

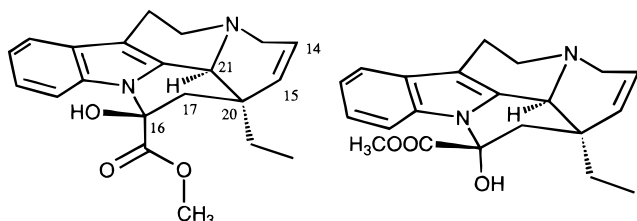
JMS Letters

Dear Sir,

Stereochemistry of Electrosprayed Ions. 14-Dehydro- and 16-*epi*-14-Dehydrovincamine

The analysis of organic molecules of natural or synthetic origin by mass spectrometry takes advantage of the production of structurally diagnostic fragment ions in the ion source or in the field-free regions. In a number of cases, the mass spectra provide useful information on the stereochemistry of the compound under investigation. As a consequence, the study of sterically controlled fragmentation processes has been the subject of a large number of studies.¹ Very often, stereoselective dissociation pathways are sensitive to the amount of internal energy deposited on the precursor ion during the ionization process. In a previous paper, we demonstrated that electrospray ionization (ESI) could be used for carrying out energy-resolved mass spectrometric (ERMS) experiments with a view to differentiating stereoisomeric pairs of indoloquinolizidines.²

In this letter, we report an ESI-ERMS study of two naturally occurring indole alkaloids, 14-dehydrovincamine (**1**) and its epimer at position 16 (**2**), which had previously been isolated from *Crioceras longiflorus* Pierre³ and *C. dipladeniiflorus* (Apocynaceae).⁴



14-dehydrovincamine **1**

16-*epi*-14-dehydrovincamine **2**

The structural elucidation of vincamine series of indole alkaloids has generally rested on electron impact (EI) ionization mass spectrometry, although chemical ionization (CI) can be used as an alternative method when the ion currents of the EI-induced M^{+} species are weak.^{5,6} Studies involving the CI of vincamine with oxygenated reagents have also been published recently.⁷ To our knowledge, previous investigations of the stereochemical differentiation of vincamine derivatives were performed using only EI.^{8,9}

The ESI mass spectra of **1** and **2** were first recorded at a potential difference of 120 V between the sampling cone and the skimmer of the ion source. They displayed at m/z 353 a very intense ion peak corresponding to the protonated molecules $1H^{+}$ and $2H^{+}$. Several fragment ions appeared at lower m/z values, some of which corresponded to the loss of small neutral species. The fragment ions at m/z 335, 324 and 293 were attributed to the loss of a water molecule, of an ethyl radical (CH_3CH_2) and of $C_2H_4O_2$ from the carbomethoxy group, respectively. Another interesting fragment ion at m/z 306 arose from the consecutive losses of water (18 u) and ethyl radical (29 u). Comparison of the two spectra showed a remarkable increase in the relative intensities of the ion peaks at m/z 335 and 306 in the case of *epi*-dehydrovincamine.

In order to compare the fragmentation behaviours of the protonated molecules $1H^{+}$ and $2H^{+}$, their ERMS analysis was undertaken. Among the various dissociation channels revealed by the mass spectra, we focused our attention on the three competitive fragmentation pathways leading to the ions

at m/z 335, 324 and 293 and on the consecutive process which generated the ion at m/z 306.

Depending on their origin, $1H^{+}$ or $2H^{+}$, the ions at m/z 335, 324 and 293 led to very different ERMS curves. The loss of water was clearly the most abundant fragmentation pathway of the $2H^{+}$ precursor ion. In contrast, in the case of dehydrovincamine **1**, the three competitive processes gave similar yields, even though the loss of the carbomethoxy group appeared to be less favourable than those of the other substituents.

Clearly, the m/z 293 and 324 ion abundances were significantly higher for **1** than for **2**. The axial positions of the corresponding substituents (16-carbomethoxyl and 20-ethyl) strongly suggested both a 1,3-diaxial interaction effect and stereoelectronic control of the fragmentation. In fact, the preferential loss of the 20-ethyl side-chain from **1** should be due mostly to the 1,3-diaxial interaction, which is expected to be more important in the case of a large axial substituent at position 16, such as carbomethoxyl, than in the case of the smaller axial hydroxyl present in **2**. The 16-carbomethoxy group in **1** experiences both steric interaction with the 20-ethyl group and the influence of the α -nitrogen atom. The tendency of axially oriented groups in the α -position to the heteroatom of a heterocyclic compound to be lost more easily than the equatorial substituents is well documented.^{10–12} The stereoelectronic control of the fragmentation has been considered to be responsible for the stereochemical effects related to the α -dissociation of cation radicals.¹² Similar conclusions could be drawn in the case of protonated molecules such as $1H^{+}$ and $2H^{+}$ and the much more abundant loss of water from the $2H^{+}$ species could be rationalized in the same terms.

The abundance of the ion at m/z 306, similarly to that of the m/z 335 fragment ion, was much larger for 16-*epi*-14-dehydrovincamine (**2**) than for its epimer **1**. This fragment could arise from two alternative fragmentation sequences according to the order of the water and ethyl losses. The calculation of the breakdown curves of the three ions involved in these two dissociation channels (i.e., ions at m/z 335, 324 and 306) allowed the order of the neutral losses to be determined. In the case of **2**, the observed increase of the m/z 306 ion relative abundance was accompanied by a symmetric decrease of the m/z 335 ion curve, whereas the ion at m/z 324 seemed to be unaffected by the formation of the ion at m/z 306. This result unambiguously indicated that the ion at m/z 306 was formed at the expense of the ion at m/z 335, or, in other words, the loss of ethyl followed that of water. In the case of alkaloid **1**, a similar trend could be observed even though the possibility of loss of water following that of an ethyl radical could not be entirely discarded. Examination of the energy dependence of the m/z 306 ion appearance, with regard to those of the ions at m/z 335 and 324, led to the same conclusion. Thus, the formation of the ion at m/z 306 from $1H^{+}$ clearly requires a greater amount of energy than that of the ions at m/z 335 and 324. In contrast, the ERMS curve of the m/z 306 ion arising from $2H^{+}$ is shifted to lower energy values than the m/z 324 ion ERMS curve.

In conclusion, ESI allows simple and precise control of the precursor ions' internal energy. Further, the ionization process leads to gas-phase species with low, near-thermal, internal energies,¹³ allowing the appearance thresholds of different fragment ions to be observed. The information extracted from the ESI-ERMS experiments can be very valuable for the detection of subtle structural characteristics of organic molecules, including stereochemical differentiation. From this point of view, the electrospray ion source represents an easy, sensitive and inexpensive tool for performing low-energy

collision-induced dissociation experiments related to structural elucidation.

We have shown here that this method, associated with a careful analysis of the spectral data (mass spectra; ERMS and breakdown curves), could be used for distinguishing the factors that influence the competitive formation of different fragment ions generated simultaneously in the ion source. The determination of a consecutive dissociation pathway among different alternative channels seems also to be possible by this analytical procedure.

Financial support from the Association pour la Recherche contre le Cancer (ARC) and the Centre National de la Recherche Scientifique (CNRS) is gratefully acknowledged. The authors thank Professor André Cavé (Faculté de Pharmacie, Châtenay-Malabry, France) for the generous gift of the samples of dehydrovincamine and epi-dehydrovincamine.

Yours

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