Abstract

An experimental study of the electronic excitation and ionic dissociation of two important classes of biomolecules – natural products (biogenic volatile organic compounds, VOCs, and volatile components of essential oils) and DNA and RNA constituents (aminoacids and bases) is here exemplified with recent results on the fragmentation of thymine and isoprene as induced by synchrotron radiation and fast electrons. Fragmentation of the thymine molecule was seen to dramatically increase as the photon energy increased from 21 to 300 eV and 450 eV. At the highest photon energy, simply and doubly charged N and O atoms were observed. The parent ion (m/z = 126) could be observed at all photon energies. The fragmentation pattern observed in the 1.0 keV electron impact mass spectrum of thymine resembled more closely the fragmentation observed with 21 eV photons. In isoprene, the dominant fragments observed at 21 eV and 310 eV photon energy as well as in the 1.0 keV electron impact mass spectrum were C5H7+ (m/z = 67), C4H5+ (m/z = 53), C3H3+ (m/z = 39) and C2H3+ (m/z = 27). Previously unreported fragments, namely H+, C+, CH+, CH2+, and CH3+ were observed at the high photon energies and at the electron impact mass spectrum.

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I. Introduction

In the last few years an extensive and systematical investigation has been dedicated to the study of processes associated with the electronic excitation and ionization of gas-phase biomolecules as induced by high energy photons and fast electrons. This investigation involves the collaborative efforts of research groups who employ advanced experimental techniques to probe the valence and core electron excitation of gas-phase biomolecules and some of the associated relaxation processes. Basically two large classes of biomolecules constitute the focus of this work: nucleic acids constituents (mainly amino acids and bases) and volatile chemical compounds either emitted by or extracted from plants (natural products). When subjected to ionizing radiation, these molecules usually dissociate into neutral and charged species. We are basically interested in describing the excitation and ionic fragmentation of these molecules following valence and core-shell excitation.

The gas-phase excitation and ionization of amino acids, DNA and RNA bases (and related compounds) has already been the focus of interesting and recent experimental studies by other research groups, either using electrons[1-3] or high energy photons[4-9]. Our group has also recently reported on the ionic dissociation of glycine, alanine, valine and proline, as induced by high energy photons[10-12].

Biogenic volatile organic compounds (VOCs), such as isoprene and monoterpenes, are emitted in significant amounts by trees and other vegetations. These volatile organic compounds are used by plants to attract pollinating insects or to repel leaf eating ones[13]. These compounds can be found in the atmosphere with concentrations ranging from between some ppb to up to several (hundreds) ppb. They can be quite reactive under atmospheric conditions and thus the emission of biogenic VOCs plays a central role in the photophysics and photochemistry of the atmosphere[14]. Volatile compounds (terpenes) can also be extracted in significant amounts from the essential oils of plants, being for instance used in the flavour and fragrance industries[15]. Compared to the amount of studies dedicated to the excitation and photoionization of amino acids and DNA and RNA bases, the number of recent experimental studies dedicated to these natural products is quite limited. The photoionization of two monoterpenes, limonene and carvone, was recently studied from the ionization threshold up to 11 eV[15]. Circular dichroism was also measured around the C 1s edge for the camphor molecule[16]. Fragmentation of the limonene molecule, following core photoionization, was recently reported[17].

Basically two classes of experimental techniques have been used in our investigation: electron energy-loss spectroscopy (with small, fixed angle or variable angle capabilities) and time-of-flight mass spectrometry. We will nevertheless give in the present paper only an overview of
recent results obtained with the isoprene and thymine molecules, using time-of-flight mass spectrometry. Both an electron beam and synchrotron radiation were independently used as ionizing agents. A more detailed discussion on the results obtained with these as well as with other molecules will be presented in forthcoming papers.

II. Experimental

The experimental apparatus used in the synchrotron radiation measurements has been described in details elsewhere[18-19]. A Wiley-McLaren type time-of-flight mass spectrometer, was used for mass/charge analysis of the resulting ionic fragments. The vacuum chamber base pressure is usually maintained in the order of \(10^{-9}\) mbar. The ionized recoil fragments produced by the interaction of the sample with the light beam are accelerated by a two-stage electric field and detected by a pair of micro-channel plate detectors mounted in a chevron configuration. Arrival of the ions gives rise to stop signals to a time-to-digital converter (TDC) model P7886, manufactured by FAST-Comtec, which allows simultaneous multihit capability with 1 ns resolution. Electrons, accelerated in the opposite direction with respect to the positive ions, are recorded without energy analysis by another pair of micro-channel plate detectors and provide the start signal to the TDC. A 766 V/cm DC electric field is applied to the first ion acceleration stage. The time-of-flight spectrometer was designed to achieve 100 % efficiency for ions with kinetic energies up to 40 eV. 

The ions produced in the ionization region are focused in a detector area by an electrostatic lens. For the measurements around the N 1s (~400 eV) and O 1s (~550 eV) edges we have used synchrotron radiation from the spherical grating monochromator (SGM) beamline of the Brazilian Synchrotron Light Facility (LNLS)[20], which provides a photon flux of approximately \(10^{11}\) photons/s/100mA/0.1% bandwidth and resolution power better than 2000. The total ion yield spectra (TIY), which are proportional to absorption spectra, were obtained by recording all ions produced after an interaction of the linearly polarized light with the molecules as scanning the photon energy around N 1s and O 1s ionization edges (NEXAFS). TIY spectra of molecular nitrogen and carbon monoxide in the N 1s and O 1s regions respectively, taken during these experiments, were used to calibrate the energy in the spectra of the amino acids. After recorded the TIY spectra, we have also obtained TOF spectra at selected energies around the N 1s and O 1s edges in order to obtain the fragmentation pattern following the inner-shell ionization process.

For the valence-shell and C 1s (~300 eV) measurements we used a toroidal grating monochromator (TGM) beamline (12-310 eV). A very efficient gas filter system has recently been added to this beamline, drastically reducing the problem of high-order contaminations[21].

The time-of-flight technique used in the electron impact measurements basically resembles the technique employed in the synchrotron radiation experiments. A pulsed, 1 keV electron beam
(10 KHz), ionizes the sample. The positively charged fragments are accelerated by a two-stage electric field and detected by a pair of micro-channel plate detectors mounted in a chevron configuration. Arrival of the ions gives rise to stop signals to a time-to-digital converter (TDC) model P7886, manufactured by FAST-Comtec, which allows simultaneous multihit capability with 1 ns resolution. The start signal to the TDC is provided by a pulse applied to an extraction grid. A pulsed 500 V/cm DC electric field is applied to the first ion acceleration stage.

The thymine sample was purchased from Sigma-Aldrich in the form of crystalline powders with minimum purity of 99%, and was used without any further purification. Due to the low vapor pressure of these compounds, it was necessary to use a heated source for sublimation. More detail on the sublimation procedure used in this work is available elsewhere[22]. The thymine experiment was conducted at 100-115 °C. Isoprene is a high vapor pressure liquid under atmospheric conditions and no heating procedure was necessary in order to introduce the sample in the collision chamber. The liquid sample was subjected to repeated freeze thaw-pump cycles before admission into the mass spectrometer. Pressure in the chamber was kept in the range of 2-5 x 10^-6 torr during experiments.

III. Results and discussion

A. Thymine

The low energy electron impact mass spectrum of thymine was first published more than 40 years ago by Rice, Dudek and Barber[23]. More recently, a careful analysis of the fragmentation of this molecule has been presented by Jochims et al.[5], using synchrotron radiation in the 6-22 eV photon energy region. The m/z = 55 fragment (CH$_3$CCHNH$^+$), corresponding to a loss of HNCO and an additional CO group, is found to be responsible for the strongest peak both in the 70 eV electron impact and the 20 eV photon energy mass spectra. In both cases, the fragments observed at m/z = 28 (HCNH$^+$) and m/z = 126 (parent ion) come next in relative intensities.

We have recently measured the mass spectra of thymine in a very large photon energy range (12 – 580 eV). The ionic fragmentation of this molecule was also induced by fast electron (1.0 keV) impact. Spectra obtained at 21 and 300 eV photon energies are compared to the spectrum obtained with 1 keV electrons in Figure 1. At 21 eV photon energy our results basically agree with previously published work. The m/z = 55 fragment dominates the spectrum, followed by the parent ion (m/z = 126) and the m/z = 28 fragment. At 300 eV photon energy, fragmentation of the molecule can also be associated with the C 1s ionization. Fragmentation is enhanced and as a result the relative contributions from both the parent ion and the m/z = 55 fragment are accordingly reduced. We assign the fragments observed at m/z = 17 and 18 as mostly due to the presence of...
water contamination in the sample. At still higher photon energies, fragmentation of the molecule has been studied following N 1s and O 1s ionization. These results are exemplified in Figure 2, in which we show the mass spectrum of thymine obtained around the N 1s edge. Although the parent ion continues to be clearly observable, fragmentation of the molecule is now greatly enhanced and the most intense peaks in the spectrum are associated with m/z = 1 (H+) and m/z = 28 (HCNH+2) fragments. Fragments like C+ (m/z = 12), N+ (m/z = 14) and O+ (m/z = 16) are pointing out to the atomization of the molecule at this photon energy. Doubly charged species are also observed at m/z = 7 (N2+) and m/z = 8 (O2+). A more detailed analysis of the photon induced fragmentation of thymine following N 1s and O 1s excitation, including the ion-ion coincidence results, will be presented in an upcoming paper[24].

Regarding the 1.0 keV electron impact mass spectrum, we observe a increasing degree of fragmentation at this energy, as compared to the 70 eV electron impact spectrum available at the NIST data base[25]. While in the latter energy the parent ion peak dominates the spectrum, the dominant peak in the 1.0 keV now corresponds to the m/z = 55 fragment. Comparing now the electron impact and photon impact mass spectra, we notice a closer resemblance between the electron impact mass spectrum and the 21 eV photon energy mass spectrum. The similarity observed between the mass spectrum obtained at this relatively high electron impact energy and the spectrum obtained at a relatively low photon energy points out to the fact that the total electron impact ionization cross section at 1.0 keV is still dominated by valence ionization processes.

B. Isoprene

The mass spectra of isoprene (2-methyl-1,3-butadiene), a very important biogenic volatile organic compound, has been obtained at 21 eV and 310 eV photon energies and the results are shown in Figure 3. In the absence of published information on the core ionization energies of isoprene, we take the ionization energies of a closely related molecule, 1,3-pentadiene[26] as a valuable approximation to the isoprene C 1s ionization energies. We may consequently take the spectrum obtained at 21 eV as basically associated with valence shell ionization while at 310 eV we are energetically above the C 1s edges. An enhancement in the degree of dissociation of the molecule can be seen as we move from 21 to 310 eV photon energy, with the observation of previously unreported fragments at m/z = 1, 12, 13, 14 and 15 (corresponding respectively to the fragments H+, C+, CH+, CH2+, and CH3+). At any rate the overall fragmentation pattern does not change much irrespective of the fact that the photon energy was increased by more than one order of magnitude. Except by the presence of the afore mentioned new fragments, the spectra at both low and high photon energies are dominated by the same fragments, namely C5H7+(m/z = 67), C6H9+(m/z = 53), C7H11+(m/z = 39) and C6H5+(m/z = 27). Basically the same fragments are observed in the electron
impact mass spectrum, obtained at 1.0 keV incident electron energy and shown in Figure 4. In a
previous study of the ionic fragmentation of another monoterpene, limonene \((\text{C}_{10}\text{H}_{16})\), at 21.21 eV
and 310 eV photon energies\[27\] it was also observed that although new, low \(m/z\) fragments were
observed in the high photon energy spectrum, basically the two mass spectra remained dominated
by the same set of ionic fragments at both photon energies.

IV. Conclusions

The experimental study of the electronic excitation and ionic dissociation of two important classes
of biomolecules – natural products (biogenic volatile organic compounds, VOCs, and volatile
components of essential oils) and DNA and RNA constituents (aminoacids and bases) has been
exemplified with recent results on the fragmentation of thymine and isoprene as induced by
synchrotron radiation and fast electrons. In the thymine case the degree of fragmentation was seen
to dramatically increase as the photon energy increased from 21 to 300 eV although the parent ion
\((m/z = 126)\) could still be observed. The fragmentation pattern observed in the 1.0 keV electron
impact mass spectrum of thymine resembled more closely the fragmentation observed with 21 eV
photons. In the isoprene case, the fragmentation pattern remained practically the same at both the
two photon energies (21 and 310 eV) and at the 1.0 keV electron impact mass spectrometer. The
main fragments observed were \(\text{C}_{5}\text{H}_{7}^+\) \((m/z = 67)\), \(\text{C}_{4}\text{H}_{5}^+\) \((m/z = 53)\), \(\text{C}_{3}\text{H}_{3}^+\) \((m/z = 39)\) and \(\text{C}_{2}\text{H}_{3}^+\)
\((m/z = 27)\).

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Figure captions:

Figure 1. Mass spectra of thymine obtained at 21 and 300 eV photon energies (synchrotron radiation) and 1 keV electron impact energy.
Figure 2: Mass spectrum of thymine obtained at 450 eV photon energy.
Figure 3. Mass spectra of isoprene obtained at 21 and 310 eV photon energies.
Figure 4. Mass spectrum of isoprene obtained with 1 keV electrons.
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