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Formation of Gas-Phase Lithium Complexes from Acetogenins and their Analysis by Fast Atom Bombardment Mass Spectrometry [1]

Olivier Laprévote, Christian Girard, Bhupesh C. Das

Institut de Chimie des Substances Naturelles, C.N.R.S., avenue de la Terrasse, 91198 Gif-sur-Yvette Cedex, France

Diego Cortes

Faculté de Médecine et de Pharmacie, Université de Rouen, 76800 Saint-Etienne du Rouvray, France

André Cavé*

Faculté de Pharmacie, U.A. C.N.R.S., rue J.-B. Clément, 92296 Châtenay-Malabry, France

Abstract: The Annonaceous acetogenins form complexes with lithium in a liquid matrix. Fast atom bombardment (FAB) combined with linked scan (*B/E* and $B^2(1-E)/E^2$) mass spectrometry of a lithium cationized acetogenin, rolliniastatin-2, was used to analyze the metastable dissociations of the complex, leading to structurally useful information.

The Annonaceous γ-lactone containing acetogenins represent a rapidly growing class of natural products, many of which exhibit biological activities such as antitumoral, cytotoxic, antibacterial or pesticidal properties.[3] Their structure is characterized by a long alkyl chain bearing a terminal γ-lactone, one or two tetrahydrofuran rings and several oxygenated substituents (hydroxy, keto, acetoxy) often located at positions adjacent to the tetrahydrofuran rings.

The major difficulty associated with the structure elucidation of a new natural acetogenin lies in the location of the different oxygenated functions along the alkyl chain. Analysis of silylated or acetylated derivatives by using high resolution electron impact ionization mass spectrometry is often necessary for solving this problem.[3] Milder ionization conditions such as chemical ionization and fast atom bombardment (FAB) allow a direct analysis of the underivatized acetogenin but very often, intense $[M+H]^+$ ion peaks are obtained at the expense of structurally useful fragment ions. In such cases, analysis of the fragment ions produced from collisional activation processes by using tandem mass spectrometry is required.[2]

Recently, we described the behaviour in FAB mass spectrometry of an acetogenin, uleicin A, showing a strong interaction with alkali metal cations, in particular with lithium.[4] Similar observation was made concerning a terminal γ-lactone containing long-chain diepoxide compound, diepomuricanin, whose structure was established by using the *B/E* linked scan spectrum of $[M+Li]^+$ ions generated by FAB.[2] We have now applied this analytical strategy, which obviates the need for derivatization or MS/MS facilities, toward the structural elucidation of the Annonaceous bistetrahydrofuran acetogenins. This paper describes the results obtained with a known acetogenin, rolliniastatin-2, whose structure is representative of this class of compounds.

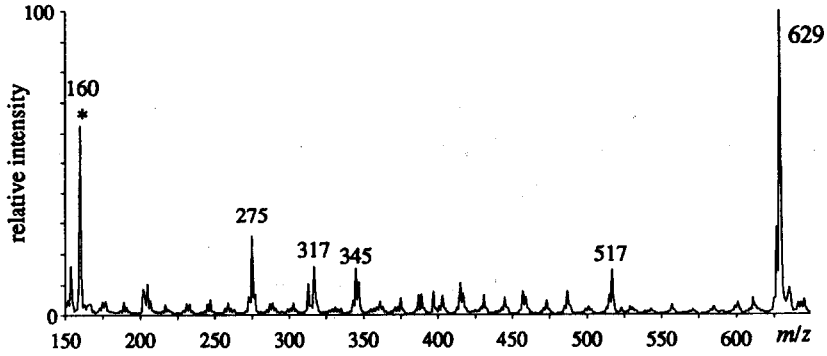


Figure 1. FAB mass spectrum of rolliniastatin-2; FAB matrix: *m*-NBA + LiCl. The peak at *m/z* 160, marked with an asterisk, corresponds to the lithium adduct of *m*-NBA.

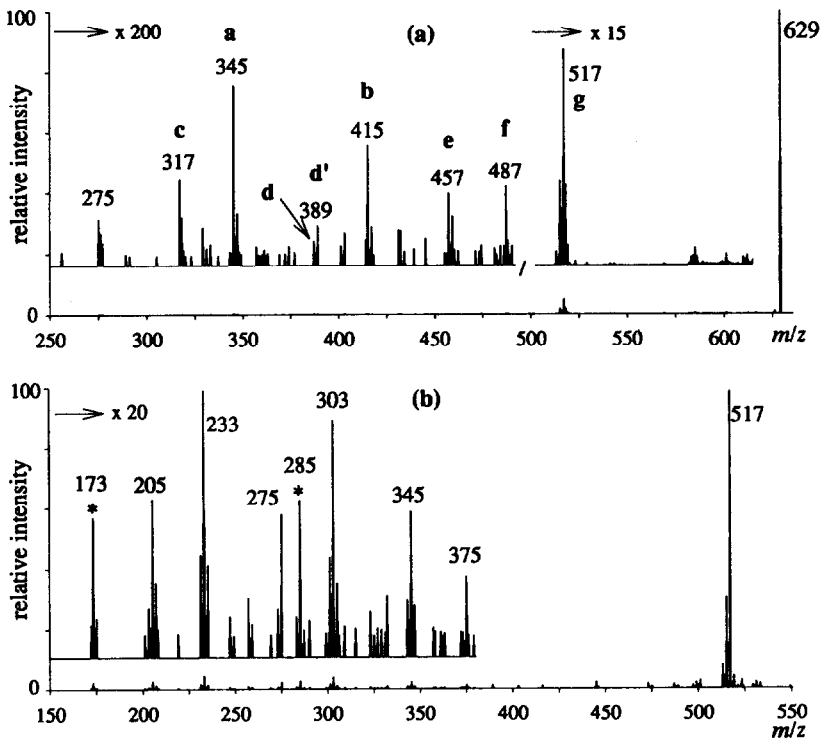
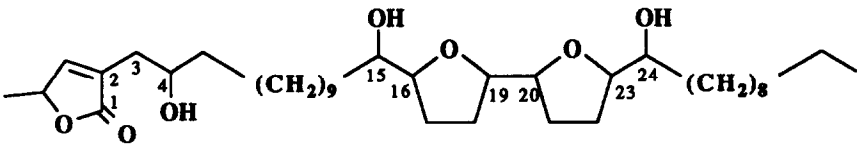


Figure 2. First field-free region reactions of lithiated rolliniastatin-2 under metastable conditions (FAB matrix: *m*-NBA + LiCl); a) *B/E* linked scan mass spectrum of $[M+Li]^+$ ions; b) neutral loss spectrum (fragment ion scan; neutral: 112 Da). The peaks at m/z 173 and 285, marked with asterisks, are due to fragments originating from the lithium adduct of monooctyl phthalate and dioctyl phthalate, respectively.

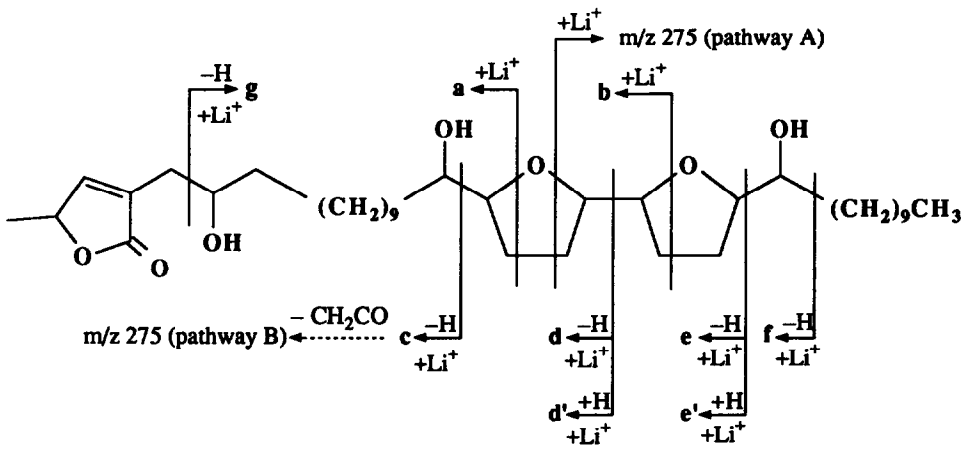


Rolliniastin-2

The FAB mass spectrum of rolliniastatin-2, using *meta*-nitrobenzylalcohol (*m*-NBA) as matrix, displayed very few fragment ion peaks. The protonated molecules at *m/z* 623 accompanied by sodium adduct ions at *m/z* 645 confirmed the molecular weight (622). While three successive losses of water from the $[M+H]^+$ ions (*m/z* 605, 587 and 569) were attributed to the presence of three hydroxyl groups in the molecule, no skeletal fragmentation was observed in this spectrum.

The mass spectrum obtained after addition of lithium chloride to the FAB matrix was dominated by the $[M+Li]^+$ ion peak at *m/z* 629 (base peak; Figure 1). Several fragment ion peaks at *m/z* 517, 345, 317 and 275 were also observed in the spectrum. However, high chemical noise made the interpretation of the spectrum difficult. Also, the possibility of consecutive decomposition processes in the ion source might lead to erroneous conclusions with regard to the structural identification of the fragment ions. These drawbacks were avoided by the use of constant-*B/E* linked scanning of the *m/z* 629 ions under metastable conditions, thus reducing the probability of consecutive fragmentations.

The first field-free region (FFR) metastable decompositions of the $[M+Li]^+$ ions generated a series of fragment ions, all of which retaining lithium (Figure 2a). In the *m/z* 250 – *m/z* 460 range of the *B/E* linked scan spectrum, the observed product ions were easily attributed to cleavages across the tetrahydrofuran rings (ions **a** and **b**) and also of their adjacent C-C bonds (ions **c**, **d**, **d'**, **e** and **e'**; see Scheme). Another fragment ion (**f**) including the hydroxyl function at C-24 originated from the cleavage of the C-24–C-25 bond. Fragmentations at the lactone site generated several other product ions in the higher mass region: *m/z* 601 (loss of CO), *m/z* 585 (loss of CO_2) and a more intense ion at *m/z* 517 (ion **g**). The ion **g** was formed by loss of 112 amu which involved a β-cleavage of the lactone ring along with a hydrogen transfer.[2]



Scheme

Thus, the location of the bistetrahydrofuran nucleus in the alkyl chain of rolliniastatin-2 could be directly established from the above fragmentation pattern. It is interesting to note that almost all the product ions (**a-f**) retained the portion of the molecule bearing the terminal γ-lactone, which was possibly implied in the lithium

complexation.[2] The characteristic loss of 112 amu by expulsion of the lactone group of the molecule has been used to confirm its presence in the structure of the **a-f** fragment ions.

A particular linked scan mode following the equation $B^2(1-E)/E^2$ = constant where B and E are the magnetic and the electrostatic fields, respectively, allowed the detection of all the fragment ions which issued from the loss of the same neutral species in the first FFR.[5] Thus, the constant neutral loss spectrum corresponding to 112 amu was expected to record all ions which possessed the γ-lactone ring. This spectrum, shown in Figure 2b, indeed displayed the complete series of product ions issued from the **a-f** ions by loss of 112 amu, thus confirming the presence of the lactone in their structures. The ions at *m/z* 275, observed in the *B/E* linked scan mass spectrum, did not register the loss of 112 amu, the corresponding fragment ions expected at *m/z* 163 being absent in the constant neutral loss spectrum. Consequently, the *m/z* 275 ion included the methyl end of the molecule (pathway A in Scheme) rather than the lactone terminal (pathway B). Surprisingly, the constant neutral loss spectrum (loss of 112 amu) displayed two additional product ion peaks at *m/z* 173 and 285, revealing the contamination of the sample by mono- and dioctylphthalates, which could lose an octene molecule (112 amu) from the lithiated molecular species (*m/z* 285 and 397, respectively).

In conclusion, the structural investigation of Annonaceous acetogenins by mass spectrometry could be carried out directly on conventional sector mass spectrometers without prior derivatization of the compounds. Complex formation between acetogenins and lithium in a liquid matrix under FAB ionization conditions allows an unambiguous molecular weight determination. The metastable dissociations occurring at the tetrahydrofuran sites of the complex may be recorded by *B/E* linked scanning, thus leading to their location on the alkyl chain. The interest of the neutral loss spectra for the structural elucidation of natural compounds must also be emphasized especially when the molecule undergoes a fragmentation by losing a part of it as a characteristic neutral species. Extension of similar studies on complex formation between natural products and alkali metal cations under FAB and on the structural significance of their gas-phase fragmentations is currently being explored in this laboratory.

References and notes

1. Part. 16 in the series "Acétogénines des Annonacées"; for part 15, see ref. 2.
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5. Jennings K. R.; Mason R. S. : 'Tandem Mass Spectrometry Utilizing Linked Scanning of Double Focusing Instruments' in *Tandem Mass Spectrometry*; McLafferty F. W., Ed.; Wiley Interscience, Inc.: New York, 1983; pp. 197-222.
6. Rolliniastatin-2 was isolated by one of us (DC) from *Annona cherimolia* seeds (Annonaceae).[7] Mass spectra were obtained with a Kratos MS80RF double-focusing mass spectrometer of Nier-Johnson geometry with an accelerating voltage of 4kV and a post acceleration voltage of 8 kV. The FAB gun voltage was set at 7 kV (1 mA emission current), using xenon as bombardment gas. The mass range from 1300 to 30 was scanned at 10 sec/decade. Experimental conditions used for the *B/E* linked scan experiments (product ion spectra) were described previously.[2] Constant neutral loss scans were performed under the control of the Kratos DS90 data system.
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