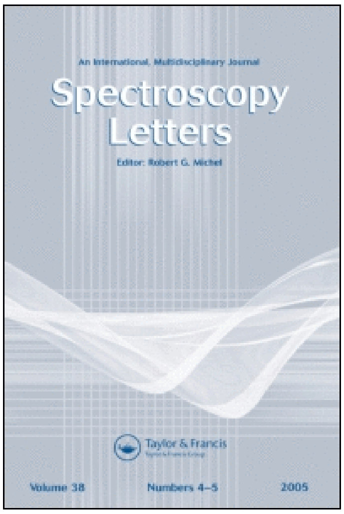

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Fragmentation Study of Globularin Through Positive and Negative ESI/MS, CID/MS, and Tandem MS/MS

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Fragmentation Study of Globularin Through Positive and Negative ESI/MS, CID/MS, and Tandem MS/MS

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Abstract: The structure of globularin was studied by a mass spectrometric methodology based on the combined use of positive and negative electrospray ionization, collision-induced dissociation (CID), and tandem mass spectrometry. The mass spectrometry investigation was achieved through in-source fragmentation of the deprotonated $[M-H]^-$, protonated $[M+H]^+$, lithiated $[M+Li]^+$, sodiated $[M+Na]^+$, and potassium-cationized $[M+K]^+$ ions. This allowed collision-induced dissociation spectra of the ionized molecular ions to be obtained to give valuable structural information regarding the nature of both the glycoside and the aglycone moieties and the effect of metal cationization on the CID spectra. Glycosidic fission and ring cleavages of both aglycone and sugar moieties were the major fragmentation pathways observed during collision-induced dissociation, where the losses of small molecules, the cinnamoyl and the cinnamate parts were also observed. Alkali metal cationization offers additional fragmentation pathways involving cross rings

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cleavage under CID conditions. Unlike the dissociation of protonated molecular ions, that of metal-cationized molecules also provides sugar fragments where the C_0^+ fragment corresponding with the glucose ion was obtained as a major daughter peak for all the studied compounds. Even with low abundance, fragment ions coordinated to K^+ were also observed from $[M + K]^+$.

Keywords: Cationization, characterization, collision-induced dissociation, ESI-MS, fragmentation, *Globularia alypum*, globularin, iridoids, MS/MS

INTRODUCTION

Iridoids are a group of natural compounds with cyclopentane pyran rings. They represent a large and still expanding group of monoterpenoid constituents with a glucose moiety attached to the C-1 in the pyrane ring that are found in a number of plant families. Iridoids constitute the more numerous family of cyclopentanoid monoterpene derivatives of which several hundred have been isolated from different plant sources.[1–5] They were reported to have biogenetic and chemotaxonomic importance as they provide a structural link between terpenes and alkaloids, and their presence has been used in some instances to support botanical classifications.[6] Iridoids are usually subdivided into four groups: iridoid glycosides, nonglycosidic iridoids, secoiridoids, and bisiridoids,[7,8] the first group being the most numerous. Iridoids are known to exhibit a number of biological activities such as antiviral,[9] antitumor,[10] antibacterial,[11] diuretic,[12] purgative,[13] choleretic,[14] antioxidant,[15] plant growth inhibitor,[16] and were also reported to be useful in the management of liver disorders.[17,18] With the increasing interest in such compounds, a multitude of analytical methods have been reported in a number of papers in order to determine their structures and their concentrations in complex matrices. Iridoids generally occur in nature as complex mixtures, and their characterization depends mainly on their spectral behavior. Indeed, plant extracts represent complex multicomponent mixtures, usually containing many low-concentration constituents. Thus, sufficient sensitive, selective, and convenient analytical methods for characterization of iridoid glycosides are required.

Structural elucidation of iridoids is generally performed by a combination of spectroscopic methods including ultraviolet (UV), nuclear magnetic resonance (NMR), and mass spectrometry (MS). Among these techniques, NMR spectroscopy could be considered as the most important tool for structural elucidation of natural products when these compounds are isolated in the milligram scale. Correlated with recent technological advances in development of high-field magnets and cryoprobe technology, the improved resolution and sensitivity of NMR techniques has increased the possibilities of accurate assignments of proton and carbon atoms and has made NMR an extremely powerful analytical technique for the determination of natural products. In addition to NMR, mass spectroscopy could be considered as one of the important physicochemical methods due to its sensitivity, rapidity, and low levels of sample consumption. Until the advent of soft ionization techniques, the use of mass spectrometry has been limited owing to the unstable nature of iridoid glucosides.[19] Recently, there have been several reports on the application of mass spectrometry for the analysis of iridoids using techniques such as chemical ionization (CI), direct chemical ionization (DCI), fast atom bombardment (FAB), and liquid chromatography/thermospray MS or electrospray ionization (ESI).[4,5,20,21] Some reports using ESI-MS with alkali adducts to determine the structure of iridoids have been also reported.[22,23]

Because the number of iridoid compounds is steadily increasing, and in order to further increase the applicability of mass spectroscopy techniques in the analysis of plant constituents, it appeared important to investigate the structural elucidation of iridoid glycosides in order to correlate the structure with their spectral behavior, thus allowing a rapid identification and analysis of such compounds. Being a part of a large group of naturally occurring monoterpene compounds widely distributed in nature, iridoid glycosides occur in complex mixtures and are difficult to separate. The investigation of their spectral behavior will probably allow their rapid recognition and structural elucidation.

In connection with our investigations on natural products isolated from Moroccan plants,[24,25] we had occasion to explore the mass spectral behavior of flavonoids.[26,27] In the current study, LC/MS (liquid chromotography) and MS/MS techniques were applied to the determination of molecular mass and structural characterization of globularin, which was chosen as a representative iridoid glucoside. This compound was isolated from a hydromethanol extract of the aerial parts of *Globularia alypum* from Morocco, according to previously reported results.[24] Whereas the structural elucidation through NMR analysis has been previously reported,[28] its MS fragmentation study has not been investigated. In this work, MS analysis of globularin was explored through ESI-MS in the negative and positive ion modes to obtain intact ionized molecules. Then, tandem MS/MS spectra were obtained by low-energy collision-induced dissociation (CID) of the deprotonated, protonated, and cationized ions and were interpreted to propose plausible fragmentation pathways for the studied compound.

MATERIALS AND METHODS

Materials

Globularin was isolated from an aqueous methanol extract of *G. alypum* aerial parts. Fresh aerial parts were air-dried in shade at room temperature, and the dried aerial parts were powdered. One hundred grams of the obtained powder were macerated during 48 hr at room temperature with 500 mL of a 3:2 mixture of distilled water–methanol. The crude preparation was filtered and concentrated under reduced pressure to provide a crude extract that was stored at −20°C until time of use. The resulting aqueous phase was extracted with hexane, and the subsequent aqueous phase was subjected to a solid phase extraction (SPE) column. Elution was performed successively by H_2O, MeOH 10%, MeOH 40%, MeOH 50%, and MeOH 100%. The fractions were concentrated under reduced pressure, lyophilized, tested for their scavenging activity, and analyzed through analytical HPLC. The MeOH 50% fraction, which was shown to be rich in natural antioxidant compounds, was explored using semipreparative HPLC. After several successive injections, samples corresponding with the same chromatographic peaks were verified by analytical HPLC, concentrated under reduced pressure, and lyophilized. This operation gave the studied purified compound.

Mass Spectrometry

LC/MS analyses were performed with a chromatographic system (Alliance) consisting of a Waters 2695 separations module equipped with an autosampler and a Waters 2487 dual lambda absorbance detector (Waters, Milford, MA, USA). The column was a 150 × 2.1 mm Interchrom UP5ODB#15E (Uptisphere 5 μm ODB) with a 10 × 2.1 mm precolumn from Interchim (Montluçon, France). Chromatography was carried out in isocratic mode with a 60/40 mixture of acetonitrile (RS-Plus quality for HPLC from Carlo Erba, Milano, Italy) and water with 0.2% acetic acid. The flow rate was 0.2 mL/min, the analyses were performed with the column, and the samples were kept at ambient temperature, and 5.0–10 μL was injected for each analysis. The effluent from the UV detector was introduced into the mass spectrometer without any splitting of the flow. The HPLC system was coupled directly to a Quattro LC MS/MS triple quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with a pneumatically assisted electrospray ionization source (ESI). Data acquisition and processing were performed using a MassLynx NT 3.5 data system (Micromass, Manchester, UK). The triple quadrupole ESI source potentials were capillary 3.25 kV (positive mode) or 3.0 kV (negative mode) and extractor 2 V. The sampling cone voltage was varied usually from 20 V to 50 V for ESI mass spectra. The specific value of the cone voltage and ion energies during CID experiment were set in order to optimize the intensity of the precursor ion. The quadrupole mass filters were set with LM and HM resolution of 15.0 (arbitrary units), which is equivalent to a 1.0 Da-mass window for transmission of both precursor and products ions. The source block and desolvation temperatures were set at 120°C and 400°C, respectively. Argon was used as collision gas at 3.5×10^{-3} torr. Nitrogen was used as drying gas and nebulizing gas at flow rates of approximately 50 and 450 L/hr.

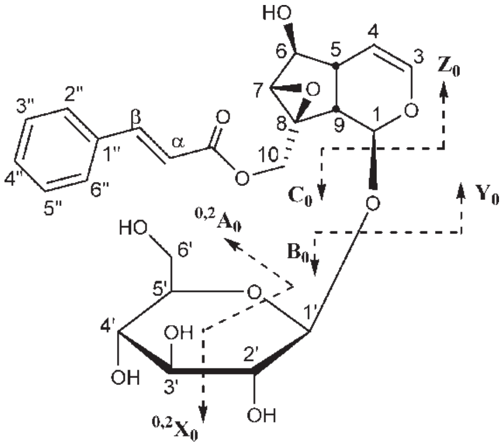
RESULTS AND DISCUSSION

Nomenclature

The classic nomenclature proposed by Domon and Castello [29] for glycoconjugates was adopted to designate the fragmentations: $^{k,l}X_j$, Y_j, Z_j represents the ions still containing the aglycone, where *j* is the number of the interglycosidic bonds broken (counted from the aglycon) and *k* and *l* denote the cleavage within the carbohydrate rings (Scheme 1).

LC/ESI/MS

After isolation, the studied sample was analyzed by analytical HPLC with mass spectrometric detection. Both positive and negative ion modes were used because of their complementary conclusions. When the analysis was conducted in the positive ion mode, protonated molecule ion and protonated aglycone in addition to product ions allowing the partial characterization of the studied iridoid glucoside were also observed. A typical ESI-MS spectrum obtained in the positive ion mode is shown in Fig. 1. Protonated ion in addition to cationized ion signals were observed at m/z 493 $[M+H]^+$, 510 $[M+NH_4]^+$, 515 $[M+Na]^+$ and 531 $[M+K]^+$. The ions $[2M+H]^+$, $[2M+NH_4]^+$, $[2M+Na]^+$, and $[2M+K]^+$ were also observed at m/z 985, 1002, 1007, and 1023, respectively. Additional peak signals were observed at m/z 331 and 313 corresponding with the loss of anhydroglucose (−162) and glucose (−180) moieties in agreement with the monoglycosidic structure of the studied compound. All these results were consistent with a molecular



Scheme 1. Structure of globularin and fragment ion nomenclature adopted for glucoside fragmentation.

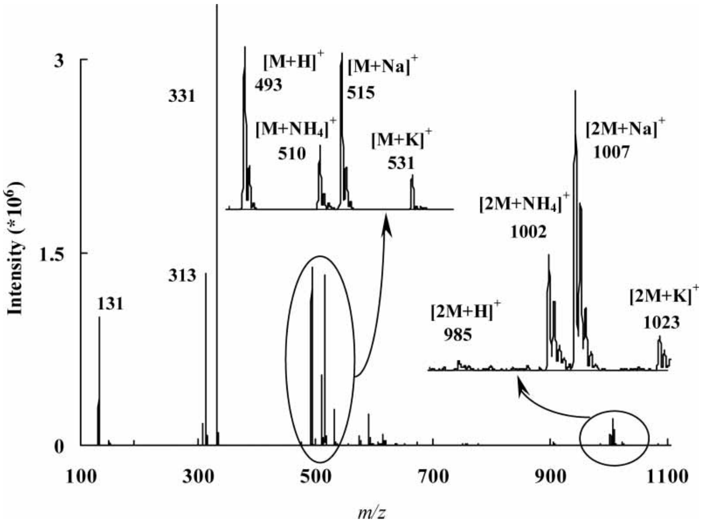


Figure 1. Positive full-scan ESI-MS spectrum of globularin.

weight of 492 Da for globularin in agreement with its chemical structure. These results were also confirmed when the analysis was conducted in the negative ion mode, where the parent ion $[M - H]^-$ was observed at *m/z* 491.

In order to characterize the fragmentation pathways of the studied compound, CID MS/MS spectra of the deprotonated, protonated, and cationized fragment ions were explored and the obtained results are discussed below.

Deprotonated Ion CID MS/MS Analysis

As indicated above, ESI-MS analysis in the negative ion mode yields the deprotonated molecule $[M - H]^-$ at *m/z* 491, which was further selected for CID MS/MS experiments. An example of the obtained MS spectra is schematized in Fig. 2A, which showed the presence of relatively abundant ion signals at *m/z* 315, 175, 161, and 147. The fragment Y_0^- was observed at *m/z* 329, only at low CID energy and whereas a low abundance, whereas the fragment Z_0^- was not observed. The abundant signals at *m/z* 147 and 161 correspond respectively with the cinnamate and probably the methylene cinnamate or the anhydroglucose moieties. The latter was further confirmed by the presence of a less intense peak signal located at *m/z* 329 corresponding with the Y_0^- ion. This was also confirmed by the presence of additional ion peak at 431 (−60), 401 (−90), and 371 (−120) corresponding with the $^{0,4}X_0^-$, $^{0,3}X_0^-$, and $^{0,2}X_0^-$ fragments, respectively.

The most striking feature of the CID spectra of the deprotonated ion generated from globularin is the presence of the fragment ion at *m/z* 315

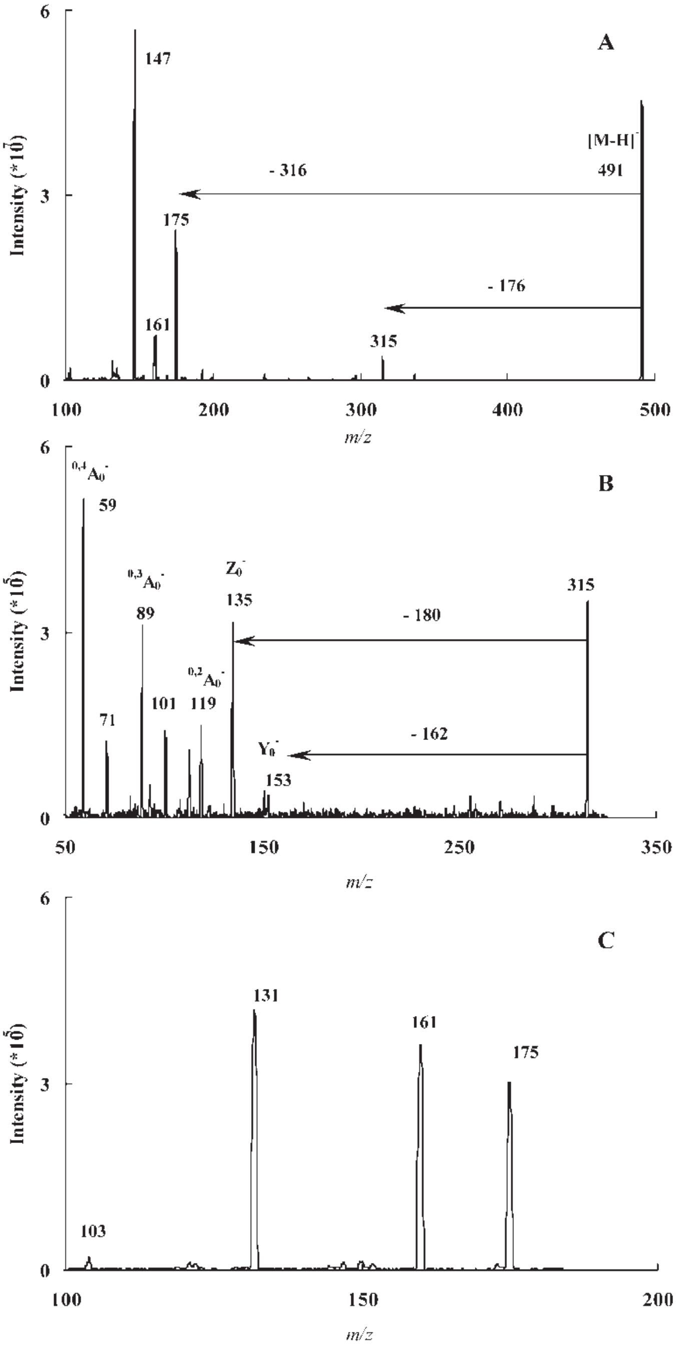


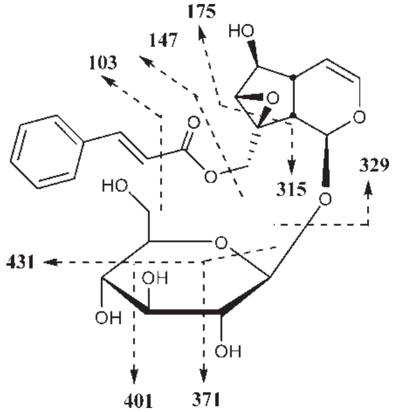
Figure 2. CID mass spectra of the $[M - H]^-$ ion generated from globularin m/z 491 (A) and of its daughter ions m/z 315 (B) and 175 (C).

obtained at lower CID collision energy. The complement of this ion was also detected at *m/z* 175. In order to elucidate the fission releasing these fragments, CID spectra of both ions were investigated. In the MS/MS experiments with the ion at *m/z* 315 (Fig. 2B) the presence of the peaks at *m/z* 153 and 135 were observed resulting from the loss of 162 (anhydroglucose) and 180 (glucose) mass units and corresponding respectively with the Y_0^- and the Z_0^- fragments. These results indicate that the glucose moiety is included in the fragment located at *m/z* 315 while the acyl part is involved in that at *m/z* 175. This was further confirmed by the presence of weak peak signals at *m/z* 255 (−60), 225 (−90) and 195 (−120) corresponding with the ${}^{0,4}X_0^-$, ${}^{0,3}X_0^-$, and ${}^{0,2}X_0^-$ fragments, respectively. The complement ions of these fragments were also observed at *m/z* 59, 89, and 119 corresponding with the ${}^{0,4}A_0^-$, ${}^{0,3}A_0^-$, and ${}^{0,2}A_0^-$ ions, respectively. Additional fragment signals were observed at *m/z* 297 ($-H_2O$), 287 ($-CO$), 271 ($-CO_2$), 101 (${}^{0,2}A_0^-$ $-H_2O$), and 71 (${}^{0,3}A_0^-$ $-2H_2O$).

In the CID spectra of the fragment at *m/z* 175 (Fig. 2C), two major peaks were observed at *m/z* 131 and 161 in addition to weak signals at *m/z* 147 and 103. The ion 147 was attributed to the cinnamate fragment, which by decarboxylation ($-CO_2$) gave the ion observed at *m/z* 103. The ion signal at *m/z* 131 is probably formed by decarboxylation (−44) from the parent ion at *m/z* 175. All these results indicated the presence of the cinnamate part in the explored ion fragment at *m/z* 175 and are in agreement with the results described above suggesting that the glucose moiety was included in the fragment at *m/z* 315. The signal at *m/z* 161 corresponds with the presence of an additional mass unit of 14 Da (CH_2) to the cinnamate ion and corresponding likelihood to the methylene cinnamate ion yielded by fragmentation between the five ring cycle and the CH_2 group. Taking into account these results, it seems that the ion 175 and its complement 315 were formed by a fragmentation involving the breaking of the pentagonal ring (Scheme 2).

Protonated Ion CID MS/MS Analysis

In the mass spectroscopic analysis of iridoid glucosides, the negative ion mode was reported to be the best analytical selective and sensitive technique.[5] However, the results obtained in the full scan mode showed that such compounds may also give good results in the positive ion mode encouraging us to investigate the CID MS/MS of the corresponding protonated ion. When the pseudomolecular ions $[M+H]^+$ at *m/z* 493 were selected as parent ion and the daughter ions were recorded at various collision energies, the ESI-MS/MS spectra showed abundant ion signals at *m/z* 331 (Y_0^+), 313 (Z_0^+), 183, 165, 149, 137, and 131 formed by the fragmentations indicated in Fig. 3A. Additional less-intense peak signals were also observed in the higher *m/z* values region at *m/z* 475 and 457 obtained by successive loss of two water molecules. Other signals at *m/z* 295 ($Z_0^+ - H_2O$), 277



Scheme 2. Main fragmentations observed in CID MS/MS spectra of the $[M - H]^-$ ion of globularin.

$(Z_0^+ - 2H_2O)$ and 407 were also observed. The latter may involve a cross ring fission through Retro-Diels-Alder (RDA) reaction followed by cleavage of the C6–C7 bond leading to the loss of a neutral fragment having a mass of 86 Da. It may be noted that the CID spectra of the $[M + H]^+$ ion do not give any peak corresponding with the sugar moiety (A_0^+ type) of the molecule. All the fragmentations were centered around the aglycone part of the molecule including the acyl part and the two rings of the iridoid skeleton (Scheme 3).

As indicated above, the first fragmentation pathway occuring in globularin was Y_0^+ corresponding with the loss of an anhydroglucose moiety giving an ion signal at m/z 331. This fragment was subsequently selected as parent ion, and its products ions were recorded at different collision energy values giving the spectrum shown in Fig. 3B. The loss of the cinnamoyl moiety was observed giving an ion signal at m/z 183, and the signal corresponding with the acyl part was observed at m/z 149. Additional fragment signals were observed at m/z 313 ($-H_2O$), 295 ($-2H_2O$), 165 (183–H_2O), 149 (cinnamate), 137 (165–CO), 131 (cinnamoyl), 119 (149–H_2O) and 109 (137–H_2O).

The ion signal observed at m/z 183 obtained through successive losses of the anhydroglucose and the cinnamoyl moieties coresponds with the iridoid skeleton ion. In order to elicit more information on its fragmentation patterns, CID MS/MS spectra of this fragment was investigated in order to facilitate the recognition of such a compounds family in a complex mixture of plant extract. Among the observed ion signals (Fig. 3C) were those corresponding with the loss of small molecules. Thus, losses of 18 (H_2O), 28 (CO), 30 (H_2CO), and/or the successive losses of these molecules from m/z 183 were observed giving rise to ions at m/z 165, 155, 153, 147, 137, 135, 123, 121, 119, 109, 97, 95, 91, 81, 79, 71, 55, and 45 (Fig. 3C), which formations involve either losses of small molecules and/or cross ring cleavages. The ion observed at m/z 137 formed by loss of 46 mass units

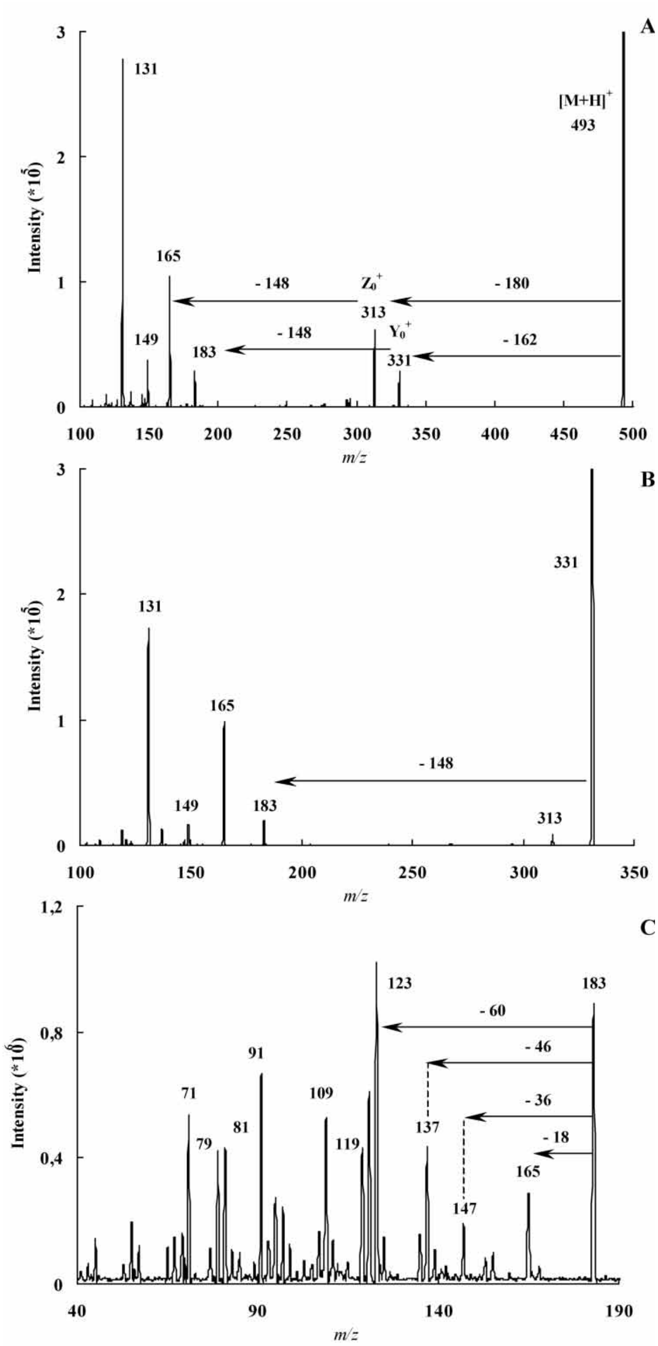
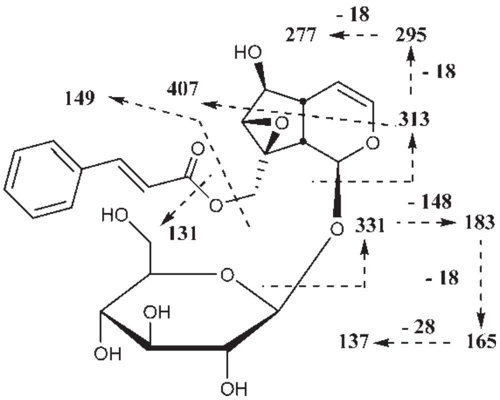


Figure 3. CID mass spectra of the $[M+H]^+$ generated from globularin m/z 493 (A) and of its daughter ions m/z 331 (B) and 183 (C).



Scheme 3. Main fragmentations observed in CID MS/MS spectra of the $[M+H]^+$ ion of globularin.

corresponds with the cleavage of the C–O bond of the pyranic ring. This is analogous to the $^{1,5}X$ type ion observed in the CID spectra of protonated oligosaccharides and results from cross ring cleavage of the C3–O and C1–C9 bonds of the pyranic ring. In a similar manner, the peak observed at m/z 123 might probably involve the cross ring cleavage of the C3–C4 and the C1–C9 linkages.

Cationized Ion CID MS/MS Analysis

In structural analysis of iridoids, few data have been reported on cationized species.[21–23] This fact prompted us to investigate the CID MS/MS spectra of the metal-cationized ions generated from globularin in order to better understand their behaviour. Alkali metals were reported to enhance the abundance of the molecular species and to induce fragmentation reactions not observed under protonating conditions.[30–32] It was therefore decided to study the ESI mass spectra and CID mass spectra of the $[M+Li]^+$, $[M+Na]^+$, and $[M+K]^+$ ions generated from globularin. It is expected that the ESI mass spectra showed abundant $[M+Alkali]^+$ ions. The presence of several oxygenated functions in the molecule makes them ideally suited for attachment of metal ions arising from the solvent.

Lithiated Ion CID MS/MS Analysis

Because the ESI-MS spectrum in the full scan mode did not show the formation of lithiated adduct of globularin, this was enhanced by addition of LiCl. The sample was then analyzed after allowing it to equilibrate, and a highly abundant signal at m/z 499 coresponding with the $[M+Li]^+$ ion was noticed. Subsequent CID spectra of this ion at various collision

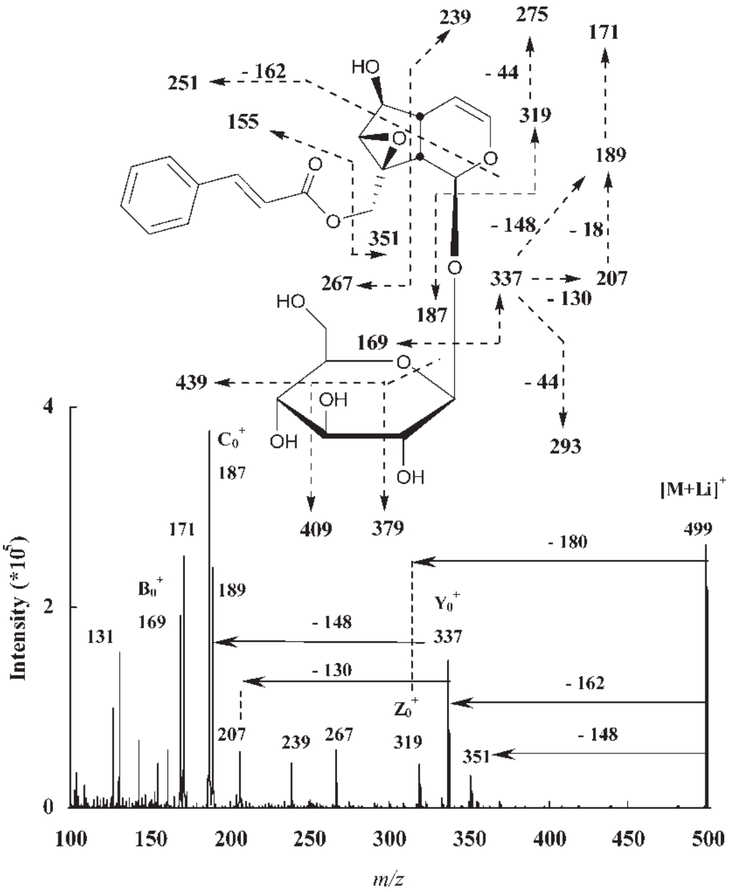


Figure 4. CID mass spectrum of the lithiated ion $[M + Li]^+$ generated from globularin (*m/z* 499) and recorded at a collision energy of 30 eV.

energies showed the occurrence of several fragmentations attested by the presence of various peak signals. A typical CID MS spectrum obtained at 30 eV is shown in Fig. 4. The ions observed at *m/z* 369, 351, 337, and 319 correspond with the loss of a cinnamoyl (−130), loss of cinnamate (−148), and with the Y_0^+ and Z_0^+ fragmentations. The lithiated counterparts of these ions, were also observed at *m/z* 155 (cinnamate), 169 (anhydroglucose, B_0^+), and 187 (glucose, C_0^+) ions, respectively. These results indicated that unlike the protonated and the deprotonated species, lithium adduct showed abundant fragmentations characteristic of both the aglycone and the sugar moieties, which were observed with a relatively high abundance. The fragmentations Y_0^+ and Z_0^+ followed by loss of the cinnamate moiety were also observed at *m/z* 207 and 189, respectively. Dehydration of the latter fragment gave the signal peak located at *m/z* 171.

Fragments involving the sugar moiety were also observed at 439 ($^{0,4}X_0^+$), 409 ($^{0,3}X_0^+$), and 379 ($^{0,2}X_0^+$). The complement ions of these fragments appeared at *m/z* 67, 97, and 127, corresponding with the $^{0,4}A_0^+$, $^{0,3}A_0^+$, and $^{0,2}A_0^+$ ions, respectively. There were also peaks at *m/z* 419, 275, and 293. The first signal corresponds with $[M + H - H_2O]^+$ and the two others correspond with elimination of CO_2 from Z_0^+ and Y_0^+ fragments, respectively. It was reported that elimination of CO_2 is characteristic of the fragmentation of lithiated species and is not observed in the case of $[M + H]^+$ CID spectra discussed above.[23]

Among the other less-intense ion peaks two complementary fragments observed at *m/z* 239 and 267 formed by losses of 260 and 232 mass units. These ions might probably involve the cross pentagonal ring cleavage. This fragmentation is thus of a useful analytical value because it gave information on the substitution occurring on the two rings of the iridoid skeleton. Another ion signal that probably involved the cross ring cleavage was observed at *m/z* 251, which was formed from the aglycone ion (Y_0^+) through RDA reaction followed by cleavage of the C6–C7 bond leading to the loss of a neutral fragment having a mass of 86 Da as reported earlier.[21,23]

The major fragmentations observed in globularin, involving glycosidic cleavage, ring cleavage of aglycone, and ring cleavage of glucose, are summarized in Fig. 4.

In order to investigate the fragmentation of the iridoid skeleton, CID spectra of the ion at *m/z* 337 (Y_0^+) were explored at various CID energy values. An example of the results obtained at 20 eV is shown in Fig. 5A. In the higher *m/z* values region, peaks corresponding with the loss of small molecules were observed at *m/z* 319 ($-H_2O$), 309 ($-CO$), 293 ($-CO_2$), and 291 ($-HCOOH$) even with a low abundance. The signal at *m/z* 239 was also observed while that at *m/z* 267 was not, suggesting that the latter involved the glucose moiety and confirming the hypothetical fragmentation indicated above. Other fragments were observed at *m/z* 207 and 189 formed probably by loss of cinnamoyl (−130) and cinnamate (−148) moieties. Losses of H_2O and CO molecules from the fragment *m/z* 189 yield the ion signals observed at *m/z* 171 and 161, respectively. Two other ion signals were observed at *m/z* 143 and 131. The first fragment was probably formed from that at *m/z* 189 by loss of 46 mass units (−HCOOH) and the second corresponds probably with the cinnamoyl ion formed by loss of a lithiated neutral counterpart.

CID fragmentations of the ion at *m/z* 207 give the results shown in Fig. 5B, which were obtained at 20 eV. The first generated ion was at *m/z* 189 obtained by loss of water molecule. Further losses of small molecules gave the ions at *m/z* 179 (−CO), 171 ($-2H_2O$) and 161 (−HCOOH). The signal ion at *m/z* 143 was also observed whereas that at *m/z* 131 was not in agreement with the hypothesis indicated above. Final CID spectra of the ion at *m/z* 189, which correspond with the basic iridoid skeleton, gave the results shown in Fig. 5C obtained at 20 eV. Among the fragmentations

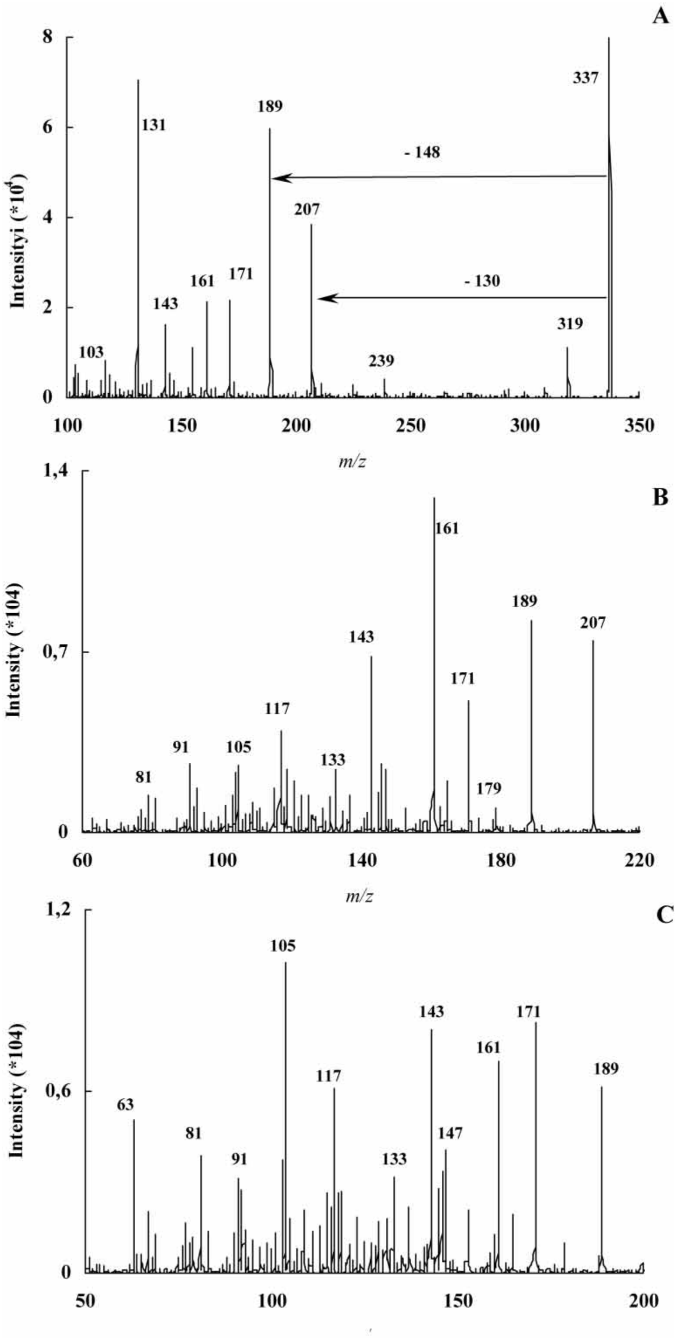
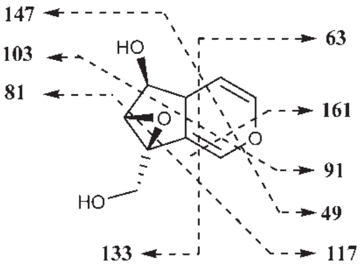


Figure 5. CID mass spectra of the daughter lithiated ions at m/z 337 (A), 207 (B), and 189 (C) recorded at a collision energy of 20 eV.



Scheme 4. Main fragmentations observed in CID MS/MS spectra of the lithiated daughter ion observed at *m/z* 189.

observed were those involving losses of small molecules giving signals at 171 ($-H_2O$), 161 ($-CO$), 153 ($-2H_2O$), 143 ($-HCOOH$). The other observed fragments could be combined by pair as they were observed with their counterparts. Thus, signals at *m/z* 133/63, 117/81, and 91/104 were observed. These ions could be rationalized by the fragmentations shown in Scheme 4. The structure of the presented molecule is only for illustration, and alternative structures are possible for some of these ions.

Sodiated Ions CID MS/MS Analysis

In order to study the fragmentations pattern of sodiated adduct, the generated pseudomolecular ions at *m/z* 515 ($[M + Na]^+$) were selected as parent ion and the daughters were recorded at various CID collision energies, and an example of the representative spectrum is shown in Fig. 6. The most striking feature of the spectrum is the high relative abundance of the ions at *m/z* 203 $[\text{glucose} + Na]^+$ and 185 $[\text{anhydroglucose} + Na]^+$ corresponding with the loss of the aglycone moiety, that is, C_0^+ and B_0^+ fragments, respectively. The Y_0^+ and Z_0^+ fragments at *m/z* 353 and 335 were also observed but with a relatively low abundance. The fragment ion corresponding with the loss of the cinnamate moiety was also observed at *m/z* 367, and its complement was observed at *m/z* 171. The fragment corresponding with the successive loss of the anhydroglucose and the cinnamate moieties was observed at *m/z* 205, which by dehydration gave the fragment located at *m/z* 187. Another fragmentation was observed giving rise to the fragment ion at *m/z* 283 with a relatively high abundance. The complement of this fragment was also observed at *m/z* 255. This fragmentation is due to the fission of the oxygenated ring of the iridoid skeleton. This fragmentation is thus of a useful analytical value because it gave information on the substitution occurring on the two rings of the iridoid skeleton. RDA reaction followed by C6–C7 cross ring cleavage was also observed giving weak ion signal at *m/z* 407.

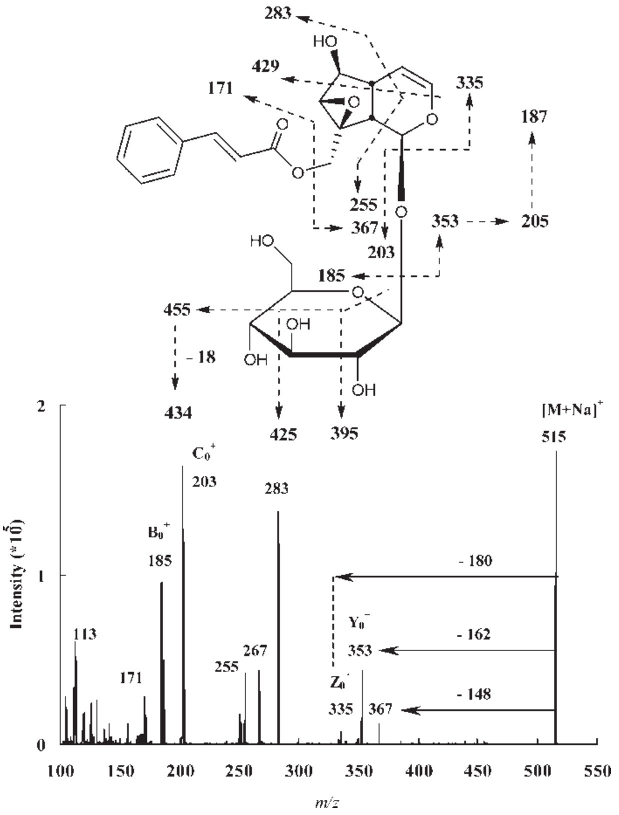


Figure 6. CID mass spectrum of the sodiated ion $[M+Na]^+$ generated from globularin (m/z 515) recorded at a collision energy of 30 eV.

In the higher mass region, fragmentations involving the carbohydrate moiety were observed. Thus, signals at m/z 497 $[M+Na-H_2O]^+$, 485 $(-H_2CO)$, 469 $(-HCOOH)$, 455 $({}^{0,4}X_0^+)$, 437 $({}^{0,4}X_0^+ - H_2O)$, 425 $({}^{0,3}X_0^+)$, and 395 $({}^{0,2}X_0^+)$ were thus attributed.

Potassium-Cationized CID MS/MS Analysis

In order to investigate the fragmentation patterns of the K^+ adduct generated from globularin, the CID spectra of the $[M+K]^+$ located at m/z 531 were recorded at different collision energy values. In contrast with the CID of $[M+Li]^+$ and $[M+Na]^+$ ions, a characteristic feature of the obtained results is the realatively low abundance of the generated product ions (Fig. 7). This is expected based on the weaker bond energies when going from lithium to potassium.[33,34] However, and opposite to published data[23] where it was reported that the $[M+K]^+$ ion decomposes upon CID

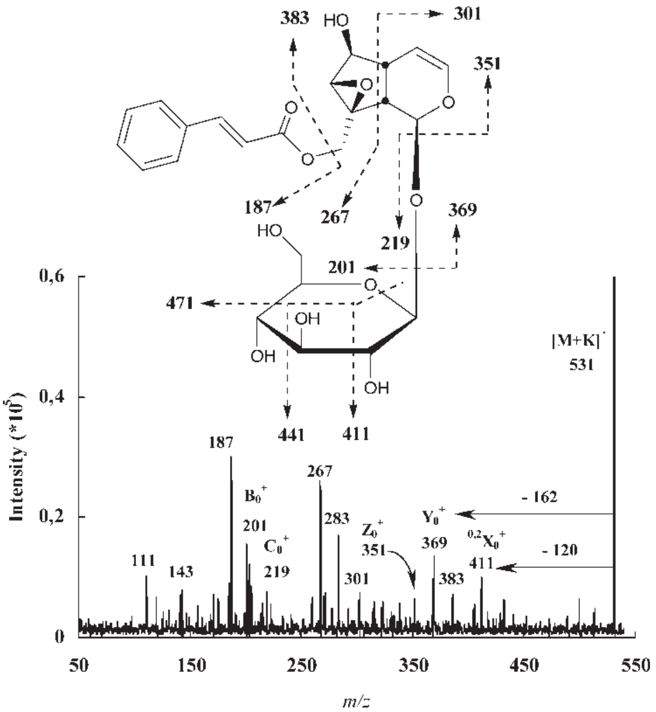


Figure 7. CID mass spectrum of the potassium-cationized ion $[M + K]^+$ generated from globularin (*m*/*z* 531) recorded at a collision energy of 30 eV.

to give only the K^+ ion, the depth examination of the spectra showed the occurrence of characterstic fragmentations.

Thus, fission involving the carbohydrate moiety was observed in the higher mass region at *m*/*z* 401, 471, 441, and 411, and corresponding respectively with the loss of 30 mass units ($-H_2CO$), $^{0,4}X_0^+$ (-60), $^{0,3}X_0^+$ (-90), and $^{0,2}X_0^+$ (-120) fragments. The Y_0^+ (*m*/*z* 369) and Z_0^+ (*m*/*z* 351) and the loss of the cinnamoyl unit (*m*/*z* 385) were also observed. The fragments B_0^+ and C_0^+ were also observed at *m*/*z* 201 and 219, respectively. The successive loss of the cinnamate and the glucose/anhydroglucose moieties giving rise to the ions located at *m*/*z* 203/221 were also observed. Finally, two complementary ion signals involving probably cross ring cleavage were observed at *m*/*z* 267 and 301. Dehydration of the latter gave the signal at *m*/*z* 283.

CONCLUSIONS

In the current study, the structure of globularin was investigated through the combined use of positive and negative electrospray ionization, collision-induced dissociation, and tandem mass spectrometry. The low-energy product ion mass spectra of $[M-H]^-$, $[M+H]^+$, $[M+Li]^+$, $[M+Na]^+$, and $[M+K]^+$ ions showed extensive fragmentation of the carbohydrate moiety, losses of the glucose residue, and fragmentation of the aglycone units, which permit exploration of the nature and the identity of substituents in the molecule.

In the negative ion mode, CID spectra were dominated by fragmentations involving the acyl part losses whereas the Y_0^- and Z_0^- fissions were weakly or not observed. In the positive ion mode, both fragmentations were observed with a predominance of the Y_0^+ and Z_0^+ fissions, whereas the fragments A_0^+ involving the sugar moiety were not observed. The results indicate that proton cation produces fewer fragmentations compared with lithium or sodium cationization, which lead to cross ring cleavages. In agreement with previously reported data, the fragmentation pathways of alkali-cationized molecules were different from protonated or deprotonated ones and provide more information.[35] Indeed, alkali metals were reported to induce tightly localized charge sites resulting in charge-remote fragmentations upon CID analysis.[36] The obtained results indicate that lithium and sodium cationization followed by CID were found to be suitable for the characterization of iridoid glucosides. The results obtained showed that lithium and sodium cationization allowed unambiguously the molecular mass determination. CID of the lithiated and sodiated species provide information on the nature and identity of the substituents in the molecule. Even with low abundance, fragment ions coordinated to K^+ were also observed from $[M+K]^+$. This behavior is expected because ion–molecule interaction energies and ligand-cat+ bond energies decrease with increasing ionic radius of the alkali metal ion.[37,38] Fragmentation mechanisms and ion structures consistent with the obtained data have been proposed. The results of this study clearly demonstrate the potential of mass spectrometry to the structural elucidation of iridoids and open perspectives for similar studies on other natural compounds.

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