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Identification of maloyl glucans from *Euphorbia tirucalli* by ESI-(−)-FT-ICR MS analyses

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ABSTRACT

Euphorbia tirucalli aerial parts are popularly used in Brazil for cancer treatment. The elution of the aqueous extract of the plant on silica gel C-18 cartridge furnished a water-soluble fraction, which was analyzed directly into the electrospray ionization (ESI) source combined with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) and characterized as a mixture of malic acid glycosides **1**–**5**. The compounds were detected in their deprotonated form $[M-H]^-$, where their exact mass (mass error lower than 1 ppm), molecular formula ($C_nH_hO_o$), double bond equivalent (DBE) and connectivity were determined from ESI-(−)-MS and ESI-(−)-MS/MS experiments. The presence of malic acid and glucose, as part of the structures, could originate from crassulacean acid metabolism (CAM) of the plant.

1. Introduction

As a treatment for cancer, *Euphorbia tirucalli*, popularly known in Brazil as aveloz, is widely used in the form of latexes (daily dosage of two drops/liter of water) and/or tinctures prepared from the aerial parts of the plant (Mwine et al., 2013). However, in a July 2011 resolution (RE2917), the Brazilian Agency for Sanitary Vigilance (ANVISA) prohibited the trade of this plant, as well as the manipulation of herbal medicines containing *E. tirucalli*, essentially because co-carcinogenic diterpenes, such as phorbol and ingenol esters, were found to be among its constituents (Fürstenberger and Hecker, 1986). Ellagitannins and flavonoids (Shwu-Jiuan et al., 2001), as well as triterpenes and sterols (Uchida et al., 2007), are regularly found in plant extracts. However, except for some ellagitannins, these classes of compounds are not water-soluble, making them unsuitable for use in medicinal preparations. In contrast, the presence of water-soluble molecules is of substantial interest as shown by the medicinal use of plants like aveloz in the form of aqueous or hydroalcoholic preparations. Although it is challenging to isolate and identify such molecules, ultra-high resolution and accurate mass spectrometry, such as Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), currently allows for the identification of complex organic mixtures without the need for any prior extraction or separation steps. FT-ICR MS is applied in all the "omics" sciences, i.e., metabolomics (De Sá et al., 2014; Ferreira et al., 2014; Martins et al., 2014; Costa et al., 2014), proteomics, and petroleomics (Tessarolo et al., 2014; Dalmaschio et al., 2014; Terra et al., 2014), thus enabling molecular-level analyses of complex mixtures. Accurate mass measurements define the unique elemental composition ($C_cH_hN_nO_oS_s$) and double bond equivalent (DBE) from singly charged ions such as $[M+H]^+$, $[M+Na]^+$, $[M+K]^+$, $[M-H]^-$, and $[M+Cl]^-$, where M corresponds to a neutral molecule (Costa et al., 2014).

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E. tirucalli is a plant with high stress tolerance, which can be explained, at least in part, by its photosynthetic system. More specifically, in response to arid conditions, C3/CAM photosynthesis is an adaptation that has evolved in the leaves and stem of *E. tirucalli*. This carbon fixation pathway provides a dual-cycle strategy by which the stomata are closed during the daytime to reduce water loss, but reopen at night to collect carbon dioxide (Zabaleta et al., 2012). CO_2 is stored as the four-carbon acid malate and then used during photosynthesis throughout the day. Hence, the presence of malate is indicative of CAM photosynthesis in this species (Hastilestari et al., 2013). Biochemically, this is important because a CAM plant produces malate and glucans. These substrates can react with one another, and the product can be identified by ultra-high resolution mass spectrometry.

Indeed, the presence of maloyl glucans was first described in *Aloe barbadensis*, a CAM plant (Esua and Rauwald, 2006). The authors used NMR and MS to identify the isolated compounds. However, during the isolation, these investigators were thwarted by the unstable, hygroscopic and amorphic nature of these compounds. Thus, as an extension of their groundbreaking work, it was our aim to further identify maloyl glucans compounds in aveloz, but without using an isolation protocol. Also, from a pharamacological perspective, it should be noted that the antiproliferative and anti-inflammatory activities of some maloyl glucans from *Aloe barbadensis* suggest that similar behavior might be found in *E. tirucalli* preparations. Therefore, as further evidence of CAM metabolism in *E. tirucalli* stems, maloyl glucans **1**–**5** (Fig. 1), esters of malic acid with glucose, are herein, for the first time, described in *E. tirucalli*. These water-soluble compounds, which were determined by negative ion mode ESI FT-ICR MS in the absence of an isolation step, allow uncovering a new perspective to understand CAM metabolism of plants.

2. Material and methods

2.1. Plant material

E. tirucalli L. stems were collected at the Federal University of Rio de Janeiro (UFRJ), Medicinal Plants Garden of the Institute of Natural Product Research, Brazil, in July, 2012, at 8:00 am, and identified by Prof. Lucy de Senna Valle, Botanical Garden of the National Museum, UFRJ. Voucher specimen (RFA-31675) has been deposited at the herbarium of UFRJ.

2.2. Extraction

Stems of the plant (100 g) were shaken with water (100 ml) in an appropriate blender for 10 min. After that, the mixture was filtered, and an aliquot (1 ml) was adsorbed in a C-18 stationary phase cartridge in which elution with 3 ml of water under compression was carried out. For the structural identification, 5 drops of methanol/ammonium hydroxide (99.9/0.1 v/v %) were added in aqueous fraction, and 10 μl were inserted in an ESI FT-ICR mass spectrometer operating at negative electrospray ionization, ESI(−) and hydrolysis with 99% trifluoroacetic acid (TFA-10 μl) was performed in additional 100 μl of the aqueous solution for 1 h under reflux. After hydrolysis, co-chromatography with authentic monosaccharides (glucose, galactose, mannose, fructose, arabinose, xylose, glucuronic acid and galacturonic acid) was performed on TLC silica gel plates (BuOH:EtOH:H_2O, 40:11:19).

2.3. Mass spectrometry – ESI-FT-ICR MS

A Model 9.4 T Solarix mass spectrometer (Bruker Daltonics, Bremen, Germany) was set to negative ion mode, ESI(−), over a mass range of m/z 200–2000. The parameters of the ESI(−) source were as follows: nebulizer gas pressure of 0.5–1.0 bar, capillary voltage of 3–3.5 kV, and transfer capillary temperature of 250 °C. The mass spectrum was processed using the Compass Data Analysis software package (Bruker Daltonics, Bremen, Germany). A resolving power, $m/\Delta m_{50\%} \cong 500{,}000$, in which $\Delta m_{50\%}$ is the full peak width at half-maximum peak height, of $m/z \cong 400$ and a mass accuracy of <1 ppm provided the unambiguous molecular formula assignments for singly charged molecular ions. Elemental compositions of the compounds were determined by measuring the m/z values. The degree of unsaturation for each molecule can be deduced directly from its DBE value according to equation DBE = $c - h/2 + n/2 + 1$, where c, h, and n are the numbers of carbon, hydrogen, and nitrogen atoms, respectively, in the molecular formula. Molecular formula, measured m/z values, mass error and ESI-MS/MS are all shown in Table 1. The tandem mass spectrometry (MS^2) experiments were performed on a quadrupole analyzer coupled to the FT-ICR mass spectrometer, Q-FT-ICR MS. The ESI(−)-MS/MS spectra were acquired using (i) infusion flow rate of 5 μL min^{-1}; (ii) capillary voltage of 3.0 kV; (iii) nebulizing temperature of 250 °C; (iv) argon as collision gas; (v) ion accumulation time of 1 s; (vi) isolation window of 1.0 (m/z units); and (vii) 25–45% of the collision energy. The

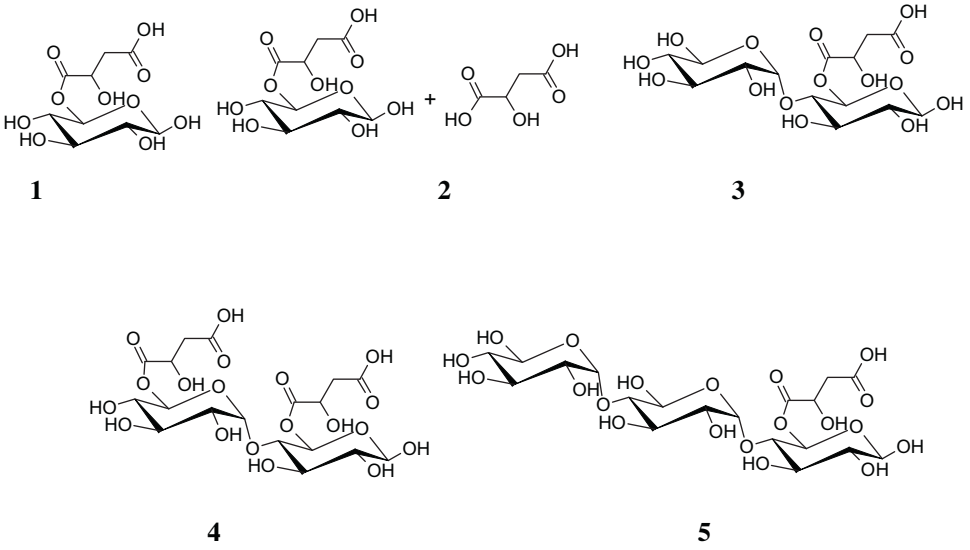

Fig. 1. Maloyl glucans from aerial parts of *Euphorbia tirucalli*.