Phytochemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

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

Berenice Corona-Castañeda [a], Daniel Rosas-Ramírez [b], Jhon Castañeda-Gómez [c], Manuel Alejandro Aparicio-Cuevas [a], Mabel Fragoso-Serrano [a], Gabriela Figueroa-González [a], Rogelio Pereda-Miranda [a,*]

[a] Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, 04510 DF, Mexico
[b] Departamento de Química de Biomacromoléculas, Instituto de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, 04510 DF, Mexico
[c] Grupo Químico de Investigación y Desarrollo Ambiental, Facultad de Ciencias y Tecnologías, Universidad del Quindío, Armenia, Colombia

ARTICLE INFO

Article history:
Received 3 September 2015
Received in revised form 18 December 2015
Accepted 4 January 2016
Available online xxxx

Keywords:
Ipomoea wolcottiana
Convolvulaceae
Resin glycosides
Glycolipids
Multidrug-resistance
Structural spectroscopy

ABSTRACT

Recycling liquid chromatography was used for the isolation and purification of resin glycosides from the $CHCl_3$-soluble extracts prepared using flowers of *Ipomoea wolcottiana* Rose var. *wolcottiana*. 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 to isolate the active glycolipids as modulators of the multidrug resistance phenotype. An ester-type dimer, wolcottine I, one tetra- and three pentasaccharides, wolcottinosides I–IV, in addition to the known intrapilosin VII, were characterized by NMR spectroscopy and mass spectrometry. In vitro assays established that none of these metabolites displayed antibacterial activity (MIC > 512 μg/mL) against multidrug-resistant strains of *Escherichia coli*, and two nosocomial pathogens: *Salmonella enterica* serovar Typhi and *Shigella flexneri*; however, when tested (25 μg/mL) in combination with tetracycline, kanamycin or chloramphenicol, they exerted a potentiation effect of the antibiotic susceptibility up to eightfold (64 μg/mL from 512 μg/mL). It was also determined that these non-cytotoxic (CI_{50} > 8.68 μM) agents modulated vinblastine susceptibility at 25 μg/mL in MFC-7/Vin$^+$ cells with a reversal factor ($RF^{+}_{MCF\text{-}7/Vin}$) of 2–130 fold.

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 (Chérigo and Pereda-Miranda, 2006). Two reports in the literature also described the chromatographic profiles (TLC) of alkaloids and glycoresins 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).

* Corresponding author.
E-mail address: pereda@unam.mx (R. Pereda-Miranda).

http://dx.doi.org/10.1016/j.phytochem.2016.01.004
0031-9422/

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 Convolvulaceae, 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-Morales 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 $CHCl_3$-soluble resin glycosides **1**–**5**, in addition to known compound **6**. Their intact oligomer 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 $CHCl_3$-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 I (**1**, Fig. 1) was conducted by ESI in the negative ion detection (Suppl. Fig. 2). 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 $C_{138}H_{224}O_{42}$ (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 ($C_{69}H_{112}O_{21}Cl$) (Escalante-Sánchez and Pereda-Miranda, 2007; Rosas-Ramírez et al., 2011; Rosas-Ramírez and Pereda-Miranda, 2015). Wolcottine I (**1**) gave a quasi-molecular ion $[M-H]^-$ at *m/z* 2552 ($C_{138}H_{223}O_{42}$) 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 ($C_{69}H_{111}O_{21}$) (macrocyclic unit A). Fig. 2 illustrates some of the diagnostic ions detected by FABMS in the negative-ion mode. Glycosidic cleavage of the sugar moieties and elimination of the esterifying groups afforded the following fragments: 1149 [1275−126 ($C_8H_{14}O$, octanoyl)]$^-$, 1019 [1149−130 (C_9H_6O, cinnamoyl)]$^-$, 873 [1019−146 ($C_6H_{10}O_4$, methylpentose)]$^-$, 691 [872−182 ($C_{12}H_{22}O$, dodecanoyl)]$^-$, 545 [691−146 ($C_6H_{10}O_4$)]$^-$, 417 [545+18−146 ($C_6H_{10}O_4$)]$^-$, 271 [417−146 ($C_6H_{10}O_4$)]; jalapinolic acid−H]$^-$. For example, in secondary ion mass spectrometry, the peak at *m/z* 419 [$C_6H_9O_5 + C_8H_{15}O + C_9H_7O$] resulting 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).

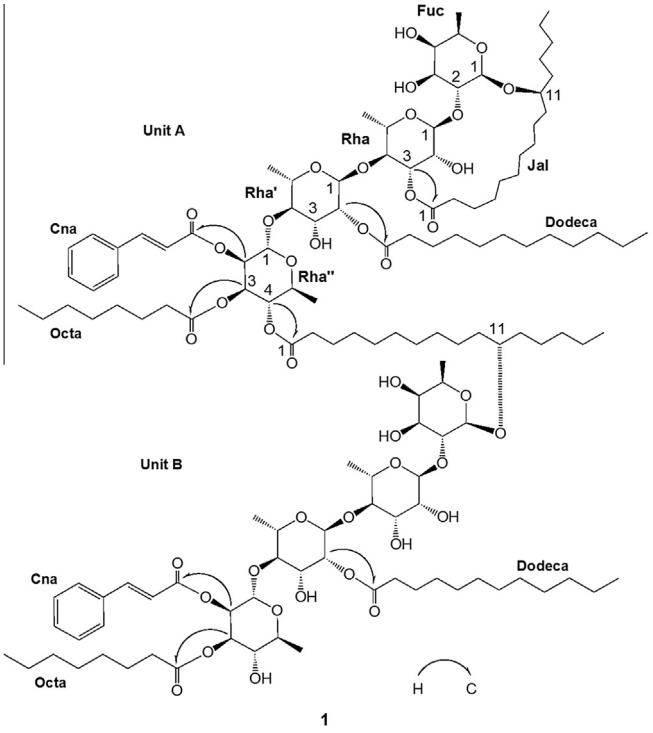

Fig. 1. Key HMBC correlations ($^3J_{CH}$) for the sites of esterification in compound **1**.

Wolcottine I (**1**) was submitted to saponification affording, as the only reaction product, operculinic acid C, (11*S*)-hydroxyhexadecanoic acid 11-*O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(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 ^{13}C NMR profiles for structural dereplication since their anomeric 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 ^{13}C NMR data with published values for the peracetylated derivative of operculinic acid C confirmed that compound **1** is an ester-type dimer related to the batatins III–VI previously isolated from the $CHCl_3$-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

Please cite this article in press as: Corona-Castañeda, B., et al. Resin glycosides from *Ipomoea wolcottiana* as modulators of the multidrug resistance phenotype in vitro. Phytochemistry (2016), http://dx.doi.org/10.1016/j.phytochem.2016.01.004