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Structural Elucidation of Acetogenins from Annonaceae by Fast Atom Bombardment Mass Spectrometry

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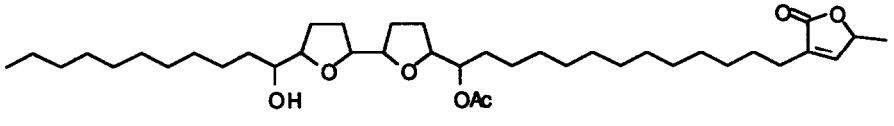
Abstract: The Annonaceous acetogenins form complexes with lithium in a liquid matrix. Fast atom bombardment (FAB) combined with linked scan (*B/E*) mass spectrometry of various lithium cationized acetogenins was used to analyze the collision-induced dissociations of these complexes. Characteristic fingerprints of the different structural types investigated have been obtained, allowing their unambiguous structural identification.

Mass spectrometry using a fast-atom beam as ionizing agent (FAB-MS) has witnessed continuing success since a dozen years because of its ability to analyze polar and involatile organic substances introduced in a liquid matrix.[1] Apart from protonation which remains the major ionizing process, a special feature of the FAB desorption is the generation of ions by attachment of alkali-metal cations to various organic substrates (glycosides, oligosaccharides, peptides, etc.). In practice, such "cationisation" of organic substances by alkali-metal salts may be effectively used for determining or confirming their molecular weight. Collisional activation of these cationized species may also lead to structurally informative results,[3-30] thus rendering tandem mass spectrometry (MS/MS) and constant-*B/E* linked scanning[2] to be applied successfully for structural investigations of peptides,[13-24] long-chain fatty alcohols and acids,[25-27] bile acids[28] and carbohydrates.[29-30] In particular, identification and recognition of a variety of modifications in fatty acids have been achieved by collision-induced dissociation (CID) of both $[M+Li]^+$ and $[M+2Li-H]^+$ ions prepared by addition of LiOH or LiI to the sample dissolved in the FAB matrix. The observed "remote-charge fragmentations" have proved to be very useful for the structural analysis of diverse compounds possessing long alkyl chains.[25-27]

An antitumoral acetogenin, uvaricin, was isolated from *Uvaria accuminata* (Annonaceae) in1982 and was assigned a new structural type.[31] Since then, more than 80 acetogenins have been isolated from the same plant family, their diverse biological activities giving rise to an increasing number of publications.[32,34] 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 THF rings. The major difficulty associated with the structural elucidation of a new natural acetogenin lies in locating the different oxygenated functions present in the alkyl chain. Analysis of silylated or acetylated derivatives by using high resolution electron ionization mass spectrometry is often necessary for solving this problem.[35]



Uvaricin

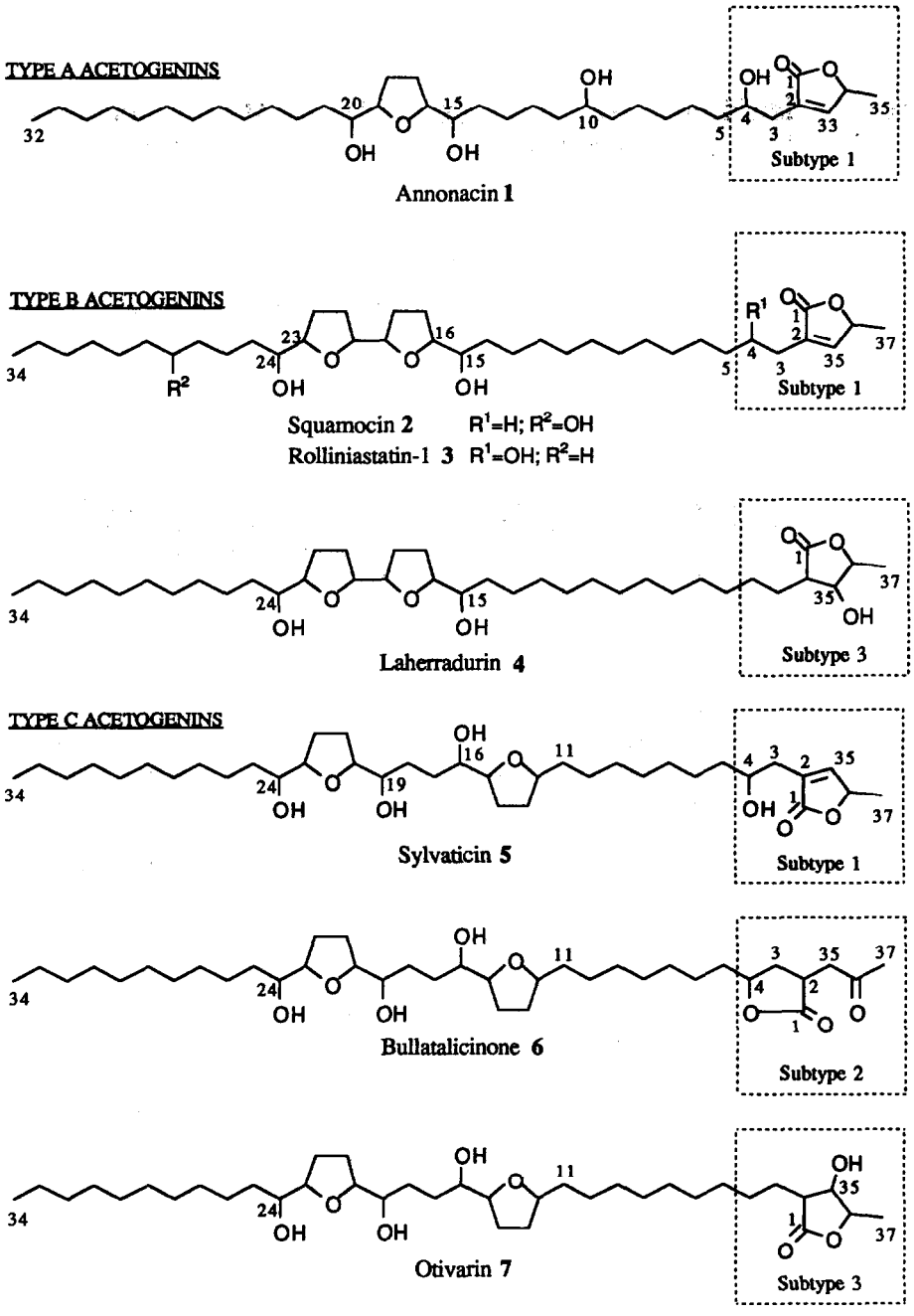

TYPE A ACETOGENINS
Subtype 1
Annonacin 1
TYPE B ACETOGENINS
Subtype 1
Squamocin 2 R1=H; R2=OH
Rolliniastatin-1 3 R1=OH; R2=H
Subtype 3
Laherradurin 4
TYPE C ACETOGENINS
Subtype 1
Sylvaticin 5
Subtype 2
Bullatalicinone 6
Subtype 3
Otivarin 7

Figure 1

Recently, we have described a different strategy involving lithium cationization of acetogenins followed by *B/E* linked scan analysis of $[M+Li]^+$ ions generated by FAB.[36] Thus, the location of the bistetrahydrofuran nucleus in the alkyl chain of rolliniastatin-2 (a stereoisomer of **3** at position 16) could be directly established from the observed fragmentation pattern. Further results, obtained by using the same method on acetogenin-related compounds possessing epoxides in place of tetrahydrofuran rings,[37,38] have prompted us to investigate all the structural types representing this chemical family. In this paper we intend to present the general fragmentation patterns obtained by using *B/E* linked scanning and collisional activation of FAB-desorbed $[M+Li]^+$ ions in order to achieve direct and unambiguous structural identification of annonaceous acetogenins.

RESULTS AND DISCUSSION

The acetogenins investigated belong to the three main structural groups (A, B and C) defined by McLaughlin[33] and Cavé[34] (Figure 1). According to a recent classification of this chemical family,[34] annonacin **1** which possesses only one tetrahydrofuran ring is a type A acetogenin. The type B is characterized by two adjacent THF rings as in squamocin **2**, rolliniastatin-1 **3** and laherradurin **4**. The third group (type C) includes the acetogenins containing two tetrahydrofuran rings separated by a four carbon chain, such as sylvaticin **5**, bullatalicinone **6** and otivarin **7**. Within each of these three main groups may be distinguished three subtypes (1, 2 and 3) corresponding to various lactone structures. The lactone type 1 is characterized by an α,β-unsaturated methyl-substituted γ-lactone (compounds **1-3** and **5**). Subtype 2, also called "iso" form, corresponds to a saturated γ-lactone substituted by an acetonyl group (compound **6**). Finally, in the acetogenins belonging to the subtype 3, the γ-lactone ring is saturated and β-substituted by a hydroxyl (laherradurin **4** and otivarin **7**).

The FAB mass spectra of the compounds **1-7** display only a few ion peaks of low relative intensities as compared to those arising from the matrix. The protonated molecules are often accompanied by sodium adducts $[M+Na]^+$ and by fragment ions produced by successive losses of water molecules from the $[M+H]^+$ species. After addition of LiCl to the matrix, the mass spectra are considerably simplified, showing in the molecular ion region only one peak corresponding to the lithiated molecules, as mentioned previously.[36-38]

Since the spectra lack fragment ions diagnostic for the location of oxygenated groups in the alkyl chain, collision-induced dissociations of $[M+Li]^+$ ions were studied as first field-free region reactions using linked scan analysis at constant *B/E*.[39]

The product-ion spectra of the lithiated molecules generated by FAB show a series of weak but reproducible fragment-ion peaks retaining the lithium cation. Two zones can be clearly recognized in these spectra. In the upper mass range, collision-induced dissociations involving the terminal lactone part of the molecules lead to three different ion series according to the lactone type. In the lower mass range, characteristic fingerprints of the tetrahydrofuran nucleus are obtained, allowing an easy distinction of acetogenins belonging to types A, B and C. We wish to describe successively the fragmentations characterizing the different lactone structures and then those arising from the tetrahydrofuran site of the acetogenins.

Fragmentations of the lactone part

The more widespread lactone structure in the annonaceous acetogenin family is that of subtype 1 as found in **1**, **2**, **3**, and **5**. Squamocin **2** differs from the rest by the absence of a hydroxyl group at position 4. Dissociation of the $2Li^+$ ion (*m/z* 629) at the lactone end leads to fragment ions generated by loss of low-mass neutrals (Fig. 2a) at *m/z* 611 ($[2Li - H_2O]^+$), *m/z* 601 ($[2Li - CO]^+$), *m/z* 585 ($[2Li - CO_2]^+$) and *m/z* 543 ($[2Li - CO_2 - CH_2{=}CH\text{-}CH_3]^+$). Another fragment ion at *m/z* 517, involving a loss of 112 amu, is formed by a β-cleavage of the lactone ring along with a hydrogen transfer to the lactone. The abundance of this fragment is considerably enhanced when the molecule is 4-hydroxy-substituted as in the case of rolliniastatin-1 **3** or sylvaticin **5** (Fig. 2b and 2d). The existence of hydrogen bonding between the lactone carbonyl and the hydroxyl has been evidenced, in solution, by ^{1}H-NMR.[32,33] At first sight, one may assume that lithium

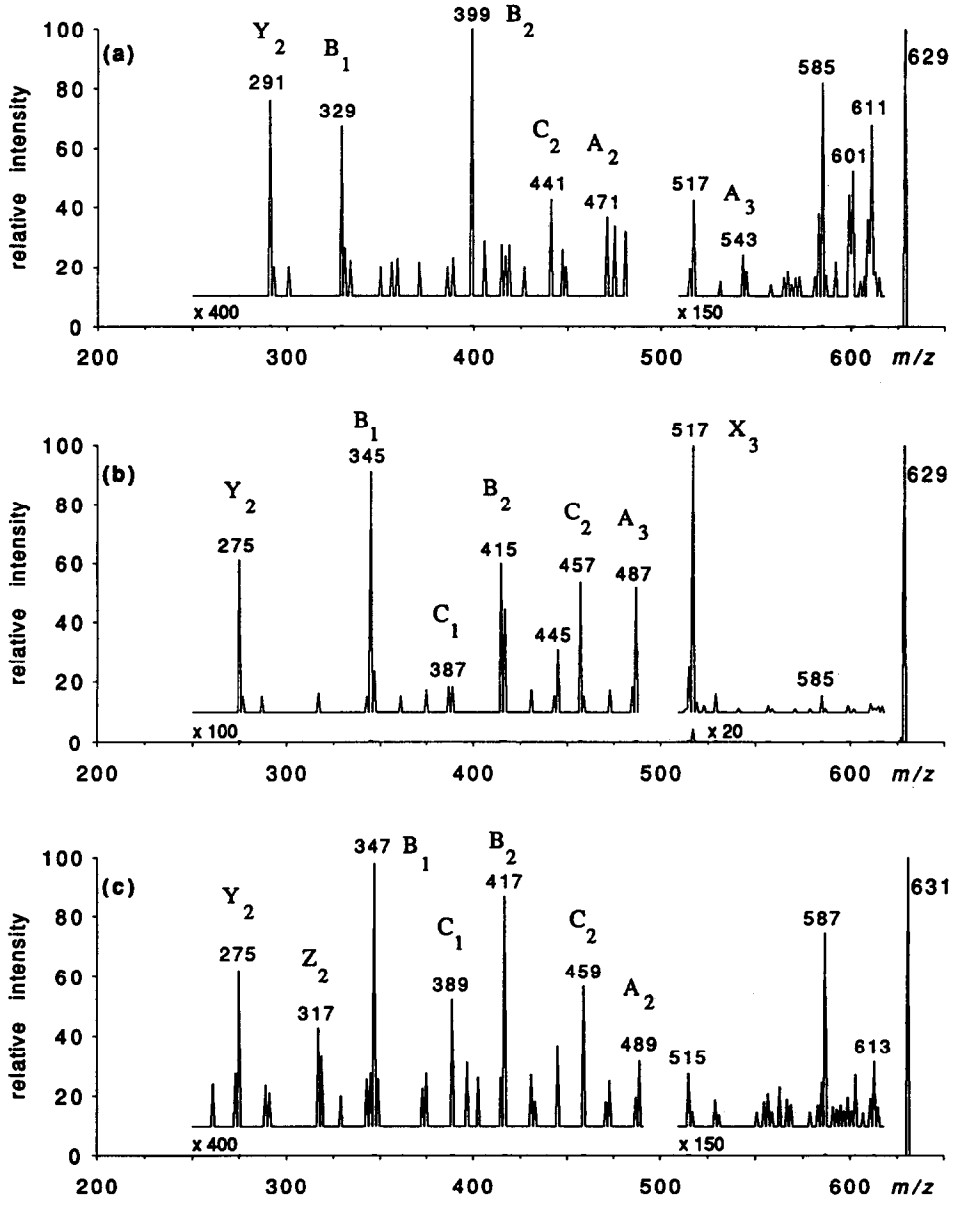


Figure 2. Constant *B/E* linked scan spectra of $[M+Li]^+$ ions generated by FAB from adjacent bis-tetrahydrofuran acetogenins: a) squamocin 2, b) rolliniastatin-1 3, c) laherradurin 4.

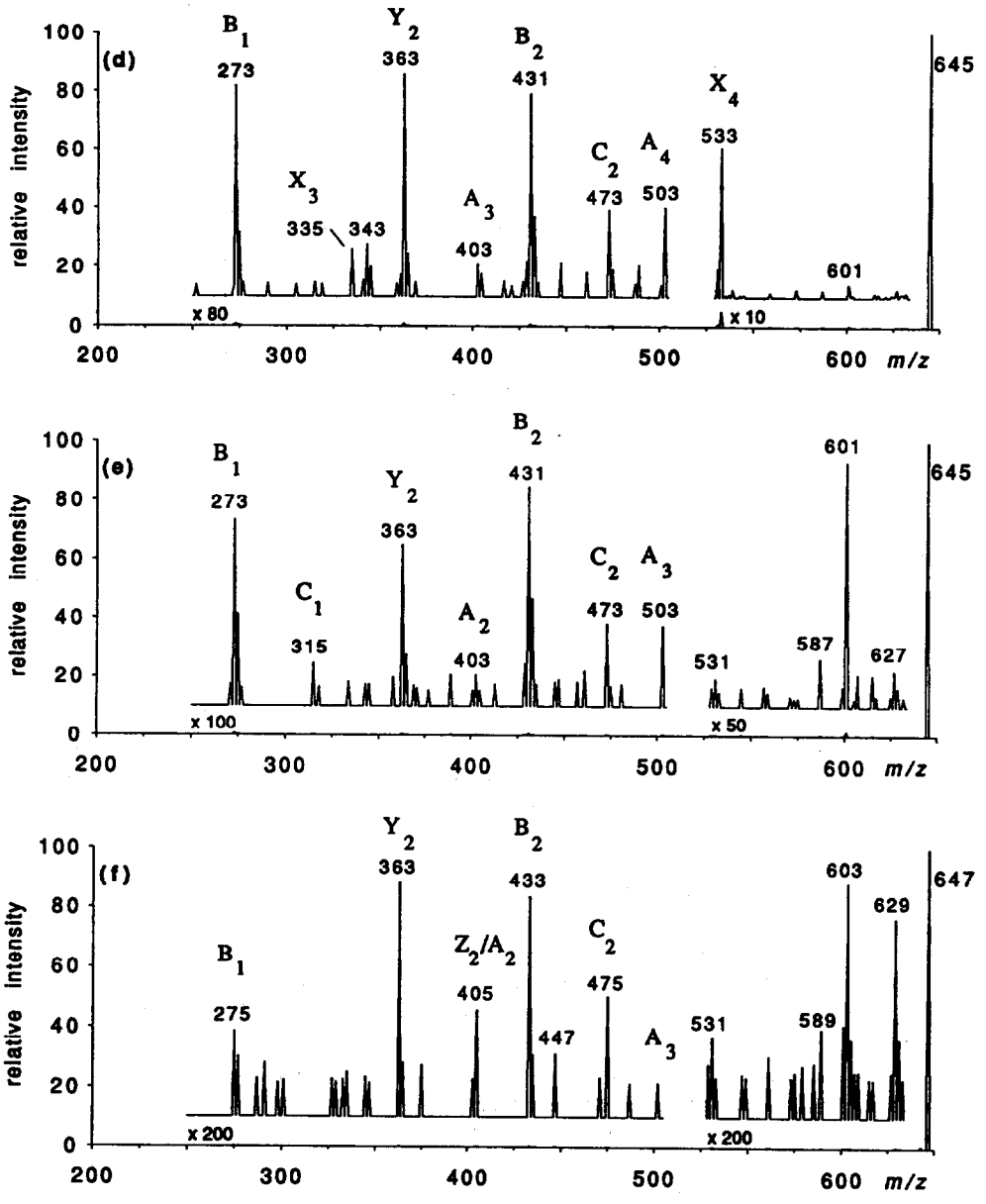
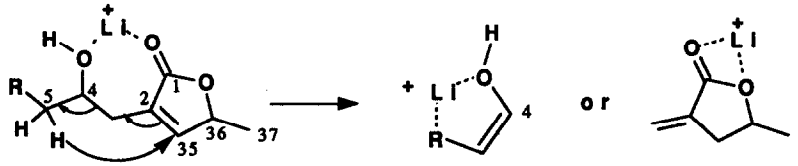


Figure 2 (continuation). Constant *B/E* linked scan spectra of $[M+Li]^+$ ions generated by FAB from non-adjacent bistetrahydrofuran acetogenins: d) sylvaticin 5, e) bullatalicinone 6, f) otivarin 7.

Table 1. Relative Intensities of the Principal Diagnostic Ions Produced by Fragmentations at the Lactone End of $[M+Li]^+$ Ions from Acetogenins (the Major Ion Peak is Assigned Arbitrarily the Value 100).

Ions	Lactone type 1		Lactone type 2	Lactone type 3
	4-CH_2	4-CHOH		
$[M + Li - H_2O]^+$	75-100	6-12	15-20	40-100
$[M + Li - CO]^+$	45-70	< 10	< 10	5-15
$[M + Li - CO_2]^+$	85-100	8-15	100	90-100
$[M + Li - 58]^+$	—	—	7-20	—
$[M + Li - 112]^+$	20-50	100	—	—
$[M + Li - 114]^+$	—	—	8-14	—
$[M + Li - 116]^+$	—	—	—	5-15

Scheme 1.



$[M+Li]^+$ $[M+Li-112]^+$ *m/z* 119

attachment to the lactone oxygens should favour the hydroxyl proton transfer to the carbonyl. Such a possibility was however excluded from the results obtained by the collision-induced dissociation of the labelled $[3\text{-}d_3 + Li]^+$ ion at *m/z* 632 which gave a peak at *m/z* 520 by the loss of 112 amu thereby indicating that no deuterium transfer was involved.

Consequently, the hydrogen transfer occurs possibly from the C-5 methylene to the C-35 position (cf. **3**) *via* a six-membered transition state (Scheme 1). Retention of lithium by the fragment containing the oxygen at C-4 suggests that other oxygen atoms of the molecule are implied in the coordination bonding.[37] Nevertheless, the presence of an ion corresponding to the lithiated lactone part cannot be excluded, the expected *m/z* value of 119 being out of the calibration range of our instrument in the *B/E* linked scanning mode.[40]

The fragmentation pathways of subtype 2 lactones as in compound **6** ($[M+Li]^+$ at *m/z* 645) are very different from those of γ-lactone of the subtype 1. The peak due to loss of 112 u is not observed in the spectrum, while the ion *m/z* 601 corresponding to the loss of 44 u is particularly enhanced (Figure 2e). The loss of the acetonyl substituent (58 u) produces a fragment ion at *m/z* 587 of low relative intensity. The fragmentation with loss of the entire lactone ring (114 mass units) leads to a weak ion peak at *m/z* 531.

The subtype 3 lactones exemplified by **7** display a spectral profile quite similar to that of the subtype 2 lactones. Although often appearing with a higher relative intensity in the considered mass range, the $[M+Li - CO_2]^+$ ion is much lower, relatively to the precursor ion $[M+Li]^+$, than in the case of subtype 2 lactones. By contrast, significant loss of a molecule of water (ion at *m/z* 629 in Figure 2f) is observed. Finally, the cleavage α to the lactone ring allows an easy characterization of the subtype 3 lactones showing a loss of 116 mass units instead of 114 u observed for the subtype 2 lactones.

Thus, examination of the upper mass range of the constant-*B/E* linked scan spectra of $[M+Li]^+$ ions allows a rapid and unambiguous recognition of a lactone structural type (Table 1).

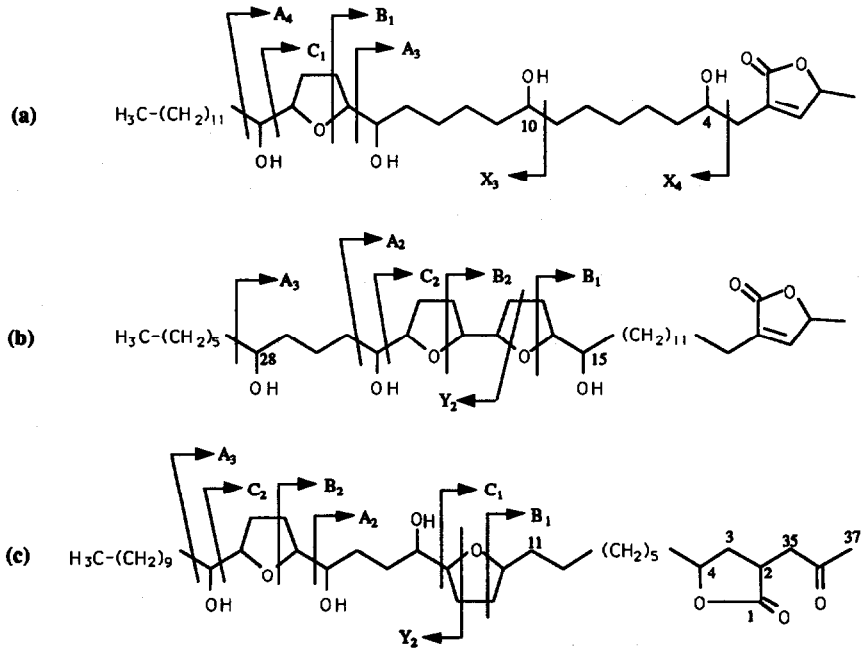
Fragmentations of the tetrahydrofuran moiety

The fragmentations of the tetrahydrofuran systems of the lithiated acetogenins **1-7**, as observed in the constant-*B/E* linked scan spectra, may be generalized from the preliminary results obtained with rolliniastatin-2, a stereoisomer of rolliniastatin-1 **3**.[36] In all these cases, the product ions retain lithium and are easily attributed to cleavages across the tetrahydrofuran rings as well as of their adjacent C-C bonds. The general fragmentation patterns of lithiated acetogenins are illustrated in Scheme 2. For clarity, we propose a nomenclature of fragment ions convenient for all the structural types of the tetrahydrofuran acetogenins as yet isolated. The ions possessing the lactone part are designated by the letters A, B and C, the ions containing the methyl-terminal chain by X, Y or Z (Scheme 2).

A and X ions are formed by a cleavage alpha to a hydroxyl group and contain the oxygen atom of this function. The ions B and Y are generated by a fragmentation across a tetrahydrofuran ring and retain a part of it. Ions C and Z are produced by cleavages alpha to a THF ring. Numeral subscripts used with these letters distinguish the different ions belonging to the same series. For example, the ion B_1 corresponds to the fragment produced by cleavage across the first tetrahydrofuran with regard to the lactone. The B_2 ion comes from the similar fragmentation of the second THF.

Although all the peaks expected from a given structural type are not always present in the spectrum, the observed fragment ion peaks can be easily interpreted according to the general fragmentation scheme discussed above. In all cases, the fragment ions containing the lactone ring (A, B and C) dominate the spectra. This is explained by the contribution of the lactone to the lithium coordination, thus leading to stable species. Such an effect has been previously noted in the case of acetogenins possessing epoxides in place of THF rings.[37] Whereas the A, B or C series of ions may be considered enough for localization of the oxygenated functions in the alkyl chain, the presence of ions belonging to the X, Y, Z series should confirm the proposed structure.

In the case of acetogenins of the group A, such as annonacin **1**, the *B/E* spectrum of the lithiated



Scheme 2. General fragmentation patterns of lithiated acetogenins under collisional activation as exemplified by **a)** annonacin **1**, **b)** squamocin **2**, **c)** bullatalicinone **6**.

molecules at *m/z* 603 is remarkably simple (Figure 3). The A_4, C_1, B_1, and A_3 fragment ions allow the location of the only THF ring of the molecule. The position at C-10 of a hydroxyl is confirmed by the corresponding X_3 ion at *m/z* 391. The substitution at C-4 by another hydroxyl is deduced from the ion peak at *m/z* 491 (loss of 112 u from $[M+Li]^+$).

The structure of group B acetogenins is also relatively easy to establish. A series of peaks due to A, C, and B ions is a first indication of the position of the bis-THF moiety. For the lithiated squamocin $2Li^+$, the ions A_2, C_2, B_2 appear respectively at *m/z* 471, 441 and 399 (Figure 2a). The presence of a second THF ring, adjacent to the previous one, is deduced from the peak at *m/z* 329 which corresponds to the ion B_1 (70 u less than the ion B_2). In this spectrum, the Y_2 ion peak is observed at *m/z* 291. A hydroxyl substitution at C-28 is confirmed by an ion peak at *m/z* 543 (ion A_3). The comparison of the *B/E* linked scan spectrum of lithiated rolliniastatin-1 $3Li^+$ relatively to that of the $2Li^+$ ion shows clearly the different location of one hydroxyl between the two compounds (Figure 2b). All the lactone-containing fragment ions are shifted to higher *m/z* values by 16 u, while the ion Y_2 is displaced from *m/z* 291 to *m/z* 275. The change of the hydroxyl position from C-28 to C-4 is confirmed by the formation of an abundant *m/z* 517 ion. In the case of laherradurin **4**, reduction of the olefinic bond of the lactone ring is demonstrated by an increase in mass (2 u) of the lactone-containing fragment ions when compared with those arising from rolliniastatin-1 **3** (Figure 2c). By contrast, the Y_2 ion peak remains unchanged at *m/z* 275.

Although the fragmentation pathways of the group C acetogenins appear to be very similar to those of the group B acetogenins, several differences in their *B/E* spectra are observed. At first sight, the CID spectrum of lithiated sylvaticin, $5Li^+$, displays a series of peaks at *m/z* 503, 473 and 431 which could be assigned respectively to the A_3, C_2 and B_2 ions arising from a tetrahydroxylated bis-THF acetogenin belonging to the group B as well as to the group C. However, the presence of two adjacent THF rings should be confirmed by a B_1 ion peak at *m/z* 361 (431 – 70) and a Y_2 ion peak at *m/z* 275. These two fragments do not appear in the spectrum. On the other hand, two intense peaks at *m/z* 273 and 363 may be attributed respectively to the ions B_2 and Y_2 of an acetogenin possessing the structural type C. The *B/E* linked scan spectrum of lithiated otivarin $7Li^+$ (*m/z* 647) confirmed this assumption. The replacement of a lactone-type 1 by a lactone-type 3 leads to an increase in mass by 2 u of all the ion peaks except that of the ion Y_2 (*m/z* 363). Thus, the collision-induced dissociation of the lithium-complexes of acetogenins provides adequate informations for their structure elucidation. However, it is noteworthy that, in the case of unknown compounds possessing a lactone of the type 3, some structural ambiguities persist. For example, the peak at *m/z* 363 corresponding to the ion Y_2 of otivarin **7** could also be interpreted as an ion B_1 [433 (ion B_2) – 70] of a tetrahydroxylated acetogenin possessing two adjacent THF rings. In such a case, the use of complementary methods, such as high resolution electron ionization mass spectrometry, is required.

With regard to the mechanisms implied in the fragmentation of the tetrahydrofuran moiety of the lithiated acetogenins, two preliminary observations must be pointed out. At first, as mentioned previously, the two oxygenated parts of the molecules, namely the lactone and the THF nucleus are involved in the lithium coordination, thus accounting for the abundance of the lactone-containing fragment ions. In addition, the

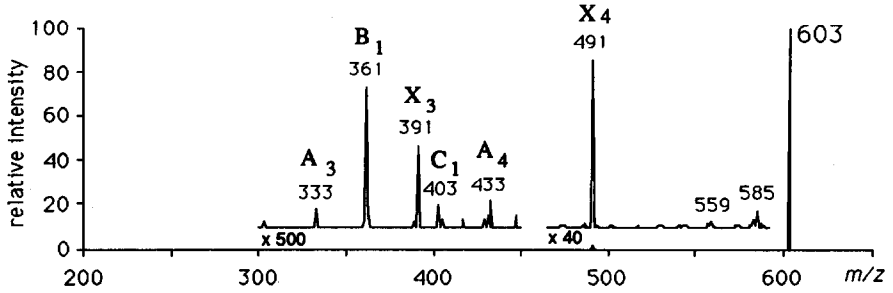
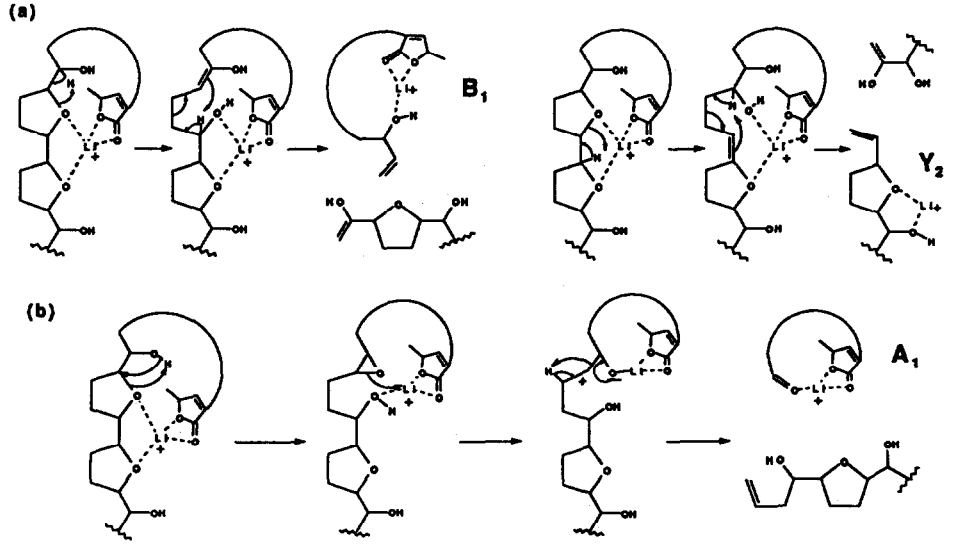


Figure 3. Constant *B/E* spectrum of lithiated annonacin $1Li^+$.

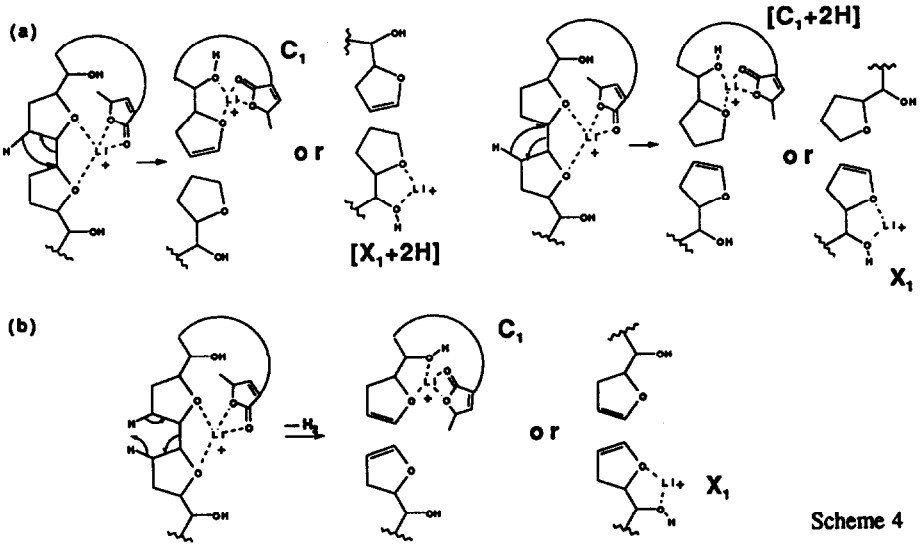
B/E linked scanning experiments carried out without using any collision gas lead to fragment ions identical to those observed under collision-induced dissociation conditions, although with much lower intensity (especially B, Y and A series). This suggests charge-induced fragmentations associated with relatively low activation energies. However, under the CID conditions, sequential fragmentations of the alkyl side chain attributable to remote-charge type fragmentations have been observed in some cases. Thus the *B/E* linked scan spectra of lithiated acetogenins recorded using a collision gas may display simultaneously both high- and low energy fragmentation pathways.

The mechanisms involved in the fragmentation of the acetogenin-lithium complexes agree well with those described by Lattimer in the case of lithium complexes of poly(ethylene glycol), PEG, and poly(propylene glycol), PPG.[8] Schemes 3 and 4 describe the fragmentation mechanisms of a model acetogenin belonging to the structural group B. Reactions leading to the ion series B and Y are shown in Scheme 3a. The first step involves 1,3-hydrogen transfers to the THF oxygen atoms which are very likely engaged in the lithium coordination. Further, internal hydrogen rearrangements in the intermediate open forms may lead to the formation of the B and Y ions. The ion Y_1 is always obtained with a very low abundance. This is likely due to an insufficient number of electron-donor atoms since the ion Y_1 contains only one oxygen while the ions Y_2, B_1 and B_2 possess respectively 2, 3 and 4 oxygen atoms. The A series ions may originate from the lithiated molecules by a mechanism similar to that proposed by Lattimer for the PPG lithium complex on the basis of deuterium labelling.[8] Transfer of hydrogen from the hydroxyl groups at C-15 or C-24 to the adjacent lithiated tetrahydrofuran oxygen atoms leads to epoxide intermediates by opening of the THF rings (Scheme 3b). Further fragmentation of the epoxides in the same way as previously described for lithiated epoxy-acetogenins generates the A ion series.[37]

The fragmentations generating the ions C and X probably involve 1,3-hydrogen transfers between two methylene groups close to the charge site (Scheme 4a).[41] Two similar rearrangements allowing fission of the C-19, C-20 bond joining the two THF units may occur. Thus, the C and [C+2H] ions should be of similar abundance in the *B/E* linked scan spectra. This is generally observed when the spectra are recorded in the absence of collision gas (metastable mode). The reinforcement of the ion C by collisional activation and the



Scheme 3



Scheme 4

formation of the X_1 ion which is never observed under metastable conditions could be explained by an additional dissociation process involving a 1,4-hydrogen elimination of the remote-charge fragmentation type (Scheme 4b). The higher internal energy level required by this fragmentation reaction agrees well with the spectral data and the experimental conditions.

CONCLUSION

Collision-induced dissociation of $[M+Li]^+$ ions generated by FAB from annonaceous tetrahydrofuran acetogenins provides structurally informative spectra which allow an easy distinction of the lactone types and a facile localization of the tetrahydrofuran rings in the hydrocarbon chain. By comparison, the CID spectra of $[M+Na]^+$ precursor ions, generated under the same ionization conditions, display only a restricted number of fragment ion peaks corresponding to successive losses of water but with lower intensities than in the case of the protonated molecules $[M+H]^+$. Energetic considerations may account for these results by the tight bonding of lithium to oxygen as compared to larger alkali cations. This could explain that the skeleton fragmentations, energetically unfavourable to the sodium complexes, become the lowest energy pathways for the lithiated molecules. On the other hand, the absence of LiOH neutral loss and the very weak intensity of the $[M+Li-H_2O]^+$ ion peak are likely due to the preferential attachment of the lithium cation to the ethereal oxygens of the THF rings. Thus, the participation of the hydroxy groups in the fragmentation processes takes place by O—H bond cleavage along with hydrogen transfer to the lithiated oxygen atom rather by C—OH bond rupture which should imply an intermediate charge location on the hydroxyl oxygen. This assumption is reinforced by the examination of the *B/E* linked scan spectra of lithium-complexes of acetogenin acetyl derivatives. In this case, successive losses of acetic acid molecules are observed without any other skeleton fragmentation.

Lastly, the affinity of acetogenins toward lithium cation remains to be determined, in so far as the complex stability may play a very important role, the skeleton fragmentations being more probable as the cation release is more difficult. For example, the highly stable potassium/crown-ether complexes and their acyclic analogs undergo C-C bond cleavages at high collision energies similar to that of lithiated acetogenin, while the protonated molecules dissociate predominantly by C-O bond cleavages.[41]

An interesting outcome of the method used in the present study is the virtual absence of consecutive decomposition processes, such as water loss from hydroxylated fragments, usually observed either under EI or positive and negative desorption/chemical ionization and low-energy multiple collision conditions.

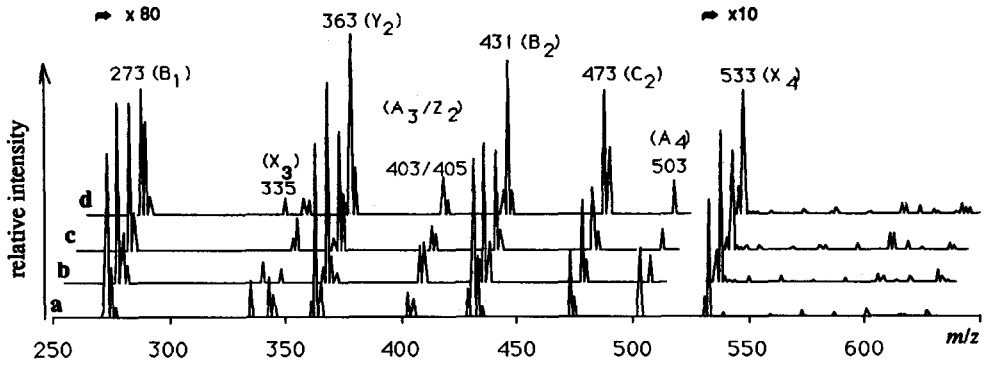


Figure 4. Mass spectrometric profiles of lithium complexes of **a)** sylvaticin **5**, **b)** uleicin A, **c)** uleicin B and **d)** uleicin C under collision-induced dissociation conditions. The precursor ion peaks are not shown.

This has led to an erroneous structural attribution to uleicins A-E which were presented previously as adjacent-bistetrahydrofuran compounds.[42] In fact, the planar structure of uleicins A, B and C has proven to be identical to that of sylvaticin **5** by collision-induced dissociation of $[M + Li]^+$ ions followed by *B/E* linked scanning (Figure 4). Uleicin D possesses the same structure as uleicins A-C but is contaminated with an isomeric acetogenin not yet identified. Uleicin E, partially transformed into its "iso" form (lactone-type 2) appears also as a mixture of at least two products.

In conclusion, the high energy collision-induced dissociation of alkali-metal ion adducts of oxygen-containing natural products can play a very important role for their structure elucidation, especially when the conventional ionization techniques lead to ambiguous results or necessitate time- and sample-consuming derivatization methods.

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39. The acetogenin samples were dissolved in ethanol at a concentration of 10 mg/ml. Typically, 4 µl of matrix (*meta*-nitrobenzyl alcohol) were transferred to the FAB probe copper tip and mixed with 1 µl of the sample solution. Lithiation experiments were carried out by adding 1 µl of a saturated aqueous solution of LiCl directly on the probe tip. Mass spectra were obtained with a Kratos MS80RF mass spectrometer of Nier-Johnson geometry with an accelerating voltage of 4 kV 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. The resolution of the mass spectrometer was 1000. *B/E* linked scan spectra were acquired under the control of the Kratos DS90 data system. The lenses were optimized for obtaining the most intense signal corresponding to the lithiated molecules. For CID (Collision Induced Decomposition) experiments, argon was introduced into the collision chamber located in the first field-free region, between the ion source and the electrostatic sector analyser. Collision gas pressure was chosen for an attenuation of the parent ion beam by about 30%.
40. The mass range available for the *B/E* linked scanning analysis is limited by the lower mass of the mass spectrometer calibration range acquired in the conventional mode. Indeed, if m_{min} is the low mass end of the calibrated mass scale and m_1 the *m/z* value of the chosen precursor ion, then the lower mass m_2 detected by *B/E* linked scanning corresponds to $(m_{min})^2/m_1$. The mass spectrometer was calibrated with Fomblin®, m_{min} being equal to 30.
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