Stereochemical Studies of Complex Molecules by Collisionally Induced Decomposition of Doubly Charged Ions: *Nuphar* Alkaloids

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Electron ionization of sulphur-containing *Nuphar* alkaloids leads to the formation of easily detectable, doubly-charged species. The study of charge-separation reactions by collision experiments allows us to determine the intercharge distances. These experimental figures agree well with the different stereochemistries of the compounds under investigation.

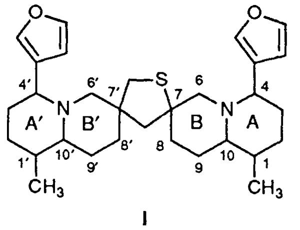
Although electron ionization mass spectrometry is one of the most widely used modern techniques for structure determination,[1] information on the stereochemistry supplied by MS measurements is generally limited. This is due to the high energy transfer to the molecules, which frequently permits them to overcome isomerization barriers, thus preventing the identification of different stereoisomers. The major drawback of this technique, in fact, is the competition between isomerization prior to decomposition and fragmentation processes which might enable the characterization of the structure from the fragmentation pattern. Metastable ion studies[2] and collision spectroscopy[3] have proved to be the most powerful MS methods for characterizing isomers, although in some cases the results are unsatisfactory. In this context, studies on doubly-charged ions may be of help in solving this important problem, as the presence of two charges makes it possible to label two atoms or groups and examine their relative positions in the fragmenting ion. Studies on structures and reaction mechanisms are, therefore, greatly facilitated.

The formation of doubly-charged species is a phenomenon often encountered during electron impact (EI) induced processes in organic molecules, and recent developments in mass spectrometry have led to new information about the energetics, stability and structure of these multiply-charged ions.[4] Literature examples include diatomic molecules,[5,6] triatomic species,[6] hydrocarbons,[7] aromatic compounds[8] and molecules containing heteroatoms.[9]

Very recently, the use of doubly-charged ions has been successfully employed in the structural elucidation of large molecules[10] but to our knowledge no data have been reported on the use of this technique for stereochemical elucidations.

Nuphar alkaloids are an important class of natural products, extensively studied in the last 20 years. In particular the sulphur-containing compounds with five heteroatoms (O_2,S,N_2) are of special interest owing to their unusual chemical structure and biological activity.[11] The general skeleton of these compounds is as shown in structure I.

The structures and stereochemistry of the alkaloids in question were determined by means of complex and extensive studies by MS, ^1H and ^{13}C NMR spectroscopy, IR spectroscopy and circular dichroism. Only two structures were confirmed by X-ray analysis.[11]



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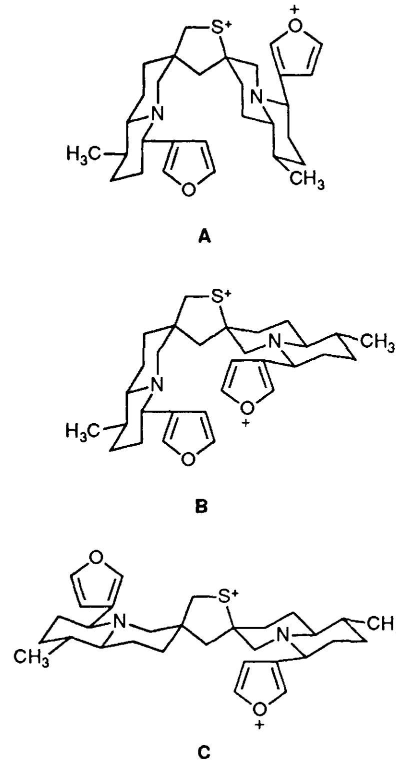


Fig. 1 Localization of the charges on the skeleton of doubly charged molecular ions of **1**–**3**

In this work we have studied the charge separation reaction of doubly-charged molecular and fragment ions originating from three stereoisomeric sulphur-containing *Nuphar* alkaloids. The results obtained permit the stereochemical characterization of the isomers and introduce a relatively simple methodology which can be proposed for stereochemical studies *via* mass spectrometry.

Results and Discussion

With respect to the different stereochemistry at C-7 or C-7′ there are three classes of alkaloids isolated from *Nuphar lutea*. For our studies we have selected the isomeric sulphides thiobinupharidine (**1**), thionuphlutine B (**2**) and neothiobinupharidine (**3**)

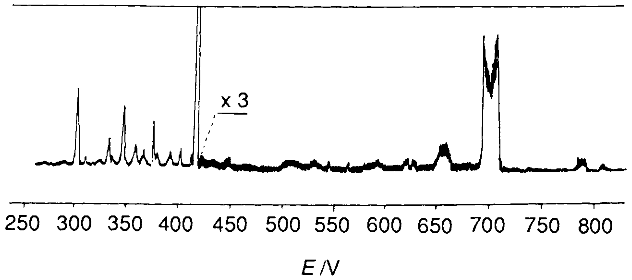


Fig. 2 Collisional activation mass spectrum of the doubly-charged molecular ion of sulphide **3**

Table 1 Kinetic energy releases and intercharge distances for the loss of furyl ion from doubly-charged sulphides **1**–**3**[a]

Sulphide	$T_{\frac{1}{2}}$/eV	R/Å
1	2.3 ± 0.1	6.5 ± 0.4
2	1.9	7.4
3	1.8	7.8

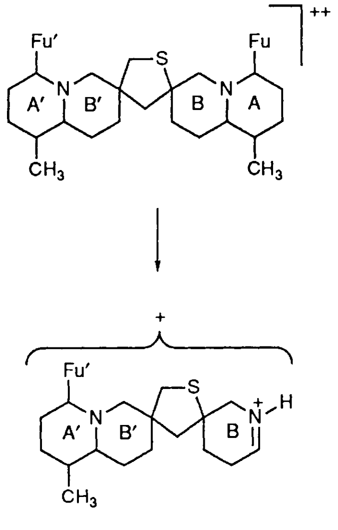
[a] The data are the average of five measurements.

which are representative of these classes. Their structures are shown in Fig. 1 in which structures **A**–**C** refer to the doubly-charged ions **1**–**3**, respectively.

The mass spectrometric behaviour of *Nuphar* alkaloids has been investigated by several authors.[11,12] The most important pathways of decomposition are related to the cleavage of tetrahydrothiophene and/or quinolizidine systems.

Unimolecular and collisional activation[2,3] studies on the molecular ion $M^{\cdot +}$ of **1**–**3** did not reveal any stereochemical difference, suggesting either that isomerization processes take place or that the competition among the different decomposition pathways is not effective for isomer characterization. Positive results have been obtained only with the corresponding sulphoxides bearing substituents at C-6 and C-6′,[12] due to specific decomposition pathways.

These results, and the presence of intense, doubly-charged ions in the EI spectra of sulphides **1**–**3**, encouraged us to undertake the study of M^{2+} species and doubly-charged fragment ions, in particular $[M - C_9H_{11}O]^{2+}$, which results from the cleavage of the AB quinolizidine system as described in eqn. (1).



(1)

Fu = furyl

Table 2 Kinetic energy releases and intercharge distances for the loss of furyl ion from $[M - C_9H_{11}O]^{2+}$ [a]

Ion	$T_{\frac{1}{2}}$/eV	R/Å
$[\mathbf{1} - C_9H_{11}O]^{2+}$	1.7 ± 0.1	8.4 ± 0.4
$[\mathbf{2} - C_9H_{11}O]^{2+}$	1.9	7.5
$[\mathbf{3} - C_9H_{11}O]^{2+}$	1.5	9.6

[a] The data are the average of five measurements.

A typical collisional activation mass-analysed ion kinetic energy (CA MIKE) spectrum[2] of the preselected doubly-charged molecular ion is shown in Fig. 2. It consists of two parts: for $E < 418$ V doubly-charged species are detected, while for $E > 418$ V charge-separation reaction products are observed. In the latter case the predominant reaction is as shown in eqn. (2).

$$[M]^{2+} \longrightarrow [M - Fu]^{+} + Fu^{+} \qquad (2)$$

The occurrence of alternative processes, as in eqn. (3), has to be

$$[M]^{2+} \xrightarrow{N} [M]^{+} + [N]^{+} \longrightarrow [M - Fu]^{+} + Fu \qquad (3)$$

$$N = \text{neutral colliding gas molecule}$$

excluded due to the high kinetic energy release associated with the furyl loss, typical of coulombic repulsions. The absence of the furyl cation peak in the mass spectrum is significant. The reasons are unclear, but it is possibly due to instrumental problems in the analysis and detection of low molecular mass ions with low kinetic energy precursors (see Experimental section). If it is assumed that the energy released in the decomposition of a doubly-charged ion is due solely to coulombic repulsion, the distance R/Å between the two charged centres in the transition state is given by the formula[2] $T_{\frac{1}{2}} = e^2/R$ where the kinetic energy release $T_{\frac{1}{2}}$, may be easily calculated from the MIKE spectrum. The values related to the decompositions described in eqn. (2) are listed in Table 1. As has already been mentioned, the $T_{\frac{1}{2}}$ values are correlated with the distances between the two charged sites. Assuming that one charge is located on the furyl moiety, as indicated by the collision-activated decomposition pathway, the R data are consistent with a localization of the other charge on the sulphur atom. The differences found in the calculated R values are consistent with the original structures shown in Fig. 1.

Since in ions **B** and **C** the distances between the two charges are the same as expected (and confirmed by experiment), for further characterization of the isomers in question we investigated the $[M - C_9H_{11}O]^{2+}$ isomeric ions [see eqn. (1)] in which one charge must necessarily be located on the quaternary nitrogen atom. In this case the predominant charge-separation reaction was the loss of the furyl ion, according to eqn. (4).

$$[M - C_9H_{11}O]^{2+} \longrightarrow [M - C_9H_{11}O - Fu]^{+} + Fu^{+} \qquad (4)$$

The pertinent results are summarized in Table 2. The data are in full agreement with doubly-charged ions with the structures shown in Fig. 3.

The two sets of data *i.e.* those related to $[M]^{2+}$ and to $[M - C_9H_{11}O]^{2+}$ decompositions are complementary to each other and allow a complete stereochemical characterization of the three isomeric sulphides **1**–**3**.

The extension of this approach to the elucidation of different stereochemical problems is now in progress in our laboratories.

Experimental

Sulphides **1**–**3** are natural products isolated as described in previous papers.[13]

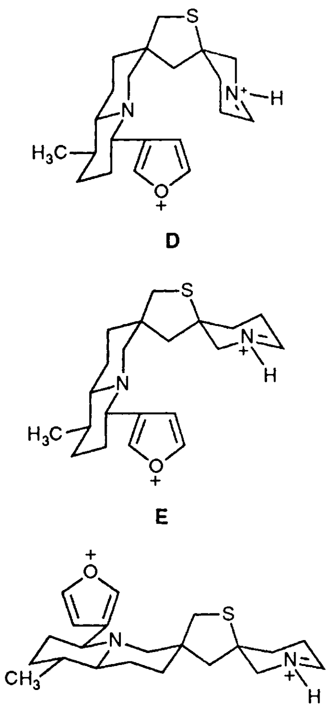


Fig. 3 Localization of the charges on the skeleton of $[M - C_9H_{11}O]^{2+}$

Mass spectrometric analyses were performed using a VG ZAB2F instrument (VG Analytical)[14] operating in EI mode (70 eV, 200 μA) with an ion-source temperature of 200 °C. In all cases the doubly-charged species was selected with the magnetic sector at one half of the mass value and with an acceleration voltage of 4 kV. The related collisionally-induced decompositions were performed in the II FFR (second field-free region) of the instrument; the MIKE spectra were then obtained by scanning the electrostatic sector voltage from 0–850 V.

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Paper 0/03098H
Received 10*th July* 1990
Accepted 25*th October* 1990