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Post-column sodiation to enhance the detection of polyacetylene glycosides in LC–DAD–MS analyses: an example from *Bidens gardneri* (Asteraceae)

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ABSTRACT

The use of liquid chromatography–electrospray ionization–mass spectrometry (LC–ESI–MS) in dereplication studies of medicinal plants is a common strategy, but the analyses of polyacetylenes by LC–ESI–MS are little explored and require huge efforts, especially if there are low concentrations in the extracts. A post-column sodiation strategy was successfully applied to enhance the detection of polyacetylene glycosides. Their molecular formulae were proposed by HRESI, whereas the polyacetylene chromophores were determined by UV data. The use of acetic acid in the mobile phase was essential to obtain satisfactory chromatographic resolution, and only the addition of sodium chloride solution allowed good mass spectra, internal calibration and undoubtedly the molar mass determination of polyacetylenes. This new approach has allowed the identification of polyacetylene glycosides from *Bidens gardneri* extract, guiding the isolation procedures, and two new compounds were obtained. The structures of the isolated polyacetylenes have been confirmed by 1D and 2D NMR, HRMS.

1. Introduction

Polyacetylenes are secondary metabolites distributed in some foods and plants used in therapeutic treatment. They are widely described in the Apiaceae family, for example, carrot roots, celery and parsley, which are common in the human diet [1]. Polyacetylenes are also reported in plants belonging to the Asteraceae family [2–4], such as species of the genera *Echinacea* [5,6] and *Bidens* [3,7], as well as in other species with wide therapeutic use such as *Panax ginseng* [8] and *Centella asiatica* [9]. These compounds are generally present in low concentrations, but their detection and identification are fundamental since they exhibit important biological properties including selective cytotoxic activity against tumor cells [1,10] and anti-inflammatory [11,12], anti-angiogenic [13,14], anti-platelet-aggregation [15], antibacterial and antifungal effects [1,16,17], among others. In addition, they have been reported to have toxic effects such as neurotoxicity [18,19] and allergenicity, including allergic skin reactions [20,21]. These facts highlight the importance of the detection, quantification and identification of polyacetylenes; furthermore, efficient analytical methods may help to guide the phytochemical procedures toward the identification of unknown compounds, thus broadening the information about polyacetylenes.

In general, gas chromatography coupled to mass spectrometry (GC–MS) is used to analyze and characterize non-glycosylated polyacetylenes, showing useful spectra for structural characterization with a high level of reproducibility, even though the molecular ion is not always visualized [1,22,23]. Liquid chromatography (LC) methods, coupled to UV and MS detectors, have also been used to analyze non-glycosylated and glycosylated polyacetylenes. UV detection is the most widespread method [1,24], since satisfactory results (related to sensitivity and spectral quality) are complicated to obtain by electrospray ionization (ESI)–MS detection, which requires a bigger effort and is generally not suitable for the analysis of polyacetylenes with low concentrations in samples [1,24]. The ESI technique appears to be an alternative method for detecting more polar natural and synthetic products from complex matrices at low concentrations [25]. Several secondary metabolites exhibit intensive and well-resolved protonated or cationated ion signals in ESI source. Besides the occurrence of natural products,

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molecular ions in ESI–MS by the loss or gain of one electron have also been described. Thus, the ions can be produced in ESI source by chemical reactions such as redox, acid–base and coordination reactions. The extension of these reactions is related to the chemical structure of the analytes, solvent composition, pH, metal competition and other factors [26]. However, ion suppression can occur in complex mixtures like plant extracts, the effect of which is more pronounced and common in ESI [25,27]. Therefore, it is possible to conclude that a specific group of secondary metabolites present in a complex mixture may show a balance between two or more different ionization mechanisms, and intensive ion suppression or even no ionization of the target compound can be observed in ESI, which influences the final quality of the mass spectra. In these last cases, the success of an investigation by dereplication can be significantly reduced. A simple literature search applying the keywords 'dereplication' and 'natural products' reveals several groups of compounds belonging to diverse structural classes such as flavonoids, terpenoids, alkaloids, lignans, carotenoids, glucosinolates and others. However, dereplication of polyacetylenes could not be retrieved and the majority of the investigations in this group are performed by the GC-MS technique.

Recently, 11 phenolic compounds have been identified by a systematic LC–ESI–MS/MS investigation of *Bidens gardneri* Bak (Asteraceae), but polyacetylenes (minority components of the extract) were not identified due to the very low ion intensities or their absence, and consequently it was difficult to find their molecular weight [28]. From a quimiotaxonomic perspective, the genus *Bidens* shows a predominance of flavonoids and polyacetylenes, as illustrated by a graphical distribution based on an extensive literature review (Fig. S1 in Supplementary material). Thus, in dereplication studies the UV spectrum may reveal the presence of polyacetylenes, but the absence of conclusive ions in the mass spectra generally prevents their identification. Considering the importance of polyacetylenes in human health, the abundance of these compounds in species belonging to the *Bidens* genus means that they are good candidates for the investigation of ionization reactions in ESI. *Bidens gardneri* was selected for investigation in this study; it is a medicinal plant in which polyacetylenes have not been identified in previous dereplication studies [28]. The aim of our study was to explore new methodologies to identify polyacetylenes, enhance their detection and guide the isolation of them. This goal is fundamental, as the outcome could have a significant impact in both chemistry and health sciences [18–21,29] since the species *Bidens* and other traditional medicinal plants and foods accumulate polyacetylenes [1,3,30].

2. Material and methods

2.1. Reagents and instrumentation

The NMR spectra (^{1}H, ^{13}C, DEPT 135°, HMQC, HMBC, COSY, TOCY and J-resolved) were acquired on a Bruker DRX500 (500 MHz) and DPX300 (300 MHz) spectrometers. Methanol deuterated (Cambrigde Isotope Laboratories) was used to prepare the samples and it was applied as internal standard. LC–DAD–MS and LC–DAD–MS/MS were performed on a Shimadzu LC-20 A apparatus equipped with a diode array detector (SPD-M20AV, Shimadzu) and coupled to an UltrOTOFq (Bruker Daltonics) ESI-qTOF mass spectrometer. A HPLC Shimadzu LC-6AD instrument equipped with a diode array detector was applied to purify the target compounds by semipreparative LC. A column C18 (Shim-pack Prep-ODS, 5 μm, 20 mm × 25 cm, Shimadzu) were used in these purifications. For chromatographic analysis, ultra-pure water (Millipore, MA, USA), NaCl (Synth, SP, Brazil), HPLC-grade acetonitrile (J.T. Baker) and formic acid (J.T. Baker) were used.

2.2. Plant material

Leaves and stems of *Bidens gardneri* were collected in Aquidauana-Pantanal (Mato Grosso do Sul, Brazil). A voucher specimen, identified by Dr. Arnildo Pott (EMBRAPA-MS), was deposited under number A. Pott 13.680 at the herbarium of EMBRAPA (Campo Grande, MS, Brazil). The plant material was obtained in agreement with the Brazilian regulations (Autor. Pat. Gen. no. 010174/2011-7).

2.3. LC–DAD–MS analyses

Two monolithic columns (Onyx C18, 15x4.6 mm^2, Phenomenex), connected in line, were used for chromatographic analyses. The flow rate was 3.0 mL min^{-1}, injection volume of 20 μL, mobile phase was acetonitrile (B) and H_2O (A) both added acetic acid 1% (v/v) and the elution profile 5–25% B in 0–25 min, 25–100% B in 25–30 min, 100% B in 30–35 min. The column eluent was split at a ratio of 8:1 and one connection was added at the effluent of column, which was connected to infusion pump (200 μl h^{-1}) to mix the effluent with sodium solution (2.0×10^{-3} M), and it was connected to mass spectrometer. The analyses were performed in positive ion mode. Nitrogen was used as nebulizer (4 bar) and collision gas (10–50 eV). The flow of drying gas was 8 L min^{-1}, drying gas temperature of 180 °C.

2.4. Guided isolation of polyacetylenes

The pulverized aerial parts (leaves and stems) of *B. gardneri* were extracted with ethanol by percolation. The ethanolic extracted was partitioned with the solvents hexane, dichloromethane and ethyl acetate, as previously described [28]. The dichloromethane and ethyl acetate fractions were analyzed by LC-DAD-MS. The ethyl acetate fraction (3.0 g) was submitted to column chromatography on Sephadex LH-20 and only 35 fractions, which were analyzed by LC–DAD–MS to identify the fractions with the interesting compounds. Only the fractions 8–10 showed polyacetylenes in their composition. The fractions 8 (208.7 mg), 9 (211.3 mg), 10 (113.4 mg) were submitted to semipreparative LC (flow rate = 9 mL min^{-1}, solvent = acetonitrile and water). The polyacetylenes **1** (11.1 mg)–**2** (9.3 mg), **3** (5.7 mg)–**4** (4.6 mg) and **5** (5.1)–**6** (4.7 mg) were obtained from fractions 10, 8 and 9, respectively.

2.4.1. 2-O-β-D-glucosyl-trideca-3E,11E-dien-5,7,9-triyn-1,2-diol (**1**)

Pale yellow solid; UV λ_{max} 250, 270, 290, 310, 330 and 355 nm; HRESIMS *m/z* 401.1221 $[M+Na]^+$ and 779.2535 $[2M+Na]^+$ (calcd for $C_{19}H_{22}O_8Na^+$ 401.1212 and $C_{38}H_{44}O_{16}Na^+$ 779.2527). ^{1}H and ^{13}C NMR spectroscopic data see Table S1 (Supplementary material).

2.4.2. 2-O-β-D-glucosyl-trideca-11E-en-3,5,7,9-tetrayn-1,13-diol (**2**)

Pale yellow solid; UV λ_{max} 238, 256, 270, 308, 326, 350 and 377 nm; HRESIMS *m/z* 399.1061 $[M+Na]^+$ and 775.22001 $[2M+Na]^+$ (calcd for $C_{19}H_{20}O_8Na^+$ 399.1056 and $C_{38}H_{40}O_{16}Na^+$ 775.2214). ^{1}H and ^{13}C NMR spectroscopic data see Table S2 (Supplementary material).

2.4.3. 3-O-β-D-glucosyl-tetradeca-6E,12E-dien-8,10-diyn-1,14-diol (**3**)

Pale yellow solid; UV λ_{max} 232, 235, 248, 260, 275, 294 and 313 nm; HRESIMS *m/z* 419.1678 $[M+Na]^+$ (calcd for $C_{20}H_{28}O_8Na^+$ 419.1682). ^{1}H and ^{13}C NMR spectroscopic data see Table S3 (Supplementary material).

*2.4.4. 1-O-β-D-glucosyl 14- hydroxyl-tetradeca-6E,12E-diene-8,10-diyn-3-one (**4**)*

Pale yellow solid; UV λ_{max} 232, 235, 248, 260, 275, 294 and 313 nm; HRESIMS *m/z* 417.1533 $[M+Na]^+$ (calcd for $C_{20}H_{26}O_8Na^+$ 417.1525). ^1H and ^{13}C NMR spectroscopic data see Table 1.

*2.4.5. 2-O-β-D-glucosyl 13-acetyl-trideca-3E,11E-dien-5,7,9-triyn-1-ol (**5**)*

Pale yellow solid; UV λ_{max} 255, 268, 298, 310, 330 and 355 nm; HRESIMS *m/z* 443.1320 $[M+Na]^+$ and 863.2736 $[2M+Na]^+$ (calcd for $C_{21}H_{24}O_9Na^+$ 443.1318 and $C_{42}H_{48}O_{18}Na^+$ 863.2738). ^1H and ^{13}C NMR spectroscopic data see Table 1.

*2.4.6. 2-O-β-D-glucosyl 13-acetyl-trideca-11E-en-3,5,7,9-tetrayn-1-ol (**6**)*

Pale yellow solid; UV λ_{max} 240, 256, 270, 307, 325, 349 and 378 nm; HRESIMS *m/z* 441.1168 $[M+Na]^+$ and 859.2423 $[2M+Na]^+$ (calcd for $C_{21}H_{22}O_9Na^+$ 441.1161 and $C_{42}H_{44}O_{18}Na^+$ 859.2425). ^1H and ^{13}C NMR spectroscopic data see Table 1.

3. Results and discussion

Fig. 1 shows the chromatographic peaks of the characteristic UV spectra of polyacetylenes, although their MS spectra reveal very low intensity ions, such as for peak **1** (an intensity of less than 50, Fig. 2A). The data confirm the difficulties of analyzing polyacetylenes by LC–MS, due to the lack or low ionization by ESI, requiring high concentrations of the target in the samples to produce satisfactory results [1].

Initially, the hydroalcoholic extract of the aerial parts of *Bidens gardneri* was fractionated in hexane, chloroform and ethyl acetate by liquid–liquid partitioning to obtain an enriched polyacetylene fraction. The hexane fraction was analyzed by GC-MS, but no polyacetylene was found (data not shown). The dichloromethane and ethyl acetate fractions were analyzed by LC–DAD–MS, and the ethyl acetate fraction only showed compounds with UV spectra compatible to polyacetylenes, which are probably glycosides. The chromatographic peaks corresponding to the polyacetylenes (Fig. 1) are **1** (14.0 min), **2** (15.1 min), **3** (17.1 min), **4** (19.5 min), **5** (26.0 min) and **6** (26.5 min). As previously stated, the mass spectra obtained by LC–DAD–MS were not satisfactory and their molecular weight (MW) could not be determined (Fig. 2A).

The polyacetylene chromophores can be determined from data of the UV spectra by comparison with the data from the literature [31]. For example, the UV spectra of compounds **1** and **5** showed bands with maximum absorptions near 250, 270, 290, 310, 330 and 355 nm, and together with their intensities, it was possible to suggest the presence of the ene-triyn-ene chromophore. The ene-triyn-ene and ene-tetrayn-ene polyacetylenes are the most common chromophores reported until now in the genus *Bidens* (Fig. S2, Supplementary material).

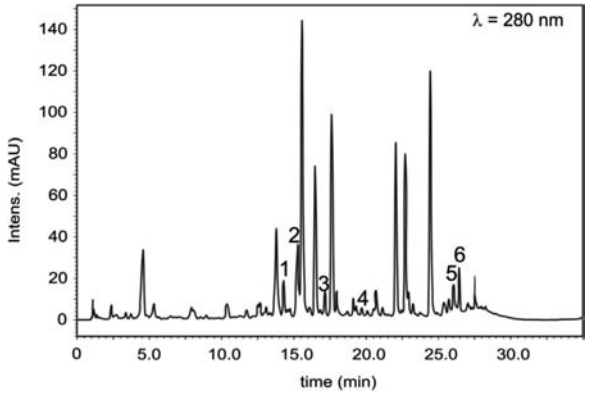

Fig. 1. LC-DAD profile of the extract from *Bidens gardneri*, showing the polyacetylenes 1–6.

Table 1
1H and 13C NMR data of compounds **4**, **5**, and **6**.

Position	**4**[a]		**5**[b]		**6**[b]	
	δ_H (*J* in Hz)[d]	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	3.83, *dt* (10.8, 6.4) 4.12, *dt* (10.8, 5.6)	65.9	3.59, *dd* (11.9, 6.0) 3.66, *dd* (11.9, 4.4)	63.8	3.75, *dd* (11.9, 4.4) 3.71, *dd* (11.9, 6.4)	64.3
2	2.75, *dd* (6.4, 5.6)	43.8	4.37, *m*	80.6	4.66, *dd* (6.4, 4.4)	71.5
3	–	210.5	6.47, *dd* (16.0, 5.4)	147.4	–	77.3
4	2.67, *td* (6.9, 2.1)	42.3	5.99, *dd* (16.0, 1.5)	109.4	–	69.9
5	2.39, *qd* (6.9, 2.0)	27.9	–	77.4	–	63.3
6	6.29, *dtd* (15.5, 6.9, 2.1)	148.2	–	65.9	–	–[e]
7	5.64, *dd* (15.5, 2.0)	110.4	–	73.9	–	74.4
8	–	80.8	–	74.5	–	75.3
9	–	73.5	–	65.3	–	61.3
10	–	74.8	–	76.4	–	77.4
11	–	80.0	5.90, *dt* (16.0, 1.7)	110.2	5.90, *dt* (16.0, 1.7)	109.5
12	5.83, *dt* (15.2, 1.8)	109.0	6.43, *dt* (16.0, 5.5)	143.0	6.53, *dt* (16.0, 5.4)	144.8
13	6.37, *dtd* (15.2, 4.7, 0.8)	147.6	4.66, *dt* (5.5, 1.7)	63.5	4.67, *dd* (5.4, 1.7)	63.4
14	4.13, *dd* (4.7, 1.8)	62.7	–	–	–	–
1′	4.25, *d* (8.0)	104.6	4.42, *d* (7.8)	103.1	4.48, *d* (7.7)	103.0
2′	3.12, *dd* (8.8, 8.0)	75.0	3.26, *t* (8.2)	74.3	3.24, *t* (8.1)	74.1
3′	3.32, *t* (8.8)	78.0	3.37, *t* (8.2)	76.9	3.37, *t* (8.1)	77.0
4′	3.24, *dd* (8.8, 7.8)	71.7	3.33[c]	70.4	3.33, *t* (8.1)	70.3
5′	3.26, *dd* (7.8, 5.8, 2.0)	78.1	3.24, *m*	77.0	3.30[c]	77.3
6′	3.65, *dd* (11.8, 5.8) 3.86, *dd* (11.8, 2.0)	62.8	3.84, *dd* (12.0, 2.2) 3.67, *dd* (12.0, 5.6)	61.5	3.87, *dd* (11.9, 2.1) 3.70, *dd* (11.9, 5.1)	61.6
Ac	–	–	2.08, *s*	19.6	2.08, *s*	19.3
C=O	–	–	–	171.1	–	-

[a] CD_3OD, 300 MHz
[b] CD_3OD, 500 MHz
[c] overlap signals with the solvent signal
[d] multipliced and *J* observed from J-Resolved spectra
[e] non-observed (the ^{13}C correlation in HMBC spectra).

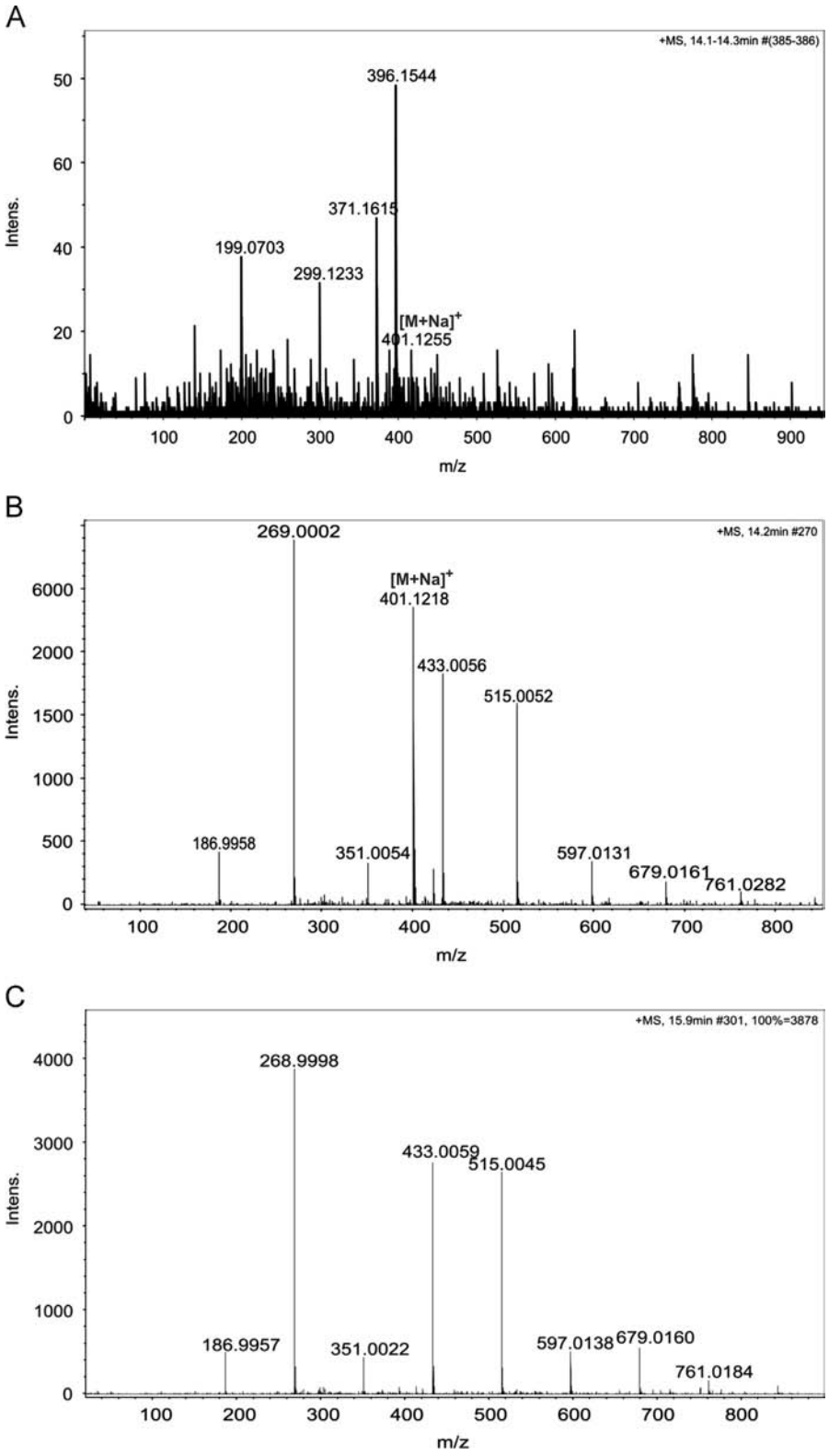

Fig. 2. Mass spectra obtained by LC–MS of chromatographic peak 1 of *B. gardneri*: without the post-column sodiation (A), after the post-column sodiation (B) [highlighted the sodiated ion of 1] and the clusters produced only between mobile phase and sodium solution (without substances of sample) (C), which are used for internal calibration.

Initially, the mass spectra of polyacetylenes from *B. gardneri* did not indicate a successful outcome, even after the change of various operating parameters (like pH and electronic parameters variations) to obtain an improvement in ionization by LC–MS. However, good mass spectra for peaks **1–6** were only obtained after the post-column addition of a diluted sodium chloride solution (Fig. 2B). In this way, the ionization of polyacetylenes was improved, reflecting greater sensitivity and better detection of them. Moreover, the use of acetic acid in the mobile phase together with the addition of sodium produced many clusters, which were used for internal calibration to allow the molecular formulae of polyacetylenes to be easily found, as well as a

satisfactory baseline that led to mass spectra of higher quality, which was different from that observed when lithium chloride was added (data not shown; Fig. S4 in Supplementary material). The internal calibration was fundamental to higher accuracy, especially when a high resolution mass spectrometer with time-of-flight (TOF) analyzer is applied. It is relevant to highlight that the presence or absence of acid in the mobile phase did not interfere with the polyacetylene ionization (in all cases the polyacetylenes showed poor ionization), but the acid was necessary to improve the chromatographic separation, since flavonoids and chlorogenic acid were present in the same fraction.

The ions observed in the mass spectra can be produced from acid–base reactions, cation or anion coordination and/or redox reactions, which depend on the chemical properties of the analytes. One or more of these reactions can occur during ionization; they can also compete with each other, such as protonation and coordination reactions [26,32]. Systematic investigations of ionization reactions with polyethers give an important overview of the effects of pH variations, the differences in potentials applied in the spectrometer and the metal presence [33,34].

Metals have previously been used to enhance the detection of target substances (for example, carotenoids, tocopherols, flavonoids and steroids [35–37]), to elucidate the glycoside flavonoids [38] and to understand the influence on the fragmentation [33,38,39], but they have not been evaluated for polyacetylene glycosides yet. The coordination reactions of analytes with different metals, including alkali, alkali earth and transition metal cations, have already described, demonstrating the influence of the coordination metal in the fragmentation [33,35,38]. Different ions such as silver, sodium, cobalt, nickel, copper, manganese, zinc, calcium, barium, potassium, lithium and others have been used for this purpose [35–38]. The coordination geometry for many metal complexes in not known and certain geometries are favored for some particular metals, as cobalt and nickel is octahedral geometry, while sodium is reported to be distorted tetrahedral [40,41]. Moreover transition metals show specific binding geometries involving d-orbitals, which have been described as producing new fragmentation mechanisms [33], as well for alkali earth metals. These studies have not been extensively applied for identifying secondary metabolites from plants by dereplication, since it is necessary to totally comprehend their fragmentation mechanisms beforehand by ESI–CID, which are not proposed for polyacetylenes yet. The coordination with alkali metals are widely more applied, exhibiting the influence of ionic radii and cation's eletronegativity on the fragmentation, as demonstrated by polyether monensin [42]. Lithiated ions can show higher fragmentation and more important ions for elucidation than sodiated ions can be observed, but sodiated ions gaive more useful information by MS/MS than other alkali metals such as rubidium and cesium [42]. Despite this, there are disadvantages to lithium addition in dereplication studies, as described below.

Different compounds such as glycosylated flavonoids, polyethers, oligonucleotides and others can coordinate to many metals. Although there are huge problems related to the possible differences in the fragmentation pathway, which have not been elucidated for each metal, and their very high affinities for Na^+ and K^+, which are present routinely in the analyses (mainly Na^+) and therefore compete by coordination with the metal added, thus complicating the mass spectra [33,35,42,43]. So the addition of other metals can make it difficult to find the mass of the analyte, since a mix of metal coordinate ions can be observed in the spectra, such as the lithium addition [42,43]. Frequently, the lithiated and sodiated ions are observed together in the mass spectra, representing a problem in dereplication studies due to their mass difference of 16 Da, which is the same difference of the sugars, for example, rhamnoside and glucoside, thus producing doubts about the identification of the compounds. For all these reasons, post-column sodiation was selected in to analyze the polyacetylene glycosides of *B. gardneri*.

Polyacetylene glycosides **1–6** showed preferentially sodiated ions, which are likely to be due to the presence of electron pairs (non-bounded) of oxygen in their chemical structures that can interact with metals; however the spatial arrangement and the number of these atoms can be crucial to the interactions, as observed for monensin [39]. The chelation site depends on the chemical structure of the analyte and can be possible more than one site. Thus the coordination reactions of polyacetylenes with sodium can take place by oxygen of sugar, as described for rutin (a diglycosylated flavonoid) [44], or even the oxygen from aglycone, as the observed for the polyacetylenes falcarinol, falcarindiol and falcarindiol-3-acetate [45]. So the post-column sodiation was successfully applied to analyze the polyacetylene glycosides of *B. gardneri*, since it enhances their detection (Fig. 2B) and can decrease the in-source fragmentation. However, the concentration of the added sodium must be low and carefully controlled, since ion suppression can happen in the ESI source if high concentrations of metal ions are applied. In addition, sodium as a metal to coordination reaction was preferentially selected due to the facility to clean the system, as previously reported by the application of ruthenium (III) chloride [46].

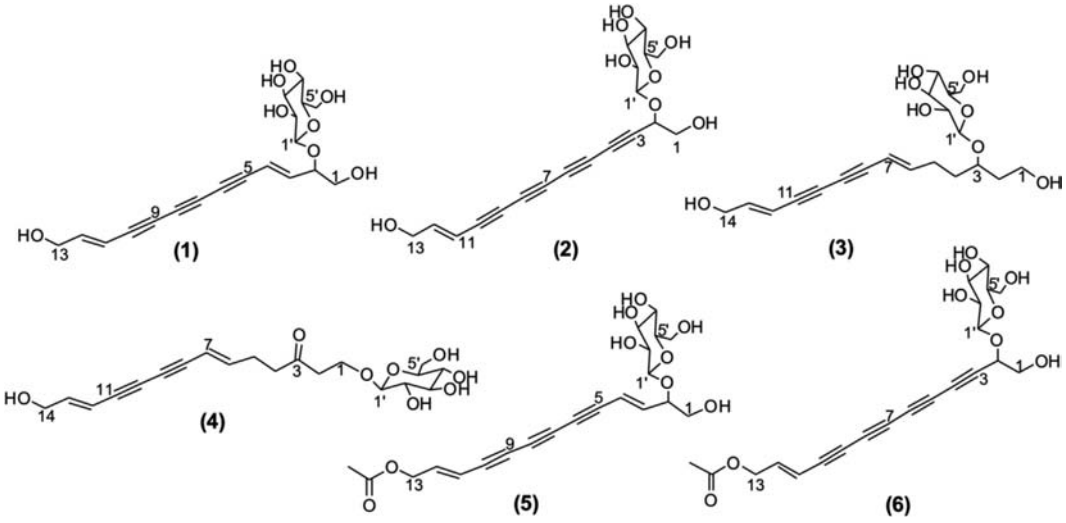

Fig. 3. Chemical structures of polyacetylenes.

After the addition of sodium solution (Fig. 2B), the polyacetylene **1** (Fig. 3) showed a good mass spectrum and its molecular formula can be determined as $C_{19}H_{22}O_8$ (observed: *m/z* 401.1218 $[M+Na]^+$, calculated for $C_{19}H_{22}O_8Na$ 401.12124, error 1.5 ppm). The cluster ions (produced from addition of sodium ions with acetic acid, a component of the mobile phase) were observed in the mass spectra and used for internal calibration (Fig. 2C). The searches were conducted in the literature using the information about the molecular formula and chromophore (ene-thriyn-ene in the chemical structure). Only one polyacetylene was found in the literature with these features, which was reported for *B. campylotheca* [47] and *B. bipinnata* [48]. This substance was isolated and confirmed to be 2-*O*-β-D-glucosyl-trideca-3E,11E-dien-5,7,9-triyn-1,2-diol (**1**) by 1D and 2D NMR (Fig. S3 and Table S1, Supplementary material), which is the same compound as that found in the literature search.

Peak **4** revealed bands in the UV spectrum with λ_{max} at 232, 235, 248, 260, 275, 294 and 313 nm, which are compatible with the ene-diyn-ene chromophore [31]. Its molecular formula was found from LC–MS ($C_{20}H_{28}O_8$). Subsequently, the search of the literature revealed only one compound with these features [49], which is probably as the the same polyacetylene present in *B. gardneri* based on their fragments observed. To validate the methodology proposed in this study, the substance **4** was isolated and characterized by 1D and 2D NMR (Table 1). Some signals were overlapped in the ^{1}H NMR spectrum, so the coupling constants and multiplicities were completely determined from the J-Resolved spectrum, which are listed in Table 1. The coupling between protons was determined by TOCSY 2D (Table S4, Supplementary material). In the olefinic proton region, four signals were observed with a *J* of 16 Hz, confirming the *trans* configuration of the double bonds. From ^{13}C NMR and DEPT 135° data, four quaternary carbons with characteristic chemical shifts of the triple bond (δ 80.8, 80.0, 74.8 and 73.5) and a carbonyl (δ 210.5) were observed. Moreover, sugar signals (δ 3–4 and 4.25 [*d*, *J*=8.0 Hz, H-1′]) were observed, which are peculiar to the β-configuration glucose unit. The position of the sugar and others were confirmed by the carbon–hydrogen correlations observed in the HMBC spectra (Fig. S3, Supplementary material). Thus, polyacetylene **4** was determined to be 1-*O*-β-D-glucosyl 14-hydroxyl-tetradeca-6E,12E-diene-8,10-diyn-3-one (**4**).

The substances of chromatographic peaks **5** and **6** were determined from the UV spectra to be ene-triyn-ene and ene-tetrayne, respectively. From their mass spectra (by LC–MS and sodium addition), the ions at *m/z* 443.1325 and 441.1169 $[M+Na]^+$ were observed, and were used to find their molecular formulae [**5**: $C_{21}H_{24}O_9$ (calculated for $C_{21}H_{24}O_9Na^+$ 443.1318, error 1.6 ppm), **6**: $C_{21}H_{22}O_9$ (calculated for $C_{21}H_{22}O_9Na^+$ 441.1161, error 1.8 ppm)]. Substances with these features could not be found in the literature, indicating the possibility that they are new polyacetylene glycosides and that they were therefore targets to be isolated.

The ^{1}H NMR spectra of isolated substances **5** and **6** showed similar signals, such as sugar signals including anomeric signals at δ 4.3 (*d*, $J \approx 8$ Hz), which confirmed the β-configuration glucose unit, as well the signals at δ 2.08 (*s*, 3 H) from a methyl of the acetyl group (Table 1). The signals observed in the ^{13}C NMR spectra at δ 19 and 171 (C=O) confirmed the presence of acetyl, and the signals at δ 60–78 (quaternary carbons) confirmed the three and four triple bonds for **5** and **6**, respectively. Four and two signals of the olefinic proton region were observed for **5** and **6**, respectively, showing coupling constants of approximately ≈ 16 Hz, which confirmed the *trans* configuration double bonds. Two methylenes were present in the aglycones, which are oxygenated due to the chemical shift value observed ($\delta \approx 63$). One oxygenated methine at δ 80.6 (C-2) and 71.5 (C-2) was observed for **5** and **6**, respectively. The upfield shift of C-2 for **6** confirmed its direct attachment to acetylene carbon, since anisotropic effects of triple bond occured. In the HMBC spectra, the correlations from H-1′ to C-2 and from H-13 to C=O were visualized, confirming the positions of sugar at C-2 and acetyl at C-13 (Fig. S3, Supplementary material). The other correlations visualized are compatible with the chemical structures of **5** and **6**. The analyses by HRESIMS (direct infusion) corroborate the molecular formulae for **5** (observed *m/z* 443.1320 $[M+Na]^+$, calculated for $C_{21}H_{24}O_9Na$ 443.1318, error 0.4 ppm) and **6** (observed *m/z* 441.1161 $[M+Na]^+$, calculated for $C_{21}H_{22}O_9Na$ 443.1318, error 1.6 ppm). Substances **5** and **6** are 2-*O*-β-D-glucosyl 13-acetyl-trideca-3E,11E-dien-5,7,9-triyn-1-ol and 2-*O*-β-D-glucosyl 13-acetyl-trideca-11E-en-3,5,7,9-tetrayn-1-ol, respectively.

4. Conclusions

This study describes a novel protocol based on post-column sodiation for the analyses of polyacetylene glycosides by LC–ESI–MS; since their analysis using this technique is complicated, it requires huge efforts and high concentrations in the samples to produce good spectra. From the LC–MS and UV data, it was possible to conduct the isolation of new and interesting polyacetylene glycosides from *Bidens gardneri* and to identify them even in low concentrations in the samples, since their detection had been enhanced. This methodology can be used for dereplication investigations and to open a new perspective on understanding the storage of polyacetylene glycosides in plants and foods.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.12.024.

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