Resin glycosides from *Ipomoea wolcottiana* as modulators of the multidrug resistance phenotype in vitro

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1. Introduction

*Ipomoea wolcottiana* Rose var. *wolcottiana* is one of the thirteen arborescent morning glories endemic to Mexico and nearby Mesoamerica (Felger and Austin, 2005). In central Mexico, it is one of the species belonging to the medicinal plant complex called with the vernacular name of “cazahuate” (from the Nahuatl language, tree “quauitl” and mange “zahuatl”; “tree for curing mange”), which also include *Ipomoea arborescens* G. Don var. *arborescens*, *Ipomoea intrapilosa* Rose, *Ipomoea murucoides* Roemer & Schultes, and *I. pauciflora* Martens & Galeotti ssp. *pauciflora*, respectively. They are a conspicuous floristic element of the Seasonal Dry Tropical Forest and have been used since Prehispanic times in traditional medicine of Mexico (Emmart, 1940; Miranda and Valdés, 1964). These arboreal morning glories share several morphological features, e.g., trees with large white flowers and funnel-shaped corollas and stems that produce milky latex. A therapeutic application that is used to treat itching, rashes and other infections is obtained by rubbing the raw flowers or leaves directly on the skin (Chérgio and Pereda-Miranda, 2006). Two reports in the literature also described the chromatographic profiles (TLC) of alkaloids and glycosides from the leaves (Perez-Amador et al., 1992b), as well as fatty acid components by GC-MS (Perez-Amador et al., 1992a) from seeds of some species of the Arborescens group of Mexican morning glories.

In traditional medicine of Mexico, *I. wolcottiana* is known with the name of “cazahuate verde” (green “cazahuate”) and it is used for the treatment of erysipelas by applying directly to the affected skin a poultice made of leaf powder mixed with rose oil. Its latex is topically applied to treat rashes and to reduce toothache (Aguilar et al., 1994). This plant is a tree (12 m tall) with a flowering period from October to March and distributed in Guatemala, El Salvador, Honduras, and the Southern states of Mexico (Felger and Austin, 2005). It can be frequently found as a pioneer species along roads and on disturbed sites (Suppl. Fig. 1).
Resin glycosides are complex mixtures of an extensive family of secondary metabolites known as glycolipids and represent unique metabolites in the plant kingdom confined to the Convulvulaceae, the morning glory family (Pereda-Miranda et al., 2010). Previous results in our research have shown that these compounds are modulators of efflux pumps that produce the multidrug-resistant (MDR) phenotype in prokaryotic (Corona-Castañeda and Pereda-Miranda, 2012; Corona-Castañeda et al., 2013; Pereda-Miranda et al., 2006a) and eukaryotic cells (Figueroa-González et al., 2012).

*Staphylococcus aureus* 1199B cells, an effluxing strain overexpressing the Nor A multidrug transporter, was used to evaluate the inhibition of ethidium bromide efflux by resin glycosides, e.g., murucoidins from *I. murucoides* (Chérigo et al., 2008; Pereda-Miranda et al., 2006a,b). Decreased expression of the P-gp by resin glycosides was also detected by immunofluorescence flow cytometry after incubation of vinblastine-resistant human breast carcinoma cells (MCF-7/Vin) with an anti-P-gp monoclonal antibody (Figueroa-González et al., 2012). Thus, incubation of MCF-7/Vin cells with resin glycosides also enhanced vinblastine susceptibility (Bautista et al., 2015; Castañeda-Gómez et al., 2013; Cruz-Moraless et al., 2012; Figueroa-González et al., 2012). For these reasons, our efforts have been focused on the chemical investigation of this type of glycolipids to increase the recognition of chemical diversity of these target molecules by isolating compounds from a collection of *I. wolcottiana* to further explore their potential as MDR modifying agents.

The present study describes the procedures used by recycling preparative HPLC for isolation and purification of five new CHCl₃-soluble resin glycosides 1–5, in addition to known compound 6. Their intact oligomeric macrocyclic structures were identified by a combination of FABMS, ESIMS, and NMR methods. These compounds exerted a potentiation effect of antibiotics and vinblastine susceptibility on multidrug resistance cells.

### 2. Results and discussion

The crude CHCl₃-soluble extract from the flowers of *I. wolcottiana* was subjected to silica gel CC to eliminate pigments and non-polar constituents in order to concentrate the polar resin glycoside-containing fraction. Bioassay-guided fractionation, using modulation of both antibiotic activity against multidrug-resistant strains of Gram-negative bacteria and vinblastine susceptibility in breast carcinoma cells, was used in compound isolation. This resulted in purification of glycolipids 1–6 as multidrug resistance phenotype modulators. Chromatographic homogeneity was achieved for these active glycolipids through recycling preparative HPLC (Sidana and Joshi, 2013). The techniques of column overload, peak shaving, and “heart cutting” were also applied in combination with recycle analysis through the use of a RP-18 stationary phase that provided maximal resolution for each component to be fractionated and purified very effectively (Pereda-Miranda and Hernández-Carlos, 2002). A refractive index detector was used to monitor this process of purification.

The spectrometric analysis of wolcottine 1 (Fig. 1) was conducted by ESI in the negative ion detection (Suppl. Fig. 1). The quasi-molecular ions [M–H]⁻ and [M+Cl]⁻ were detected at *m/z* 2552.4955 and 2588.4815, which designated a molecular formula of C₁₃₆H₂₂₂O₄₂ (Suppl. Fig. 3) for compound 1. Fragment [M/2 +Cl]⁻ indicated the rupture of the ester type bond and represented the high-mass fragment ion for the two monomeric units at *m/z* 1311.7355 (C₉₀H₁₁₁O₃₂Cl) (Escalante-Sánchez and Pereda-Miranda, 2007; Rosas-Ramírez et al., 2011; Rosas-Ramírez and Pereda-Miranda, 2015). Wolcottine 1 (1) gave a quasi-molecular ion [M–H]⁻ at *m/z* 2552 (C₁₃₆H₂₂₂O₄₂) in negative FABMS (Suppl. Fig. 4) and a readily detectable ion resulting from the cleavage of the ester-type dimer linkage at *m/z* 1275 (C₉₀H₁₁₁O₂₁) (macrocyclic unit A). Fig. 2 illustrates some of the diagnostic ions detected by FABMS in the negative-ion mode. Glycolipidic cleavage of the sugar moieties and elimination of the esterifying groups afforded the following fragments: 1149 [1275–126 (C₆H₄O, octanoyl)]⁻·1019 [1149–130 (C₆H₄O, cinnamoyl)]⁻, 873 [1019–146 (C₉₀H₁₀₂O₄)]⁻, 691 [873–182 (C₁₂H₁₅O₂, dodecanoyl)]⁻·545 [691–146 (C₉₁H₁₀₂O₄)]⁻, 417 [545+18–146 (C₉₁H₁₀₂O₄)]⁻, 271 [417–146 (C₉₁H₁₀₂O₄)]⁻ j alapinic acid–H⁻. For example, in secondary ion mass spectrometry, the peak at *m/z* 419 [C₆H₄O₅+ C₈H₁₅O₂ + C₄H₈O] resulted from cleavage of the anomeric linkage at Rha” in unit B suggested that an octanoyl residue substituted this sugar unit (Escalante-Sánchez and Pereda-Miranda, 2007; Rosas-Ramírez et al., 2011). Therefore, the dodecanoyl moiety was located at Rha” as confirmed by the ion at *m/z* 857, which resulted from cleavage of the anomeric linkage at Rha” in unit A (Fig. 2).

Wolcottine 1 (1) was submitted to saponification affording, as the only reaction product, operculonic acid C, (11S)-hydroxyhexadecanoic acid 11-O-α-L-rhamnopyanosyl-(1 → 4)-O-α-L-rhamnopyanosyl-(1 → 2)-O-β-D-fucopyranoside, which was unambiguously characterized by comparison of HPLC retention time and melting point, with an authentic sample and its peracetylated derivative (Rosas-Ramírez et al., 2011). These products were used to generate 13C NMR profiles for structural dereplication since their anomic signals are easily distinguishable and used as a fingerprint for pattern recognition (Pereda-Miranda et al., 2006b; Rosas-Ramírez and Pereda-Miranda, 2015). Comparison of melting point, optical rotation, and 13C NMR data with published values for the peracetylated derivative of operculanic acid C confirmed that compound 1 is an ester-type dimer related to the batatinis III–VI previously isolated from the CHCl₃-soluble resin glycoside mixture of sweet potato (*I. batatas*) (Rosas-Ramírez et al., 2011). A combination of 1H NMR spectra (Suppl. Fig. 5) and 2D homonuclear techniques (DQF-COSY and TOCSY) allowed all C-bonded protons to be sequentially assigned within each ring system of the two tetrasaccharides which were arbitrarily designated as units A and...