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Liquid chromatography–electrospray ionization mass spectrometry for analysing plant hormone conjugates

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Abstract

A series of synthetic gibberellin glycosyl esters and abscisic acid glucosyl ester were investigated using LC–electrospray ionization (ESI) MS (negative- and positive-ion modes). From the spectra obtained, diagnostic ions were chosen to monitor HPLC separations on RP-18. The abundant $[M + Na]^+$ ion of the positive-ion ESI spectra enables sugar esters to be recognized from their molecular masses. Under negative-ion ESI conditions, the $[M - sugar]^-$ ion as the base peak allows one to search for a group of different sugar esters of a distinct aglycone. Under selected-ion monitoring conditions (positive-ion ESI), less as $3 \cdot 10^{-2}$ ng of abscisic acid glucosyl ester could be detected. The accessibility of ESI-MS results together with characterization by retention properties obtained by HPLC qualify LC–ESI-MS as a powerful method for the analysis of intact plant hormone conjugates, especially glucosyl ester, at endogenous levels.

Keywords: Liquid chromatography–mass spectrometry; Plant hormones; Gibberillin glycosyl esters; Abscisic acid glucosyl ester

1. Introduction

The combination of HPLC with modern MS techniques provides new prospects for the investigation of polar and high-molecular-mass compounds without prior derivatization or hydrolysis. This is especially important for certain groups of compounds such as sugar ester of organic acids, which cannot be determined by other methods, e.g., GC–MS, without destruction. For the determination of glycosyl esters of acidic plant hormones, atmospheric pressure chemical ionization, and frit fast atom bombardment techniques combined with HPLC have been used [1–5]. Electrospray ionization (ESI) represents another recent technique for the soft ionization of fragile compounds, which can be combined with HPLC. In this work, we investigated a series of gibberellic acid and abscisic acid glycosyl esters by ESI-MS in order to evaluate the usefulness of the LC–ESI-MS combination.

2. Experimental

The synthesis and characterization of the substances used (Fig. 1) have been described elsewhere [6,7].

ESI mass spectra (centroid data) were measured on a Finnigan (San Jose, CA, USA) TSQ

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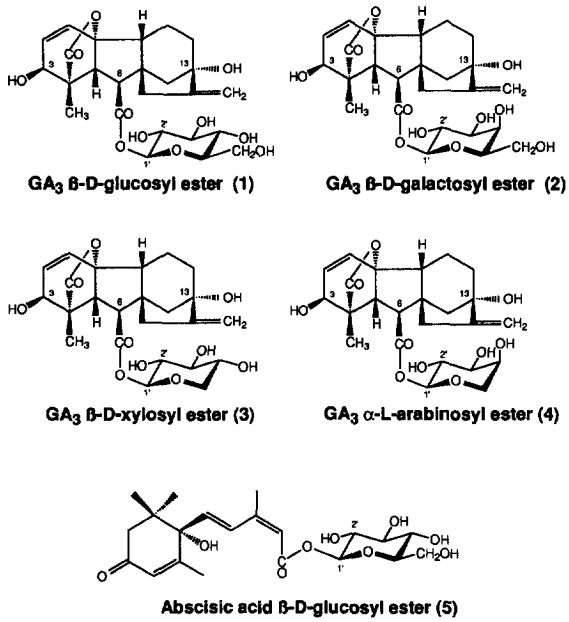


Fig. 1. Structures of compounds **1–5**.

7000 instrument (electrospray voltage 4.5 kV, sheath gas nitrogen) using a ConstaMetric 4100 HPLC system (Thermo Separation Products, Fremont, CA, USA) fitted with a column (100 × 2 mm i.d.) of LiChrospher 100 RP-18, 5 μm (Merck, Darmstadt, Germany) and with acetonitrile (containing 0.3% acetic acid)–water (containing 0.3% acetic acid) (16:84 as the mobile phase at a flow-rate of 0.2 ml min^{-1}).

Selected-ion monitoring (SIM) experiments for the sensitivity tests were carried out with the five most abundant ions of **1–5** (1 *m/z* scan width, 1 s scan rate). The integrated areas of the ion chromatograms obtained with 1.0-μl injections of a dilution sequence were calculated by the TSQ 7000 software.

3. Results and discussion

GA_3 glucosyl ester (**1**), GA_3 galactosyl ester (**2**), GA_3 xylosyl ester (**3**), GA_3 arabinosyl ester (**4**) and ABA glucosyl ester (**5**) were subjected to LC–ESI-MS (positive- and negative-ion modes). As an example, the spectra of positive and negative ions of ABA glucosyl ester (**5**) are shown in Fig. 2. The most important ions in the spectra obtained for **1–5** are summarized in Tables 1 and 2. The negative-ion spectra are characterized by an abundant ion **b** (base peak), which represents the aglycone moiety. The molecular ion $[M-H]^-$ is weak. As shown previously [6], the ion **a** originates from a fragment in which all sugar carbon atoms except C-1′ and C-2′ have been cleaved off (Table 1).

The spectra of positive ions are characterized by adduct ions with Na^+ and K^+. The $[M+Na]^+$ ion exhibits sufficient abundance to be used for calculating the molecular mass such as *m/z* 531 for **1** and **2**, *m/z* 501 for **3** and **4** and *m/z* 449 for **5** (Table 2). Another important fragment is the Na^+ adduct of the aglycone (*m/z* 369 for **1–4** and *m/z* 287 for **5**). The formation of such adducts is a general and reproducible observation, which is due to the ubiquitous presence of small amounts of Na^+ and K^+ originating from the system (e.g., glassware, solvents). For all glycosyl esters of GA_3 the base ion appears at *m/z* 283 [6].

The mixture of the above glycosyl esters was subjected to HPLC on RP-18 with acetonitrile–water (16:84) containing 0.3% acetic acid. In order to follow the separation we chose an array of diagnostic ions, which in addition to the RIC signal permit the differentiation of these compounds. An example using positive-ion ESI-MS is shown in Fig. 3. Trace B (*m/z* 369) shows all glycosyl esters with GA_3 as parent molecule. Likewise, ABA glucosyl esters can be recognized by the ion at *m/z* 287 (trace A). From the ions at *m/z* 531 and 501 (traces C and D), pentosyl or hexosyl esters of GA_3 can be distinguished.

With respect to the sensitivity of the method, we checked the response of LC–ESI-MS in the SIM mode with abundant ions. The highest sensitivity was found for ABA glucosyl ester (**5**) with positive ionization (*m/z* 287), where less than $3 \cdot 10^{-2}$ ng could be detected. For the GA_3 glycosyl esters **1–4** as much as about 100 ng and 10 ng were found to be necessary for detection with positive-ion SIM (*m/z* 283) and negative-ion SIM (*m/z* 345), respectively.

In conclusion, the combined LC–ESI-MS technique (negative- and positive-ion modes) pro-

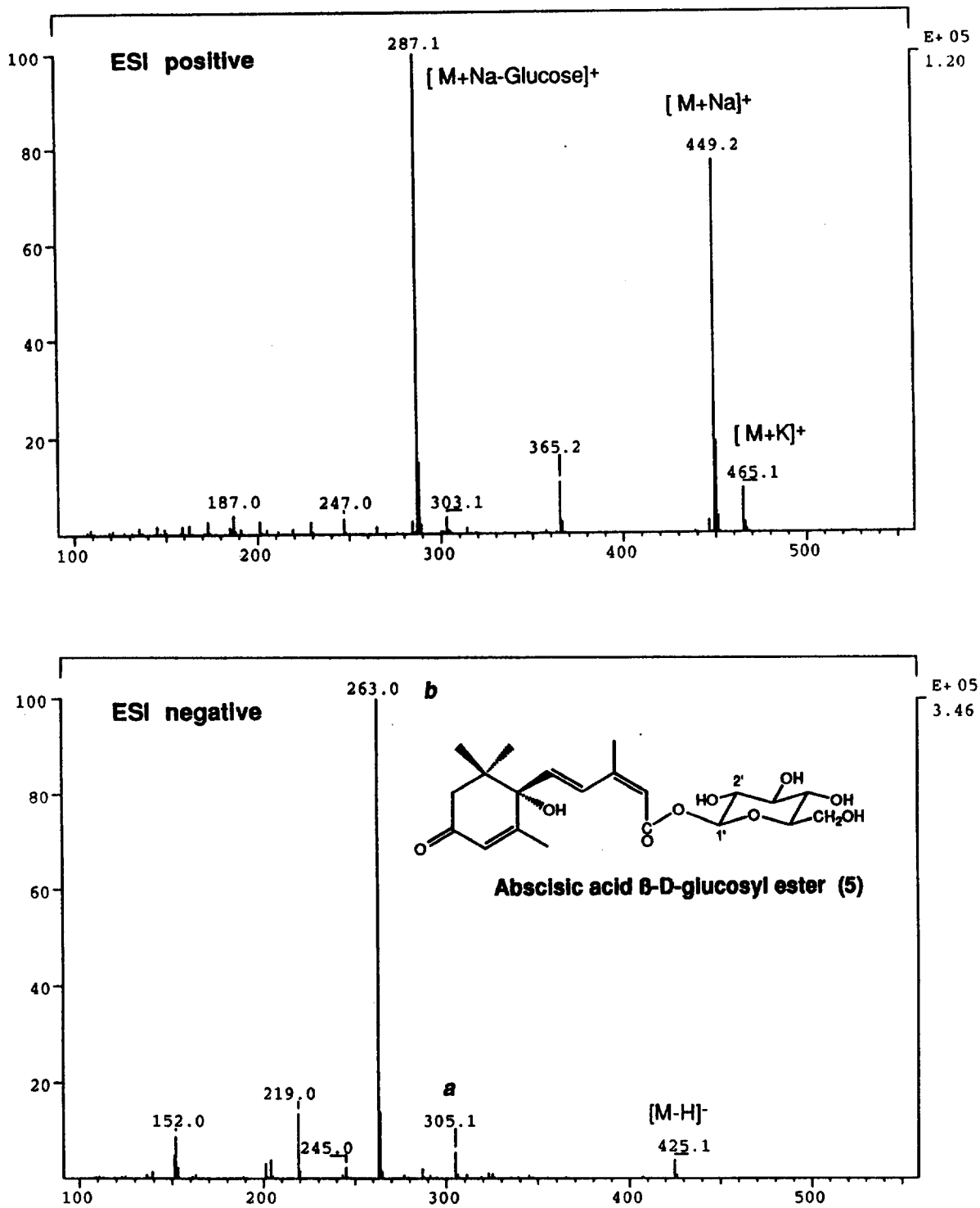
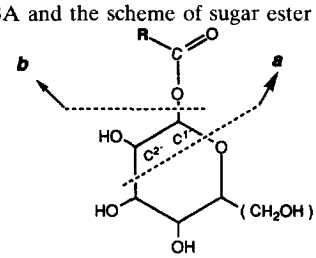


Fig. 2. ESI mass spectra of ABA glucosyl ester (**5**) positive and negative ions.

Table 1
Diagnostic ions (m/z) and their abundances (%) (in parentheses) in negative-ion ESI mass spectra of glycosyl esters of GA_3 and ABA and the scheme of sugar ester fragmentation



Compound	$[M-H]^-$	a	b
GA_3GlcE (**1**)	507 (1)	387 (26)	345 (100)
GA_3GalE (**2**)	507 (5)	387 (24)	345 (100)
GA_3XylE (**3**)	477 (8)	387 (8)	345 (100)
GA_3AraE (**4**)	477 (4)	387 (25)	345 (100)
ABAGlcE (**5**)	425 (4)	305 (10)	263 (100)

vides efficient conditions to obtain significant mass spectra of intact, underivatized glycosyl esters of gibberellins and of abscisic acid at endogenous levels.

In combination with appropriate retention times, the appearance of the diagnostic ions allows one to search for and to identify these glycosyl esters.

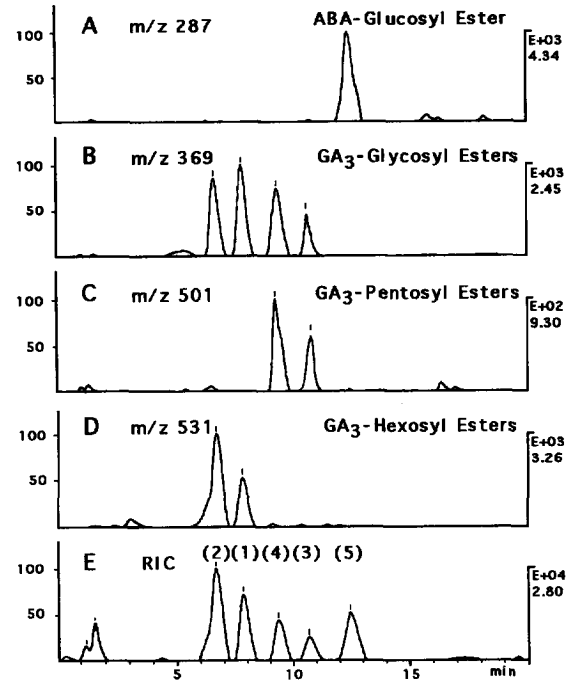


Fig. 3. LC–MS traces (positive-ion ESI, SIM mode) of a mixture of GA_3 glucosyl ester (**1**), GA_3 galactosyl ester (**2**), GA_3 xylosyl ester (**3**), GA_3 arabinosyl ester (**4**) and ABA glucosyl ester (**5**) separated on LiChrospher 100 RP-18 (5 μm) (100 × 2 mm I.D.) with acetonitrile–water (16:84) containing 0.3% HOAc acetic acid as mobile phase at a flow-rate of 0.2 ml min^{-1}. Amounts injected, 0.5 μg of each.

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Table 2
Diagnostic ions (m/z) and their abundances (%) (in parentheses) in positive-ion ESI mass spectra of glycosyl esters of GA_3 and ABA

Compound	$[M+K]^+$	$[M+Na]^+$	$[M+Na-sugar]^+$	$[M+H-sugar]^+$	$[M+H-sugar-H_2O]^+$	$[M+H-sugar-H_2O-HCOOH]^+$
GA_3GlcE (**1**)	547 (15)	531 (90)	369 (30)	347 (2)	329 (58)	283 (100)
GA_3GalE (**2**)	547 (20)	531 (80)	369 (10)	347 (20)	329 (95)	283 (100)
GA_3XylE (**3**)	517 (4)	501 (10)	369 (10)	347 (8)	329 (42)	283 (100)
GA_3AraE (**4**)	517 (15)	501 (45)	369 (20)	347 (10)	329 (43)	283 (100)
ABAGlcE (**5**)	465 (10)	449 (75)	287 (100)	265 (2)	247 (4)	–

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