

Characterization of Geshodin-Metal Complexation by Electrospray-Mass Spectrometry

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Abstract:

Selected naturally occurring glycosylated flavonoids, iridoids, naphthalenic and anthraquinones were characterized by tandem mass spectrometry via metal complexation. Metal ions solutions of Group I, Group II and some transition metal ions were mixed with selected compounds to generate $(M + L + H)^{n+}$, $(M + 2L + H)^{n+}$, where M is the metal ion and L is the selected compound. Collision induced dissociation (CID) of the $(M + L + H)^{n+}$ complexes resulted in diagnostic mass spectra in contrast to the CID mass spectra of the protonated and sodiated compounds. Fragmentation pattern observed depended on the type of ion, i.e. group I, or group II or transition metal that formed the complex. Selected cross-ring cleavages of the glucose residue were observed from transition metal complexes of naphthalenic compounds. In this presentation characterization of glycosylated naphthalenic compound-metal complexation by ES-MSⁿ is presented.

Keywords: Glycosylated naphthalenic compounds, Electrospray, mass spectrometry, metal complexation

Introduction:

The development of mass spectrometry over the past 50 years has evolved into one of the most essential techniques with numerous biological and chemical applications. The earlier model of mass spectrometers (MS) had limitations due to the need of the molecules to be volatile and of low molecular mass. The development of soft ionization techniques, such as fast atom bombardment (FAB), thermospray ionization (TSP), atmospheric pressure ionization (API), mainly electrospray (ES) and atmospheric pressure chemical ionization (APCI) and matrix assisted laser desorption ionization (MALDI) have extended the usefulness of the MS. The MS now has ability to characterize not only polar, but also thermal labile and higher mass range compounds. Recently, atmospheric pressure ionization modes (APCI and ES) and MALDI, in conjunction with tandem mass spectrometry (MS/MS) and high performance liquid chromatography (HPLC), have found universal application [1-3]. LC-MS with APCI and ES has been found to be a powerful tool for identification of natural products such as polyphenols, with electrospray tandem mass spectrometry (ES-MSⁿ) at the forefront in polyphenol compounds research.

In comparison with all these other methods, electrospray tandem mass spectrometry (ES-MSⁿ) provides more abundant and complementary fragment information to characterize the structures of many natural products such as flavonoids, glycosides, iridoids and naphthalenic compounds

[3]. However, metal complexation has been found to enhance intensity of mass spectra and even gives more specific fragmentation pathways [2-5].

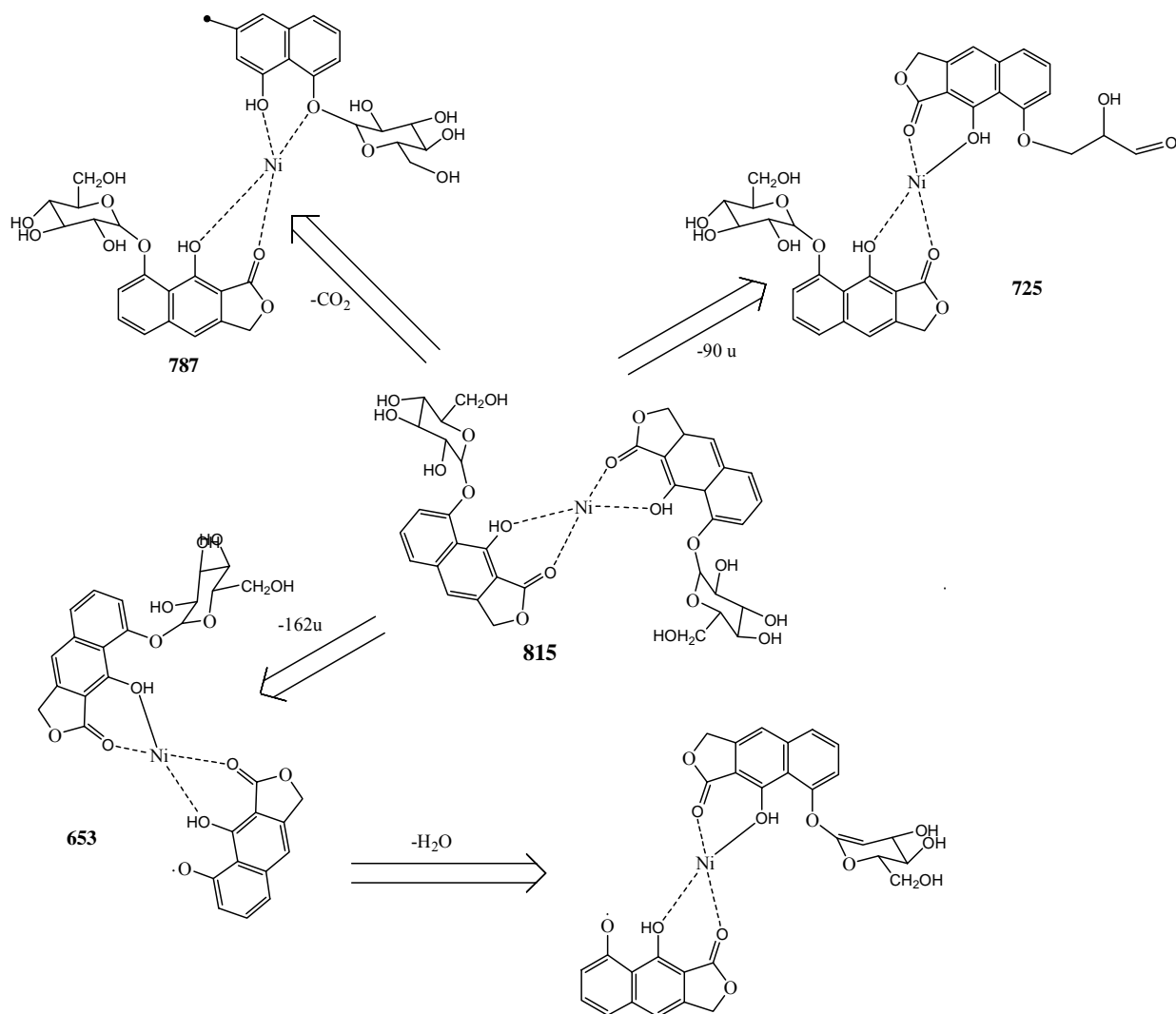
These compounds exist in large numbers and discovery of new active constituents in medicinal plant extracts warrants the use LC-MS, a technique that has proved useful to unlocking the secret of their effectiveness [6, 7]. The high degree of variety in the structures of these compounds makes accurate identification a prerequisite in confirmation of their chemo-preventive properties. Their non-volatile and thermally labile nature poses limitations to methods of analysis such as GC and/or GC-MS. Tentative identifications can only be achieved with additional analytical techniques such as ultra violet (UV), infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy. However, a good measure of work has been done on the determination of active ingredients (polyphenols) of medicinal plant extracts.

Rhamnus prinoides has potential use as a commercial hopping agent in the beer industry and its stem and leaves have been used as a bittering agent in traditional brewing process for centuries [8, 9]. An extract of this plant (A naphthalene glycoside, Geshoidin) is responsible for this purpose. Nindi [8] and co-workers have investigated Geshoidin using electrospray single quadrupole mass spectrometry (ES-SQMS). These workers used an in source fragmentation and hence had limitations. However, through the LCQ^{Deca}, tandem mass spectrometry will be employed here. An ion trap type of mass analyzer would be used and hence would generate more informative fragmentation patterns for the compounds of study such as Geshoidin.

In this study metal cationization in combination with electrospray mass spectrometry was used for structural elucidation of the naphthalenic group of compounds found in the plant of interest.

Results and Discussions

Recently, the use of metal complexation strategies for electrospray-mass spectrometry analysis of flavonoids, iridoids and other group of compounds of medicinal plants has gained popularity [2-5, 10-12]. Previously a number of researchers used Electrospray mass spectrometry and/or LC-ES-MS to characterize flavonoids, glycosylated iridoids and geshoidin [8]. In this work a series of metals of group I and II and some transition metals (Li, Na, K, Cs, Mg, Ca, Mn, Co, Ni, Cu, Zn) and Sn were used to form complexes. These complexes, $[L + M]^{n+}$ and $[2L + M]^{n+}$ (where L = organic molecule, M = metal ion and n = charge on complex) were analyzed by electrospray-tandem mass spectrometry. Previously, it has been reported that certain structural features are required for metal chelation (active or binding sites). However, for flavonoids the positions suggested are the 4-keto and 5-OH groups, 4-keto and 3-OH groups, and 3'-OH and 4'-OH groups. Generally, the metal ion binds to two adjacent carboxyl or hydroxyl oxygens. This tool, i.e. metal complexation and electrospray-tandem mass spectrometry has demonstrated potential in the studies of flavone, flavonol and flavanone aglycones [2-4, 11]. The possible chelation sites for the compounds of this study are given in Scheme 1. The nomenclature used for the cross-ring cleavage of the glucose molecule (for glycosylated compounds) was adopted from Davis and Broadbelt [2].



Scheme 1: Proposed fragmentation pattern of the Ni-Geshoidin $[2L + Ni]^{2+}$ complex. The resulting six membered ring at the metal chelation site shows that the formed complexes were relatively stable.

Geshoidin-Metal Complexation

When geshoidin (**1**) was mixed with metal ions of study two complexes were observed, the $[L + M]^{n+}$ and $[2L + M]^{n+}$, where M is the metal ion and L is geshoidin. Table 1 shows the summary of ES-MS/MS of Geshoidin-metal complexes. Figure 1 a-d shows examples of typical ES-MS/MS spectra of geshoidin-metal complexes.

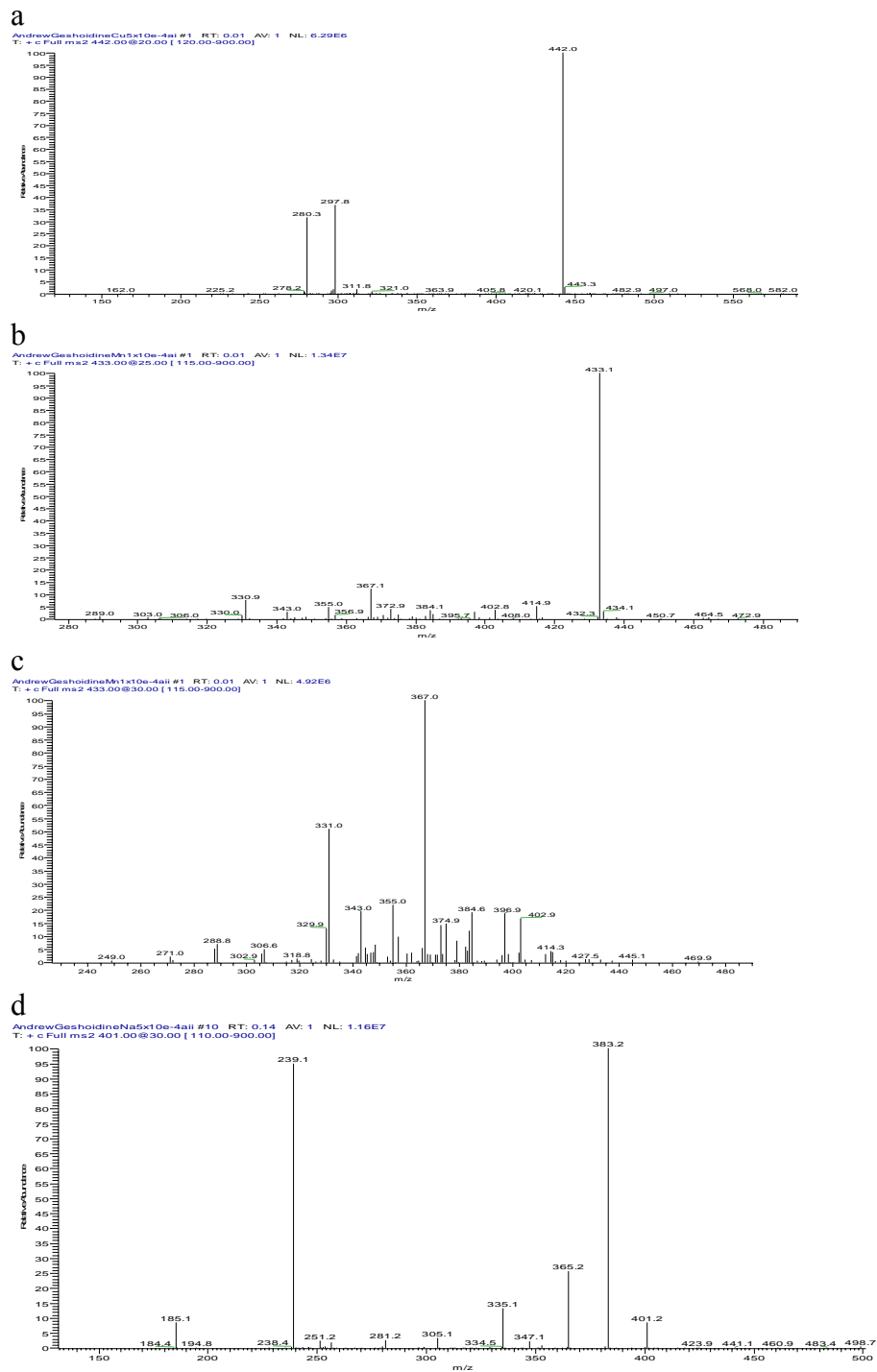


Figure 1: Spectra for Geshoidin metal complexes; a). ES-MS² for Cu
b). ES-MS² for Mn c). ES-MS³ for Mn d). ES-MS² for Na

Table 1: Geshoidin: a= MS/MS on Molecular ion, over a range of Energy (20-45) b= MS³ on molecular ion

Metal	Parent	CID PRODUCT IONS, (<i>m/z</i> [%]) ^a											
		-H ₂ O	-CO	-CO ₂	-2CO	-CH ₂ O	-(CH ₂ O+2H ₂ O)	^{0,4} A	^{0,3} A	^{0,2} A	-G	-(G+ H ₂ O)	A
Li	763	745(2)	-	-			-	-	-	-	-	-	-
	385	367(35)					319(4)			265(7)	223(28)		169(19)
Na	779	761(13)									617(18)		199(5)
	401	383(42)					335(17)				239(30)		185(10)
	401 ^b										239(100)		
K	795	777(15)	767(15)					735(9)			633(2)		579(24)
	417	399(18)		373(40)							255(100)		201(6)
Cs	889	871(33)						829(21)			727(60)		673(44)
	511							451(4)			349(30)		295(4)
Mg	780			736(31)							617(7)		
	402	384(90)					336(16)				240(55)		185(4)
	402 ^b										240(57)		
Mn	811	793(8)	783(42)	767(15)	755(16)						649(13)		595(15)
	433	415(5)					367(13)	373(4)					
	433 ^b					403(25)	367(100)		343(40)		271(7)		
Co	815	797(6)							725(4)		653(5)		
	437					407(28)	371(70)				275(2)		
Ni	815		787(30)	771(17)	759(4)				725(7)		653(7)		
	437					406(23)	371(73)	377(23)			275(32)		
Cu	442								280(32)				
	442 ^b								280(70)				
Zn	821		793(4)	777(16)									
	443	425(17)					377(100)				281(3)		
Sn	497			453(30)								317(2)	335(5)

Table 2: A summary of geshoidin-metal complexes studied, the tick (√) shows that the fragment observed. Two general trends with respect to metal groups (alkali/alkaline-earth and transition metals)

Metal	Parent	CID PRODUCT IONS, (m/z [%]) ^a											
		-H ₂ O	-CO	-CO ₂	-2CO	-CH ₂ O	-(CH ₂ O+2H ₂ O)	^{0,4} A	^{0,3} A	^{0,2} A	-G	-(G+ H ₂ O)	A
Li	763	√	-	-			-	-	-	-	-	-	-
	385	√					√			√	√		√
Na	779	√									√		√
	401	√					√				√		√
	401 ^b										√		
K	795	√	√					√			√		√
	417	√		√							√		√
Cs	889	√						√			√		√
	511							√			√		√
Mg	780			√							√		
	402	√					√				√		√
	402 ^b										√		
Mn	811	√	√	√	√						√		√
	433	√					√	√					
	433 ^b					√	√	√	√		√		
Co	815	√							√		√		
	437					√	√				√		
Ni	815		√	√	√				√	√	√		
	437					√	√	√			√		
Cu	442								√				
	442 ^b								√				
Zn	821		√	√									
	443	√					√				√		
Sn	497			√								√	√

Alkali and alkaline-earth metals

The fragmentation patterns for the group I metal ions (Li, Na, K and Cs) and magnesium complexes are characterized by losses of water (18 u), glucose (162 u) and aglycone (216 u) molecules (Table 1). The ES-MS/MS study of the lithium complex, $[L + M]^+$ produced five fragments ions at m/z 367, 319, 265, 223 and 169. The peak at m/z 367 corresponded to loss of a water molecule from the sugar and m/z 319 resulted from the loss of 66 u ($\text{CH}_2\text{O} + 2\text{H}_2\text{O}$). The $^{0,2}\text{A}$ cross-ring cleavage of geshoidin sugar residue was resulted in m/z 223 (-120 u). The m/z 223 and 169 ions were due to loss of the glucose and aglycone molecules, respectively. This fragment at m/z 169 is an indication that the metal ion was attached on the aglycone side instead of the sugar. The $[2L + \text{Li}]^+$ ion was very weak and hence MS/MS experiments were not performed. Similarly, the sodium ion also formed $[L + \text{Na}]^+$ and fragmented in a similar manner as the lithium complex, with the exception that the $^{0,2}\text{A}$ cross-ring cleavage was not observed. The MS/MS of $[L + \text{Na}]^+$ produced an ion at m/z 239 (100 %), corresponding to a loss of glucose moiety. This observation has earlier been reported by Nindi et. al. [8]. The ES-MS/MS of the $[2L + \text{Na}]^+$ complex resulted in loss of water (m/z 761) and glucose (m/z 617) and a single aglycone (m/z 580). An ES-MS³ on the $[L + \text{Na}]^+$ produced m/z 239 (100 % abundance) fragment ion.

The potassium ion complex, $[L + \text{K}]^+$ produced four fragment ions at m/z 399, 373, 255 (100 %) and 201 corresponding to the losses of H_2O (-18 u), CO_2 (-44 u), glucose and aglycone respectively. It is interesting to note that the potassium is attached to the sugar not the aglycone molecule (m/z 199). The ES-MS/MS spectrum of the potassium dimer, $[2L + \text{K}]^+$ produced five ions at m/z 777, 767, 735, 633 and 579 corresponding to losses of water (-18 u), CO (28 u), $^{0,4}\text{A}$ cross-ring cleavage of glucose (-60 u), of glucose and aglycone respectively. The $[L + \text{Cs}]^+$ produced three fragment ions at m/z 451, 349 and 295 corresponding to $^{0,4}\text{A}$ cross-ring cleavage of glucose (-60 u), of glucose and aglycone respectively. The ES-MS/MS spectrum cesium dimer, $[2L + \text{Cs}]^+$ produced fragments at m/z 871, 829, 727 and 673 corresponding to loss of water (H_2O), $^{0,4}\text{A}$ cross-ring cleavage of glucose (-60 u), glucose and aglycone. Magnesium ion complex formed was similar to the sodium counterparts. The fragment ions observed were due to m/z 384 (90 %), 336 (16 %) and 240 (57 %) which correspond to losses of H_2O , ($\text{CH}_2\text{O} + 2\text{H}_2\text{O}$) and glucose, respectively. For the dimer, only m/z 736 and 240 fragment ions were observed that were due to losses of CO_2 and glucose respectively. The ES-MS/MS spectrum of both magnesium complexes, $[L + \text{Mg}]^{2+}$ and $[2L + \text{Mg}]^{2+}$ were identical to those of their sodium counterparts. This observation could be due to the possible isobaric overlap from $[L + \text{Na} + \text{H}]^+$ species; hence these results could have been influenced by sodium. Ions from calcium complexes were very weak and therefore were not useful.

Transition metal complex analyses

Both transition metal complexes of geshoidin, $[L + M]^{2+}$ and $[2L + M]^{2+}$ fragmentation patterns were significantly different from those observed from the group I and II metals ions. Generally, the fragment ion due to loss of 66 u ($\text{CH}_2\text{O} + 2\text{H}_2\text{O}$) was the most abundant among complexes of this group. The ES-MS/MS spectrum of $[L + \text{Mn}]^{2+}$ produced ions at m/z 415, 367 and 373 corresponding to losses of water, 66 units ($\text{CH}_2\text{O} + 2\text{H}_2\text{O}$) and $^{0,4}\text{A}$ cross-ring cleavage (60 u) respectively. The ES-MS/MS spectrum of $[2L + \text{Mn}]^{2+}$ were produced ions at m/z 783, 767, 755, 649 and 595 corresponding to loss of H_2O , CO (28 u), CO_2 (44 u) and 2 molecules of CO (56 u), glucose and aglycone respectively. The ES-MS³ spectrum of the $[L + \text{Mn}]^{2+}$ complex resulted in fragment ions at m/z 403, 367 (100 %), 343

and 271 were observed corresponding to losses of 30 u (CH_2O), ($\text{CH}_2\text{O} + 2\text{H}_2\text{O}$), ^{0,2}A ring-cleavage (-120 u) and glucose. The MS³ observation, however suggests that the geshoidin molecule was bound to the metal through the aglycone portion. The $[\text{L} + \text{Co}^{2+}]$ complex produced m/z 407 and 371, which were due to losses of (CH_2O) and ($\text{CH}_2\text{O} + 2\text{H}_2\text{O}$) respectively. The abundance of the dimer, $[\text{2L} + \text{Co}^{2+}]$ was very low, however fragmentation observed were at m/z 797, 725 and 653 which are due to dehydration, ^{0,3}A cross-ring cleavage and loss of a glucose molecule respectively. The ES-MS/MS spectrum of $[\text{L} + \text{Ni}]^{2+}$, consisted of ions at m/z 406, 371, 377 and 275 which were due to losses of CH_2O (-30 u), ($\text{CH}_2\text{O} + 2\text{H}_2\text{O}$) and ^{0,4}A cross-ring cleavage and loss of a glucose moiety respectively. The nickel dimer of $[\text{2L} + \text{Ni}]^{2+}$ underwent losses of CO , CO_2 , 2CO , ^{0,3}A cross-ring cleavage and glucose resulting in ions at m/z 787, 771, 759, 725 and 653 respectively. Scheme 1 shows the proposed fragmentation pattern of $[\text{2L} + \text{Ni}]^{2+}$.

Both the ES-MS/MS and ES-MS³ spectra of the copper monomer, $[\text{L} + \text{Cu}]^{2+}$ produced a fragment at m/z 208 which was the result of the ^{0,3}A cross-ring cleavage of glucose (-90 u). The zinc complex $[\text{L} + \text{Zn}]^{2+}$ produced three fragment ions at m/z 425, 377 (100 %) and 281 which corresponded to losses of water, ($\text{CH}_2\text{O} + 2\text{H}_2\text{O}$), which was the most abundant and loss of a glucose moiety. The $[\text{2L} + \text{Zn}]^{2+}$ produced only two fragments at m/z 793 and 777 which were due to losses of CO and CO_2 respectively. The tin monomer complex, $[\text{L} + \text{Sn}]^{2+}$ under ES-MS/MS produced three fragment ions at m/z 453, 317 and 335 which were due to losses of CO_2 (-44 u), 180 units (glucose + H_2O) and aglycon respectively.

Experimental:

All samples were extracted in the University of Botswana, Chemistry Department Geshoidin (**1**) (Previously extracted from *R. prinoides* leaves) were from Professor Abegaz's research lab. HPLC grade methanol was used to dissolve all analytes. Metal ions were prepared from their respective chlorides [MCl , MCl_2 or $\text{MCl}_2 \cdot x\text{H}_2\text{O}$, with the exception of Cu^{2+} which was from $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$].

Sample Preparation

The metal stock (1×10^{-3} M) and sample solutions were prepared by dissolving in methanol without further purification. The metal and sample solutions were mixed in Eppendorf glass sample vials, using methanol/water (1:1) solution as solvent, obtaining a final metal concentration of (5×10^{-4} M). The mixed solutions were directly introduced into the mass spectrometer.

ES-MS

The solutions of compounds were introduced to the electrospray source by direct infusion using a Harvard apparatus syringe pump 22 (South Natick, Massachusetts, U.S.A). The mobile phase additive consisted of 25 mM formic acid in water/methanol (1:1). The spectra were obtained using a Thermo Electron Finnigan LCQ^{DECA} quadrupole ion trap mass spectrometer, using XCalibur software (Thermo Electron, San Jose, California, U.S.A). The ES flow rate was 10 $\mu\text{L}/\text{min}$ and the ion-injection time was typically 60 seconds for both full scans and MSⁿ experiments. All the samples were analyzed in the positive-ion mode. The following ES conditions were used for metal complex analyses; spray voltage + 5 kV, sheath gas flow rate 45 arbitrary units, auxiliary gas flow rate 10 units, capillary temperature 220 °C,

capillary voltage 10 V, tube lens -15 V, ion gauge (0.75×10^{-5} Torr), convention gauge 0.87 Torr. The isolation window for MSⁿ was 1.0 Da.

Conclusions

The study of the collision induced dissociation patterns of metal complexes, allowed successful characterization of the selected secondary metabolites. Metal complexes, $[L + M]^{n+}$ and $[2L + M]^{n+}$ were successfully formed. The abundances and intensities of the product ions were greatly enhanced by metal chelation. Influence of a metal on the fragmentation was noticed to be associated with the metal group (that is group I, group II or transition metal). The later observation is explicitly displayed in Table 2.

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