(20)	236(3)	-	204(100)	234(7)	250(45)	220(100)	234(100)
- CHO·	205(47)	-	251(18)	-	-	249(35)	-	233(25)
-H_2C=NH	205(47)	235(4)	-	-	-	-	-	-
- CH_4N	204(55)	234(5)	-	-	-	-	-	-
- MeOC	-	221(22)	237(23)	-	219(100)	235(31)	-	-
- CO, - OH·	-	-	-	-	-	-	-	217(2)
- H_2O, - CO	-	-	234(60)	-	-	232(15)	-	-
- H_2O, - CO, - CO	-	-	-	-	-	204(3)	-	-

First of all it must be taken into account that the ESI-induced protonation reasonably can occur on both heteroatoms present in the molecular structure and consequently protonated molecules **a** and **b** can be generated. The most basic site present in the molecule is surely the N atom. However it should be emphasized that, in general, the protonation reactions are not governed by thermodynamic data only, but kinetic effects can also play a fundamental role.[12]



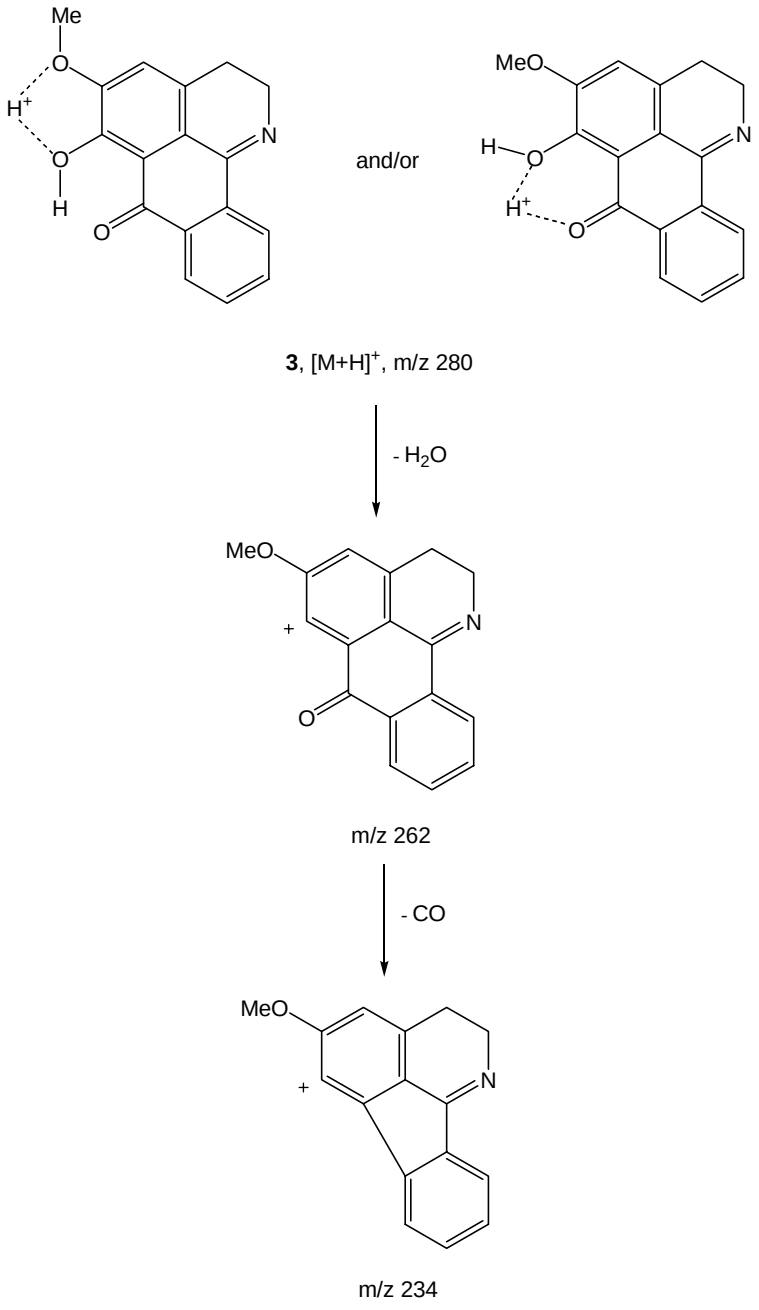
Scheme 1

The species **a** and **b** can well justify the observed fragmentation pathways. Species **b**, through cleavage of the NH-CH_2 bond and H rearrangement can activate the primary $Me^{\bullet}$ loss. This exception of the even electron rule can be well justified by the high stability of the odd electron fragment ion (**j** of Scheme 1) so formed. Ion **b** can lead, through H rearrangement, to species **b'**, that, by cleavage 2 with H rearrangement,

looses a neutral HCN molecule, giving rise to the abundant ion at m/z 207. The same process takes place without H rearrangement, giving rise to the ion at m/z 205, or with H rearrangement on the neutral moiety, leading to the ion at m/z 204. Both **b** and **b'** ions can be considered the precursors for the CO loss (usually observed in dibenzophenone species), leading to the ion at m/z 206.

Molecules protonated on the carbonyl oxygen (**a** of Scheme 1) can be the responsible of two radical losses: the first through species **a'**, due to $OH^{\bullet}$ loss, the second due to direct $CHO^{\bullet}$ loss, analogous to the CO one observed from **b** and **b'**. In both cases the radical losses can be well justified by the high stability of odd electron product ions, due to their aromaticity and the consequent availability to distribute the positive charge and the unpaired electron. In the case of compound **2**, the observed collisional behaviour suggests that the molecular species originate from protonation occurring preferentially on the N atom, reasonably for a mesomeric effect of the methoxy group. Indeed, the electronic delocalization afforded by an electron-donating as OMe group through quinolin-7-one aromatic system in **2** can stabilize this positive charge on the heteroatom. This has been verified by theoretical studies of proton affinity (PA) carried out with similar oxoisoaporphine derivatives in order to determine the more stable protonated form.[13] As it can be seen by the data reported in Table 1, the primary fragmentation processes are analogous to those described for protonated **1** in the **b** form, while the processes originating from O-protonated molecules (**a** ones of Scheme 1), i.e. $OH^{\bullet}$ and $CHO^{\bullet}$ losses, are completely suppressed. For **2** a new fragmentation channel is activated due to the presence of the methoxy group. It is to emphasize that the $Me^{\bullet}$ loss can originate in this case also from the methoxy group: the increase of the abundance of the related peak is a good evidence of the existence of parallel processes. Compound **3** exhibits a specific behaviour. Aside the $Me^{\bullet}$ and the Me-$OC^{\bullet}$ losses and the inhibition of decomposition processes originating by N-protonated molecules, new fragmentation routes become present, consisting in H_2O and sequential CO losses. These results may suggest that for this species the protonation has taken place on oxygen atoms, as shown in Scheme 2, and the proton is stabilized by both the oxygen atoms, through the formation of O-H^{+}-O bridged systems. In this case the protonated molecule behaves following the even electron rule. Compounds **4 – 8** behave in different way with respect to **1 - 3** but their collisional spectra give further support to the hypothesis done for **1 - 3**. Thus $[M+H]^{+}$ of compound **4**, the analog of **1** in oxidated form, shows only one decomposition channel, due to the CO loss. The $Me^{\bullet}$ loss originating by the cleavage of CH_2-NH bond in **1** is completely absent, due to the aromaticity now present in the pyridine substructure. Also the decomposition pathways related to the O-protonated molecules are in this case inhibited, suggesting that the protonation has taken place on the nitrogen atom. Analogous results are obtained for **5** with the formation of fragments due to CO loss and $Me^{\bullet}$, $MeOC^{\bullet}$ losses for the presence of the methoxy group. Compound **6** exhibits a behaviour analogous to that observed for **3**, confirming the hypothesis reported in Scheme 2. Also in this case H_2O, [H_2O and CO] losses are present, together with fragments related to the

presence of methoxy group ($Me^{\bullet}$, $MeOC^{\bullet}$). Further evidence of the validity of the proposal of Scheme 2 is given by the behaviour of compound **7**. In this case any water loss is not observed and the only fragment is due to CO loss, which in principle can originate either from the dibenzoquinone or from the phenol substructures. Finally compound **8** shows fragments due to CO and $CHO^{\bullet}$ primary losses and sequential losses of H_2O, CO, suggesting that also in this case the protonation has taken place on the carbonyl group.

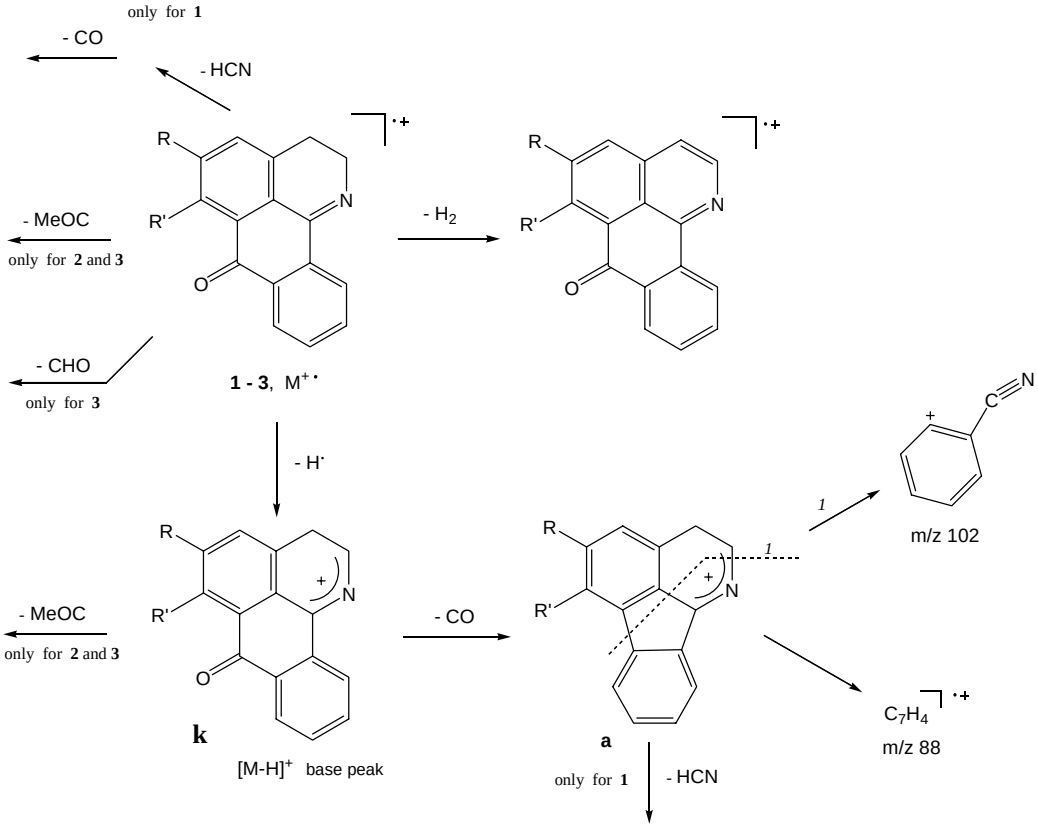


Scheme 2

The most abundant species detected in the EI spectra of **1 – 7** are reported in Tables 2 and 3. Compounds **1 - 3** lead to the formation of abundant molecular ions but interestingly show an easy loss of $H^{\bullet}$, leading to the base peak for **1** and **2**.

Table 2 – EI spectra of compounds **1** - **3**

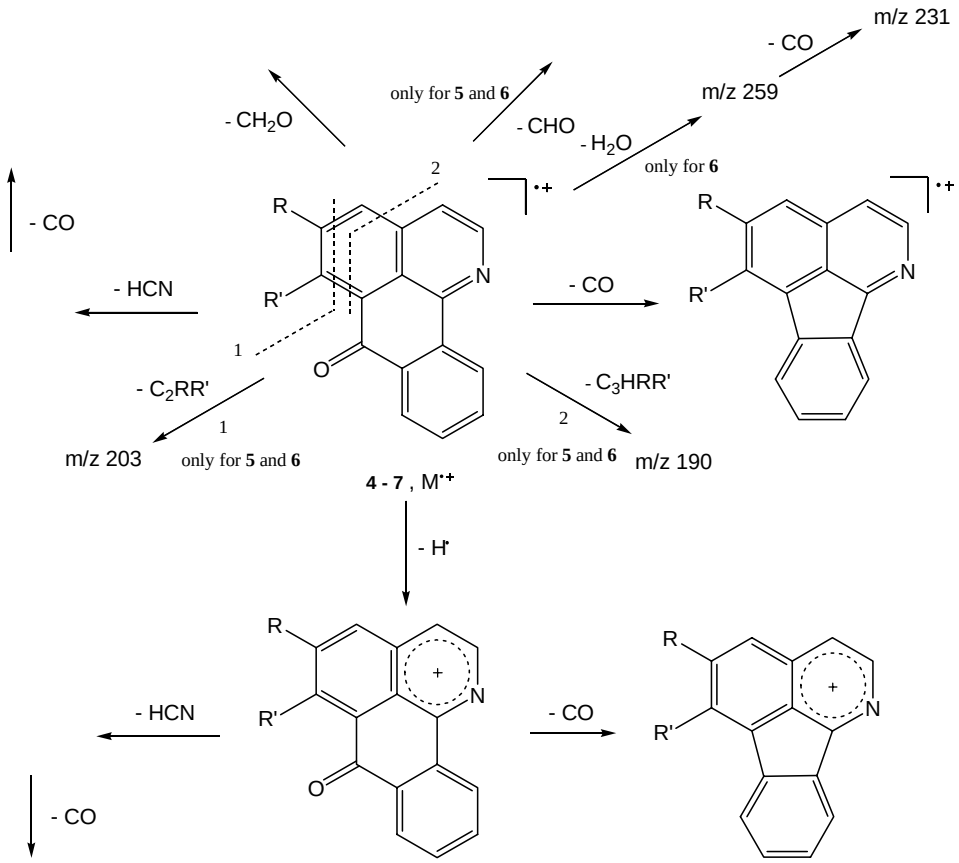
	1	**2**	**3**
Ionic species	m/z (rel.ab.)	m/z (rel.ab.)	m/z (rel.ab.)
$M^{+\bullet}$	233 (66)	263 (73)	279 (100)
$[M-H]^{+}$	232 (100)	262 (100)	278 (71)
$[M-H_2]^{+\bullet}$	231 (5)	261 (3)	277 (3)
$[M-HCN]^{+\bullet}$	206 (6)	236 (11)	-
$[(M-H)-CO]^{+}$ (a)	204 (14)	234 (1)	250 (18)
$[a-HCN]^{+}$	177 (21)	-	-
$[(M-HCN)-CO]^{+\bullet}$	178 (13)	-	-
$[M-CHO]^{+}$	-	-	250 (18)
$[M-MeOC]^{+\bullet}$	-	220 (12)	236 (29)
$[(M-H)-MeOC]^{+}$	-	219 (35)	235 (31)
$[C_7H_4N]^{+}$	102 (23)	102 (7)	102 (19)
$[C_7H_4]^{+\bullet}$	88 (25)	88 (8)	88 (6)



Scheme 3

This behaviour can be explained by the formation of species **k** of Scheme 3, with an easy charge delocalization. Furthermore a primary H_2 loss is also observed, leading to the $M^{+\bullet}$ species of the corresponding oxidated compounds. Leaving aside the $[M-H]^+$ ion, all the other EI-generated fragments are of quite low abundance, indicating a high stability of both $M^{+\bullet}$ and $[M-H]^+$ species.

Common fragments are detected at m/z 102 and 88, due to $C_7H_4N^+$ and $C_7H_4^{+\bullet}$ species respectively, originating by cleavages of the molecule skeleton (see Scheme 3). Primary HCN loss is also observed. The CO loss takes place only from the $[M-H]^+$ ion, leading to species **a** of Scheme 3, precursor for a further HCN loss only in the case of **1**. Compounds **2** and **3** show some specific behaviour, related to the hydroxyl group present in positions 6. Thus, $MeOC^{\bullet}$ losses from both $M^{+\bullet}$ and $[M-H]^+$ are observed; furthermore for compound **3** an abundant ion at m/z 250 is present. It could originate either from $[M-H]^+$ ion through CO loss or by $CHO^{\bullet}$ loss from the phenol substructure. The low intensity of the analogous fragment observed for **2** (2%) indicates the latter hypothesis as the most reasonable one.



Scheme 4

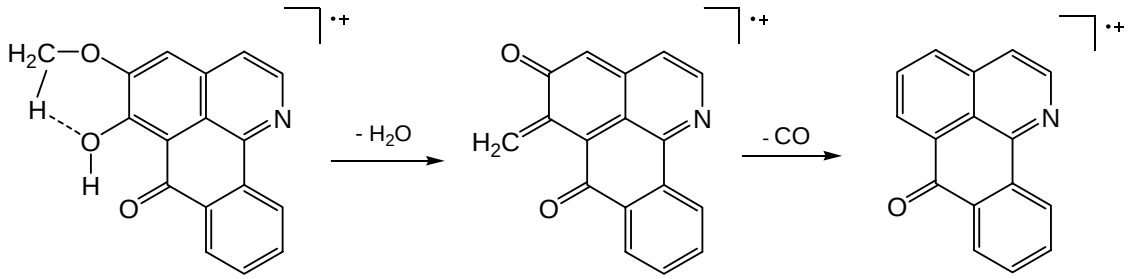
Compounds **4 - 7** lead to $M^{+\bullet}$ representing the base peak of the EI spectra. The $[M-H]^+$ ion is still present, as usually observed in aromatic compounds, due to the easy positive charge delocalization. Aside the primary CO and HCN losses, some new decomposition routes are activated, as those originating from cleavages 1 and 2 of Scheme 4, implying the substituted benzene ring. Furthermore some specific fragmentations are

observed: as an example, only for compound **6** a primary H_2O loss is present, and it reasonably occurs for the interaction of hydroxyl group with the methoxy hydrogens, as shown in Scheme 5, analogously to what observed for protonated molecules (see Scheme 2).

Table 3 – EI spectra of compounds **4** - **8**

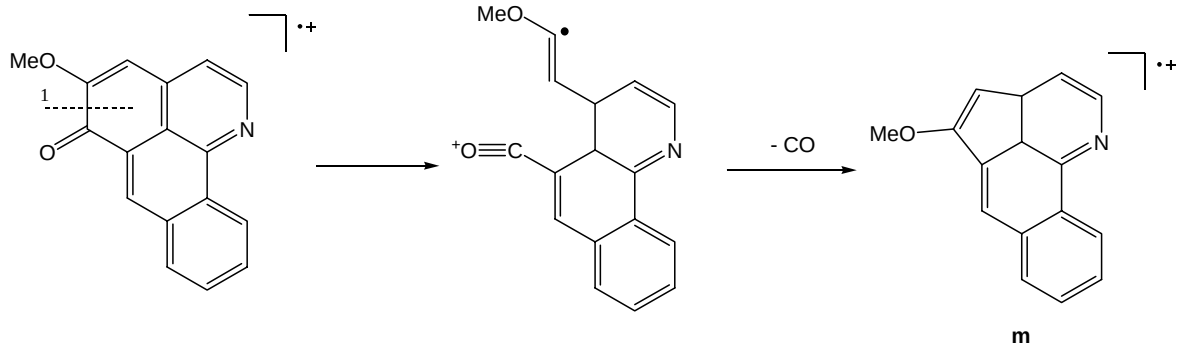
	4	**5**	**6**	**7**	**8**
Ionic species	m/z (rel.ab.)	m/z (rel.ab.)	m/z (rel.ab.)	m/z (rel.ab.)	m/z (rel.ab.)
$M^{+\bullet}$	231 (100)	261 (100)	277 (100)	247 (100)	261 (45)
$[M-H]^+$	230 (17)	260 (30)	276 (19)	246 (12)	260 (5)
$[M-HCN]^{+\bullet}$	204 (8)	234 (1)	250 (1)	220 (5)	234 (2)
$[M-CO]^{+\bullet}$	203 (55)	233 (3)	249 (14)	219 (36)	233 (10)
$[(M-H)-HCN]^+$	203 (55)	233 (3)	249 (14)	219 (36)	233 (10)
$[(M-H)-CO]^+$	202 (27)	232 (26)	248 (81)	218 (12)	232 (100)
$[M-CH_2O]^{+\bullet}$	-	231 (41)	247 (20)	-	231 (4)
$[M-C_2RR']^{+\bullet}$	-	203 (46)	203 (30)	-	203 (45)
$[M- C_3HRR']^{+\bullet}$	-	190 (52)	190 (18)	-	190 (20)
$[M-H_2O]^{+\bullet}$	-	-	259 (19)	-	-
$[(M-H_2O)-CO]^{+\bullet}$	-	-	231 (59)	-	-
$[(M-CO)-HCN]^{+\bullet}$	176 (15)	-	-	-	-

The ion so formed shows an easy CO loss, which can originate from two different sites. The sequential losses of CO and HCN are observed only for **5**, suggesting that this behaviour is characteristic for the unsubstituted compounds only.



Scheme 5

A major difference is at first sight evident: for **8** the base peak is no more due to $M^{+\bullet}$, but to $[M-CO]^{+\bullet}$ species. This can be explained by a weakening of the $(H_3CO-)C-CO$ bond, with the consequent cleavage 1 and further CO loss (see Scheme 6), leading to the highly stable ion **m** of Scheme 6.



Scheme 6

The easy occurrence of this cleavage inhibits the decomposition processes 1 and 2 of Scheme 4, observed for **5** and **6** with a consequent lowering of related fragment abundance.

CONCLUSIONS

Electrospray shows the easy production of abundant protonated molecules and by collisional experiments informations on the stability of the title compounds in acidic media can be obtained. In particular the experimental data show that the protonation takes place on either oxygen or nitrogen atoms, activating specific fragmentation routes.

The odd electron molecular ions produced in electron ionization conditions decompose through fragmentation channels well related to the structures of neutral molecules, allowing the characterization of isomeric compounds.

EXPERIMENTAL

Chemicals

Methanol and Chloroform were purchased from Sigma-Aldrich (Milan, Italy).

Samples

Compounds **1 - 8** were analytically pure samples synthesized according to literature.[7]

Mass Spectrometry

Electrospray (ESI) mass spectra were obtained by a Thermo Finnigan LCQ Deca instrument. 10^{-6} M methanol solutions of **1 - 8** were directly infused in the source at a flow of 10 μL/min. The spray voltage was 4 kV, the sheath gas (N_2) was set at 50 (a.u.), the entrance capillary voltage and temperature were respectively 15 V and 270 °C. Collisional experiments were performed inside the ion trap by selecting the ion of interest and inducing its collision with the He bath gas (2.8 x 10^{-5} Torr) by applying a supplementary RF voltage on the two end caps in the range of 0-5 V. The electron ionization mass spectra were obtained by a Fisons MD800 GC/MS system (70 eV, 200 μA) operating with a transfer line and source temperatures of 220 °C and 200 °C respectively. Chloroform solutions of **1 - 8** were injected in a

DB5-MS column (30 mt length, 0.25 mm, 0.25 μm film tickness) starting at 100 °C for 5 min with a 10 °C/min ramp up to 300 °C.

ACKNOWLEDGEMENTS

E.S.-S. thanks Fondecyt Grant. N° 1070623 for financial support, and Dr. B. Cassels for his useful and motivational comments to continue this research.

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