Revista Brasileira de Farmacognosia 27 (2017) 434–439

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original Article

# HPLC-MS profiling of the multidrug-resistance modifying resin glycoside content of *Ipomoea alba* seeds

Jhon Castañeda-Gómez[a], Daniel Rosas-Ramírez[b], Sara Cruz-Morales[c], Mabel Fragoso-Serrano[c], Rogelio Pereda-Miranda[c,*]

[a] Grupo Químico de Investigación y Desarrollo Ambiental, Programa de Licenciatura en Ciencias, Facultad de Educación, Universidad Surcolombiana, Neiva, Colombia
[b] Departamento de Química de Biomacromoléculas, Instituto de Química, Universidad Nacional Autónoma de México, Mexico City, Mexico
[c] Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Mexico City, Mexico

ARTICLE INFO

*Article history:*
Received 18 February 2017
Accepted 30 May 2017
Available online 23 June 2017

*Keywords:*
HPLC–MS profiling
Glycolipid
Resin glycoside

ABSTRACT

High performance liquid chromatography profiling with mass spectrometry detection was applicable to identify known and novel multidrug-resistance glycolipid inhibitors from the complex resin glycosides mixture of *Ipomoea alba* L., Convolvulaceae, seeds. Albinosides X and XI were purified by recycling liquid chromatography and their structural elucidation was accomplished by nuclear magnetic resonance. Albinoside XI exerted a strong potentiation of vinblastine susceptibility in multidrug-resistant human breast carcinoma cells.

© 2017 Sociedade Brasileira de Farmacognosia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

## Introduction

*Ipomoea alba* L., Convolvulaceae, or moon vine, is a night-blooming morning glory native to tropical Americas (McDonald, 1994). In Mexico, the decoction of the leaves has been used for the treatment of paralysis and swelling of tissues (Lim, 2014). This species is an ornamental plant but it has also been reported its invasive potential (Foxcroft et al., 2008).

Previously, eleven resin glycosides were isolated from moon vine, calonyctins $A_1$ and $A_2$ from EtOH-soluble extracts of dried leaves (Fang et al., 1993) and albinosides I–IX from $CHCl_3$-soluble extracts of seeds (Cruz-Morales et al., 2012, 2016). Resin glycosides are complex mixtures of oligosaccharides of monohydroxy and dihydroxy C-14 and C-16 fatty acids, which represent distinctive secondary metabolites restricted to the morning glory family (Pereda-Miranda et al., 2010). These mixtures of glycolipids are a source of efflux pumps modulators responsible for the multidrug resistant (MDR) phenotype in Gram-positive (Pereda-Miranda et al., 2006) and -negative bacteria (Corona-Castañeda and Pereda-Miranda, 2012), as well as in mammalian cancer cells (Figueroa-González et al., 2012). MDR phenotype is considered a major cause for the failure of anticancer agents. Thus, the use of efflux pump modulators co-administered with cytotoxic drugs results in a susceptibility equivalent to that of a sensitive cell (Figueroa-González et al., 2012). Hence, in this context, the present HPLC-MS profiling of the resin glycoside content of *Ipomoea alba* seeds was undertaken to identify novel multidrug-resistance glycolipid inhibitors.

## Materials and methods

### *General experimental procedures*

The experimental procedures, including HPLC, GC–MS, MS and NMR instrumentation, were previously described (Cruz-Morales et al., 2012, 2016).

### *Chemicals and cell lines*

RPMI 1640 medium and fetal bovine serum were purchased from Gibco (Life Technologies, Carlsbad, CA) and sulforhodamine B (SRB), reserpine, and vinblastine from Sigma-Aldrich (St. Louis, MO). Breast (MCF-7 and MDA-MB-231), cervix (HeLa), and colon (HCT-15 and HCT-116) carcinoma cell lines were acquired from the American Type Culture Collection. The resistant counterpart MCF-7/Vin has been subcultured during seven years (Figueroa-González et al., 2012).

### *Plant material*

Moon vine seeds (*Ipomoea alba* L., Convolvulaceae; item # 01052-PK-P1) were purchased from Park Seed (Greenwood, SC)

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

http://dx.doi.org/10.1016/j.bjp.2017.05.003
0102-695X/© 2017 Sociedade Brasileira de Farmacognosia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

in March 2015. Eight seeds were germinated and two seedlings were grown to maturity under the conditions previously described (Cruz-Morales et al., 2016). Vouchers were deposited at the Arkansas State University Herbarium (STAR 027009).

*Extraction and isolation*

Maceration of dried powdered seeds (300 g) was performed at room temperature with $CHCl_3$. Precipitation of the resin glycoside with MeOH was achieved from the $CHCl_3$-soluble extract to obtain a white solid (4.5 g), which was washed with hexane. The precipitated solid was analyzed by HPLC using a reversed-phase Symmetry $C_{18}$ column (Waters; 7 μm, 19 × 300 mm), an isocratic elution with MeOH–$CH_3CN$–$H_2O$ (5:4:1), and a flow rate of 4 ml/min with a sample injection of 500 μl (concentration: 0.1 mg/μl). The resulting chromatogram showed eleven resolved peaks (Fig. 1). Eluates with $t_R$ values of 83.3 min (20.9 mg) and 147.1 min (18.3 mg) were collected by the heart cutting technique (Pereda-Miranda and Hernández-Carlos, 2002). Each subfraction was independently reinjected and purified by recycling HPLC to achieve homogeneity after ten consecutive cycles by application of peak shaving technique (Pereda-Miranda and Hernández-Carlos, 2002) until overlapped minor impurities were separated: Symmetry $C_{18}$ column (Waters; 7 μm, 19 × 300 mm) with an elution of MeOH–$CH_3CN$–$H_2O$ (10:7:3) and a flow rate of 6 ml/min. These procedures allowed the purification of **1** (15.5 mg; $t_R$ 8.6 min) and **2** (13.5 mg; $t_R$ 10.7 min).

*Albinoside X (**1**):* White solid; mp 128–131 °C; $[\alpha]_{589}$–11.7, $[\alpha]_{578}$–12.0, $[\alpha]_{546}$–13.7, $[\alpha]_{436}$–20.6, $[\alpha]_{365}$–26.2 (*c* 1.0, MeOH); $^1$H and $^{13}$C NMR see Table 2 positive ESIMS: *m/z* 997 $[M+Na]^+$; negative FABMS: *m/z* 973 $[M–H]^-$, 891 $[M–H–C_5H_6O$ (tigloyl)$]^-$, 809 $[891–C_5H_6O$ (tigloyl)$]^-$, 663 $[809–C_6H_{10}O_4$ (methylpentose unit)$]^-$, 517 $[663–C_6H_{10}O_4$ (methylpentose unit)$]^-$, 389 $[517+H_2O–C_6H_{10}O_4$ (methylpentose unit)$]^-$, 243 $[389–C_6H_{10}O_4$ (methylpentose unit)$]^-$; HRESIMS *m/z* 973.5081 $[M–H]^-$ (calcd for $C_{48}H_{77}O_{20}$ requires 973.5014).

*Albinoside XI (**2**):* White solid; mp 138–140 °C; $[\alpha]_{589}$–13.3, $[\alpha]_{578}$–13.8, $[\alpha]_{546}$–15.7, $[\alpha]_{436}$–22.9, $[\alpha]_{365}$–29.5 (*c* 1.0, MeOH); $^1$H and $^{13}$C NMR see Table 2; positive ESIMS: *m/z* 1025 $[M+Na]^+$; negative FABMS: *m/z* 1001 $[M–H]^-$, 919 $[M–H–C_5H_6O$ (tigloyl)$]^-$, 837 $[919–C_5H_6O$ (tigloyl)$]^-$, 691 $[837–C_6H_{10}O_4$ (methylpentose unit)$]^-$, 545 $[691–C_6H_{10}O_4$ (methylpentose unit)$]^-$, 417 $[545+H_2O–C_6H_{10}O_4$ (methylpentose unit)$]^-$, 271 $[417–C_6H_{10}O_4$ (methylpentose unit)$]^-$; HRESIMS *m/z* 1001.5392 $[M–H]^-$ (calcd for $C_{50}H_{81}O_{20}$ requires 1001.5327).

*Alkaline hydrolysis of compounds 1–2*

Solutions of compounds **1** and **2** (10 mg for each sample), in 5% KOH–$H_2O$ (1 ml) were refluxed at 95 °C for 3 h. Then, the mixtures were acidified to pH 5.0 and extracted with $CHCl_3$ (2× 2.5 ml) and $Et_2O$ (2× 2.5 ml). The $CHCl_3$-soluble layer was washed, dried over anhydrous $Na_2SO_4$, evaporated, and analyzed by GC–MS. Tiglic acid was detected as the only volatile product for compounds **1** and **2** ($t_R$ 6.95 min): *m/z* $[M]^+$ 100 (30), 73 (100), 55 (22). The aqueous layer was extracted with *n*-BuOH (10 ml) and concentrated. Hydrolysis of compound **1** afforded **12** (4.5 mg), and compound **2** yielded **13** (4.3 mg).

*Albinosinic acid H* (**12**): white solid; mp 96–98 °C; $[\alpha]_D$–32.1 (*c* 1.0, MeOH); negative ESI-MS *m/z* 827 $[M–H]^-$. FABMS *m/z* 827 $[M–H]^-$, 681 $[M–H–C_6H_{10}O_4$ (methylpentose unit)$]^-$, 535 $[681–H–C_6H_{10}O_4$ (methylpentose unit)$]^-$, 389 $[535–H–C_6H_{10}O_4$ (methylpentose unit)$]^-$, and 243 $[389–H–C_6H_{10}O_4$ (methylpentose unit), aglycone$]^-$.

*Albinosinic acid I* (**13**): white solid; mp 102–105 °C; $[\alpha]_D$–30.6 (*c* 1.0, MeOH); negative ESI-MS *m/z* 855 $[M–H]^-$. FABMS *m/z* 855 $[M–H]^-$, 709 $[M–H–C_6H_{10}O_4$ (methylpentose unit)$]^-$, 563 $[709–H–C_6H_{10}O_4$ (methylpentose unit)$]^-$, 417 $[563–H–C_6H_{10}O_4$ (methylpentose unit)$]^-$, and 271 $[417–H–C_6H_{10}O_4$ (methylpentose unit), aglycone$]^-$.

*Sugar analysis*

Compounds **12** and **13** (3 mg of each one) in 10 ml HCl 4 N were independently refluxed at 90 °C for 1 h. Then, mixtures were diluted with 2.5 mL $H_2O$ and extracted with ether (2 × 5 ml). The organic layer was evaporated, re-suspended in $CHCl_3$ (3 ml), and alkylated with $CH_2N_2$. The aqueous layer was neutralized with KOH 1 N and extracted with *n*-BuOH (10 ml), then washed with $H_2O$ (2 × 5 ml) and concentrated. The thiazolidine derivatives of each mixture were prepared according to previously described procedures (Cruz-Morales et al., 2016). These mixtures were treated with chlorotrimethylsilane (Sigma Sil-A) and analyzed by GC-MS (Cruz-Morales et al., 2016).

*Identification of aglycones*

The derivatized ($CH_2N_2$) residues obtained from acid hydrolysis of **12** and **13** were analyzed by normal-phase HPLC (μPorasil, 3.9 × 300 mm, 10 μm), using an elution [hexane–$CHCl_3$–$Me_2CO$ (6:3:1)] and a flow rate of 1 ml/min to identify methyl (11*S*)-hydroxytetradecanoate ($t_R$ 18.0 min) from compound **1** and methyl (11*S*)-hydroxyhexadecanoate ($t_R$ 15.5 min) from compound **2** by comparison with authentic samples (Cruz-Morales et al., 2016).

*Cytotoxicity and modulation of multidrug-resistance assays*

Cytotoxicity of compounds **1** and **2** was determined by using the SRB assay. Cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and cultured at 37 °C in 5% $CO_2$ in air (100% humidity). MCF-7/Vin$^+$ cells were cultured in medium containing 0.192 μg/ml vinblastine. A stock of MCF-7/Vin cells was also maintained in vinblastine-free medium (MCF-7/Vin$^-$). Cells at log phase were treated in triplicate with test samples (0.2–25 μg/ml) and incubated for 72 h. For the reversal effects, sensitive MCF-7 and MDR MCF-7/Vin cells were seeded into 96-well plates and treated with various concentrations of vinblastine (0.00064–10 μg/ml) in the presence or absence of glycolipids (25 μg/ml) for 72 h. The ability of glycolipids to potentiate vinblastine cytotoxicity was measured by calculating the $IC_{50}$ (Figueroa-González et al., 2012).

## Results and discussion

*HPLC profiling of the resin glycoside content and isolation of 1 and 2*

Preparative reversed-phase HPLC-RI (refractive index detection), using the heart-cutting technique (Pereda-Miranda and Hernández-Carlos, 2002), was used to separate eleven major constituents from the resin glycoside fraction of moon vine seeds (Fig. 1). Profiling of this resin glycosides based on MS allowed dereplication to be performed after fractionation, which included drying and redissolution of the eluted fractions (Potterat and Hamburger, 2013) in order to identify known and novel compounds (Smyth et al., 2012). Each collected peak was analyzed by ESIMS and FAB-MS in both positive and negative modes. Albinosides I–IX (**3–11**) were unambiguously identified by comparison of the fragmentation ions observed in negative mode FAB-MS (Cruz-Morales et al., 2012, 2016), as well as the adduct ion $[M+Na]^+$ detected in ESI (Table 1). This HPLC profiling indicated the presence of

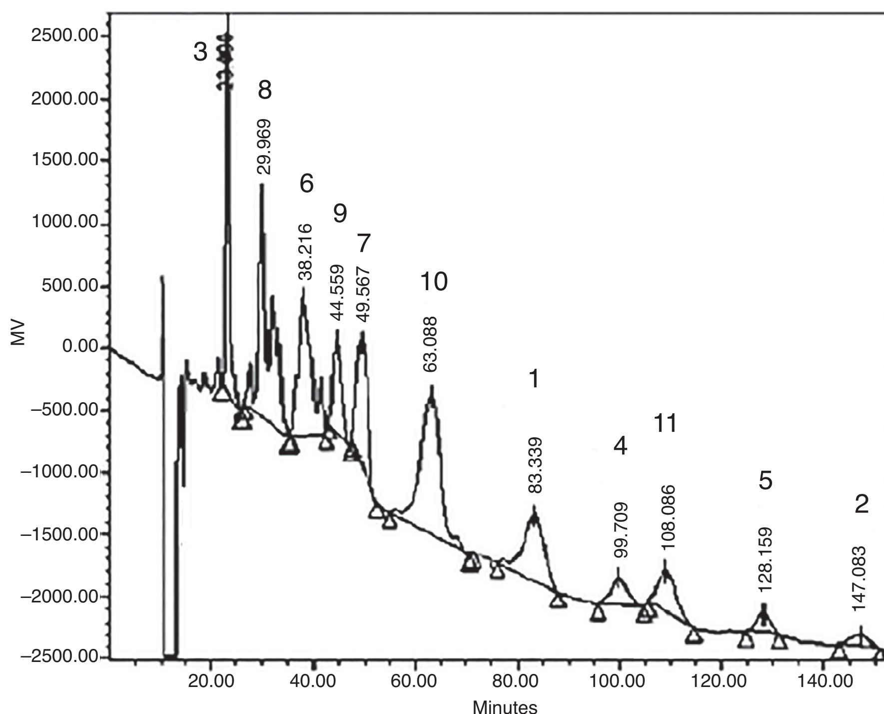



**Fig. 1.** HPLC chromatogram for the resin glycoside mixture from $CHCl_3$-soluble extract. Instrumental conditions: Symmetry $C_{18}$ column (waters: 19 × 300 mm, 7 mm); isocratic elution with MeOH–$CH_3$CN–$H_2O$ (10:7:3); flow rate: 4.0 ml/min; sample injection: 500 μl [0.1 mg/μl]. Compounds: **1** ($t_R$ = 83.3 min, albinoside X); **2** ($t_R$ = 147.1 min, albinoside XI); **3** ($t_R$ = 23.1 min, albinoside I); **4** ($t_R$ = 99.7 min, albinoside II); **5** ($t_R$ = 128.2 min, albinoside III); **6** ($t_R$ = 38.2 min, albinoside IV); **7** ($t_R$ = 49.6 min, albinoside V); **8** ($t_R$ = 29.9 min, albinoside VI); **9** ($t_R$ = 44.6 min, albinoside VII); **10** ($t_R$ = 63.1 min, albinoside VIII); **11** ($t_R$ = 109.1 min, albinoside IX).

two previously unknown glycolipids with $t_R$ = 83.3 and 147.1 min (Fig. 1), which were named as albinoside X (**1**) and albinoside XI (**2**), respectively. Each new peak was recycled in HPLC to achieve chromatographic homogeneity, allowing the purification of compounds **1** and **2**.

*Structural elucidation of 1 and 2*

Albinoside X (**1**) presented a deprotonated molecule ion peak at *m/z* 973.5081 ([M−H]$^-$; $C_{48}H_{77}O_{20}$, calcd error: δ = −0.80 ppm) in HRESIMS, while albinoside XI (**2**) showed a [M−H]$^-$peak at *m/z* 1001.5392 ($C_{50}H_{81}O_{20}$, calcd error: δ = −0.70 ppm). The difference of 28 mass units between **1** and **2**, and the observation of an identical pattern for the glycosidic cleavages, allowed to recognize the same linear tetrasaccharide core in both natural products. The presence of 11*S*-hydroxytetradecanoic acid as the aglycone for compound **1** was confirmed by the diagnostic FAB-MS negative ions at *m/z* 827 [M−H]$^-$, 681, 535, 389 and 243 (aglycone), which corresponded to four consecutive eliminations of methyl pentose units ($C_6H_{10}O_4$, 146 uma), for its saponification derivative, albinosinic acid H (**12**); while for the albinosinic acid I (**13**), negative ions at *m/z* 855 [M−H]$^-$, 709, 563, 417, and 271 confirmed the aglycone as 11*S*-hydroxyhexadecanoic acid for natural product **2**.

Saponification of both natural products liberated 2-methyl-2-butenoic acid as the acylating group. The same sugar composition was obtained by acid hydrolysis of both compounds **12** and **13**, L-rhamnose, D-quinovose, and D-fucose in the ratio 2:1:1. The NMR spectra of both natural products **1** and **2** were very similar (Table 2). For **1**, four anomeric protons were observed at $\delta_H$ 4.77 (1H, d, *J* = 7.6 Hz; $\delta_C$ 103.2, Qui-1); 5.89 (1H, brs; $\delta_C$ 100.4, Rha-1); 5.29 (1H, d, *J* = 7.6 Hz; $\delta_C$ 107.1, Fuc-1), 5.64 (1H, brs; $\delta_C$ 99.9, Rha′-1) in the HSQC spectra. The HSQC spectra registered for compound **2** was almost identical with four anomeric signal centered at $\delta_H$ 4.75 (1H, d, *J* = 7.6 Hz; $\delta_C$ 103.0, Qui-1); 5.88 (1H, brs; $\delta_C$ 100.2, Rha-1); 5.30 (1H, d, *J* = 7.6 Hz; $\delta_C$ 106.9, Fuc-1), 5.64 (1H, brs; $\delta_C$ 99.8, Rha′-1). The glycosylation sequence was established by the long-range heteronuclear coupling correlations ($^3J_{CH}$) in the HMBC spectra. For instance, key correlations were observed in compounds **1** and **2**: (a) the connectivity between H-1 of the quinovose (**1**: $\delta_H$ 4.77; **2**: $\delta_H$ 4.75) and C-11 of the fatty acid (**1**: $\delta_C$ 81.7; **2**: $\delta_C$ 80.9); (b) H-2 of quinovose (**1**: $\delta_H$ 4.44; **2**: $\delta_H$ 4.45) with C-1 of rhamnose (**1**: $\delta_C$ 100.4; **2**: $\delta_C$ 100.2); (c) H-4 of rhamnose (**1**:

**Table 1**
Albinosides identification with ESI-MS and FAB-MS fragmentation.

| Compound | $t_R$ (min) | Formula | MW | ESI [M + Na]$^+$ | FAB [M−H]$^-$ | Fragment ions (FAB negative mode, *m/z*) |
|---|---|---|---|---|---|---|
| **1** (Albinoside X) | 83.3 | $C_{48}H_{78}O_{20}$ | 974.508 | 997 | 973 | 891, 809, 663, 517, 389, 243 |
| **2** (Albinoside XI) | 147.1 | $C_{50}H_{82}O_{20}$ | 1002.539 | 1025 | 1001 | 919, 837, 691, 545, 417, 271 |
| **3** (Albinoside I) | 23.1 | $C_{51}H_{86}O_{26}$ | 1114.532 | 1137 | 1113 | 1013, 971, 825, 679, 533, 389, 243 |
| **4** (Albinoside II) | 99.7 | $C_{54}H_{88}O_{24}$ | 1120.558 | 1153 | 1119 | 1037, 891, 745, 517, 389, 243 |
| **5** (Albinoside III) | 128.2 | $C_{57}H_{96}O_{24}$ | 1164.620 | 1187 | 1163 | 1081, 935, 853, 707, 561, 417, 271 |
| **6** (Albinoside IV) | 38.2 | $C_{49}H_{82}O_{24}$ | 1054.513 | 1077 | 1053 | 971, 907, 825, 679, 533, 389, 243 |
| **7** (Albinoside V) | 49.6 | $C_{54}H_{90}O_{25}$ | 1138.569 | 1161 | 1137 | 1037, 891, 809, 791, 745, 663, 517, 389, 243 |
| **8** (Albinoside VI) | 29.9 | $C_{45}H_{76}O_{22}$ | 968.470 | 991 | 967 | 867, 679, 551, 533, 389, 243 |
| **9** (Albinoside VII) | 44.6 | $C_{45}H_{76}O_{21}$ | 952.477 | 975 | 951 | 851, 809, 663, 535, 517, 389, 371, 243 |
| **10** (Albinoside VIII) | 63.1 | $C_{48}H_{78}O_{21}$ | 990.496 | 1013 | 989 | 907, 825, 679, 533, 389, 243 |
| **11** (Albinoside IX) | 109.1 | $C_{50}H_{82}O_{21}$ | 1018.527 | 1041 | 1017 | 935, 853, 707, 561, 417, 271 |

**Table 2**
$^{1}H$ NMR and $^{13}C$ NMR spectra data of albinosides X–XI (**1**–**2**) (400 MHz and 100 MHz, $C_5D_5N$, $\delta$ ppm, $J$ in Hz).

| Position | 1 | | 2 | |
|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| Qui-1 | 4.77 (d, $J$ = 8.0 Hz) | 103.2 | 4.75 (d, $J$ = 8.0 Hz) | 103.0 |
| 2 | 4.44 (dd, $J$ = 9.0, 8.0 Hz) | 77.1 | 4.45 (dd, $J$ = 9.0, 8.0 Hz) | 76.8 |
| 3 | 4.33 (dd, $J$ = 9.0, 9.0 Hz) | 79.6 | 4.33 (t, $J$ = 9.0, 9.0 Hz) | 79.4 |
| 4 | 3.63 (dd, $J$ = 9.0, 9.0 Hz) | 78.0 | 3.65 (dd, $J$ = 9.0, 9.0 Hz) | 77.7 |
| 5 | 3.67 (dq, $J$ = 9.0, 6.0 Hz) | 73.2 | 3.67 (dq, $J$ = 9.0, 6.0 Hz) | 72.9 |
| 6 | 1.59 (d, $J$ = 6.0 Hz) | 19.7 | 1.61 (d, $J$ = 6.0 Hz) | 19.1 |
| Rha-1 | 5.89 (brs) | 100.4 | 5.88 (brs) | 100.2 |
| 2 | 4.79 (dd, $J$ = 3.0, 2.0 Hz) | 70.7 | 4.80 (dd, $J$ = 3.0, 2.0 Hz) | 70.5 |
| 3 | 5.76 (m*) | 79.7 | 5.75 (t, $J$ = 8.8 Hz) | 79.5 |
| 4 | 4.32 (dd, $J$ = 9.9, 9.9 Hz) | 86.1 | 4.33 (dd, $J$ = 9.9, 9.9 Hz) | 85.9 |
| 5 | 4.83 (dq, $J$ = 9.9, 6.5 Hz) | 68.9 | 4.84 (dq, $J$ = 9.6, 6.5 Hz) | 68.6 |
| 6 | 1.92 (d, $J$ = 6.5 Hz) | 19.3 | 1.93 (d, $J$ = 6.5 Hz) | 19.3 |
| Rha′-1 | 5.64 (brs) | 99.9 | 5.64 (brs) | 99.8 |
| 2 | 5.88 (dd, $J$ = 3.0, 2.0 Hz) | 74.3 | 5.88 (dd, $J$ = 3.0, 2.0 Hz) | 73.9 |
| 3 | 4.15 (m*) | 81.9 | 4.34 (dd, $J$ = 9.0, 3.0 Hz) | 79.4 |
| 4 | 3.66 (dd, $J$ = 9.9, 9.9 Hz) | 75.9 | 3.68 (dd, $J$ = 9.9, 9.9 Hz) | 75.6 |
| 5 | 3.86 (dq, $J$ = 9.9, 6.5 Hz) | 72.6 | 3.85 (dq, $J$ = 9.9, 6.5 Hz) | 72.3 |
| 6 | 1.49 (d, $J$ = 6.5 Hz) | 18.8 | 1.49 (d, $J$ = 6.5 Hz) | 18.6 |
| Fuc-1 | 5.29 (d, $J$ = 7.6 Hz) | 107.1 | 5.30 (d, $J$ = 7.6 Hz) | 106.9 |
| 2 | 4.33 (dd, $J$ = 9.2, 7.6 Hz) | 74.2 | 4.33 (dd, $J$ = 9.6, 7.6 Hz) | 73.7 |
| 3 | 4.25 (dd, $J$ = 9.2, 3.0 Hz) | 80.9 | 4.25 (dd, $J$ = 9.2, 3.0 Hz) | 81.7 |
| 4 | 5.63 (brs) | 74.9 | 5.64 (brs) | 74.7 |
| 5 | 3.93 (q, $J$ = 6.0 Hz) | 70.9 | 3.93 (q, $J$ = 6.0 Hz) | 70.7 |
| 6 | 1.32 (d, $J$ = 6.4 Hz) | 17.7 | 1.31 (d, $J$ = 6.4 Hz) | 17.4 |
| conv-1 | | 173.2 | | |
| 2a | 2.82 (ddd, $J$ = 10.4, 7.6, 2.0 Hz) | 35.2 | | |
| 2b | 2.43 (ddd, $J$ = 10.4, 7.6, 2.0 Hz) | | | |
| 11 | 3.79–3.82 (m*) | 81.7 | | |
| 14 | 0.90 (t, $J$ = 7.2 Hz) | 14.8 | | |
| jal-1 | | | | 173.0 |
| 2a | | | 2.88 (ddd, $J$ = 16.4, 10.4, 6.0 Hz) | 35.6 |
| 2b | | | 2.43 (ddd, $J$ = 16.4, 10.4, 6.0 Hz) | |
| 11 | | | 3.82–3.88 (m*) | 80.9 |
| 16 | | | 0.85 (t, $J$ = 6.8 Hz) | 14.7 |
| tga-1 | | 168.2 | | 168.0 |
| 2 | | 129.7 | | 129.4 |
| 3 | 6.99 (dq, $J$ = 7.2, 1.6 Hz) | 138.3 | 6.99 (dq, $J$ = 7.2, 1.6 Hz) | 138.0 |
| 4 | 1.49 (d, $J$ = 7.2 Hz) | 14.9 | 1.49 (d, $J$ = 7.2 Hz) | 14.6 |
| 5 | 1.85 (brs) | 13.1 | 1.81 (brs) | 12.7 |
| tga′-1 | | 169.0 | | 169.0 |
| 2 | | 129.6 | | 129.3 |
| 3 | 7.13 (dq, $J$ = 7.2, 1.2 Hz) | 138.8 | 7.13 (dq, $J$ = 7.2, 1.2 Hz) | 138.5 |
| 4 | 1.47 (d, $J$ = 7.2 Hz) | 15.1 | 1.45 (d, $J$ = 7.2 Hz) | 14.5 |
| 5 | 1.87 (brs) | 13.0 | 1.86 (brs) | 12.7 |

Chemical shifts $\delta$ are in ppm relative to TMS.
Chemical shifts marked with an asterisk (*) indicate overlapped signals.
*Abbreviations*: qui, quinovose; rha, rhamnose; fuc, fucose; conv, 11-hydroxytetradecanoyl; jal, 11-hydroxyhexadecanoyl; tga, tigloyl.

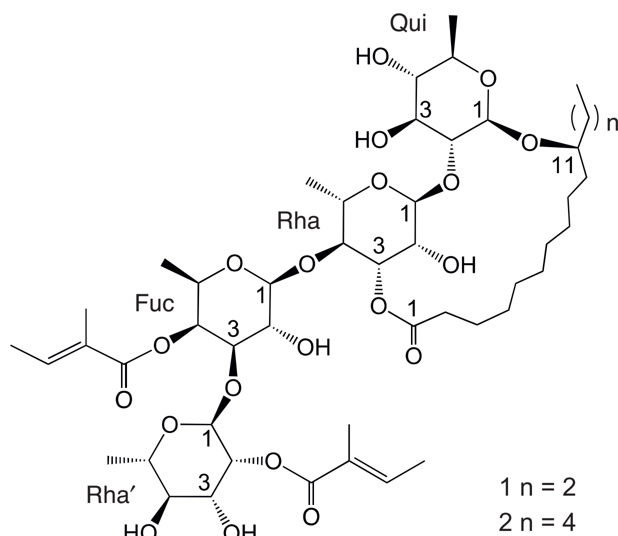
$\delta_H$ 4.32; **2**: $\delta_H$ 4.33) with C-1 of fucose (**1**: $\delta_C$ 107.1; **2**: $\delta_C$ 106.9); and (d) H-3 of fucose ($\delta_H$ 4.25) with C-1 of Rha′ (**1**: $\delta_C$ 99.9; **2**: $\delta_C$ 99.8). Accordingly, the glycosidic acid for compound **1**, named as albinosidic acid H (**12**), corresponded to (11*S*)-hydroxytetradecanoic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-β-D-fucopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-6-deoxy-β-D-glucopyranoside, while the structure for albinosidic acid I (**13**), the glycosidic acid for compound **2**, was characterized as (11*S*)-hydroxyhexadecanoic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-β-D-fucopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-6-deoxy-β-D-glucopyranoside. For the natural products, the macrolactonization site was located by the $^{3}J_{CH}$ correlations between H-3 (**1**: $\delta_H$ 5.76; **2**: $\delta_H$ 5.75) of Rha with C-1 (**1**: $\delta_C$ 173.2; **2**: $\delta_C$ 173.0) of the fatty acid in the HMBC spectrum. The tigloyl group linkages were at the same location on the oligosaccharide core for **1** and **2**. Thus, H-2 ($\delta_H$ 5.88) of Rha’ correlated with the C-1 (**1**: $\delta_C$ 168.2; **2**: $\delta_C$ 168.0) signal

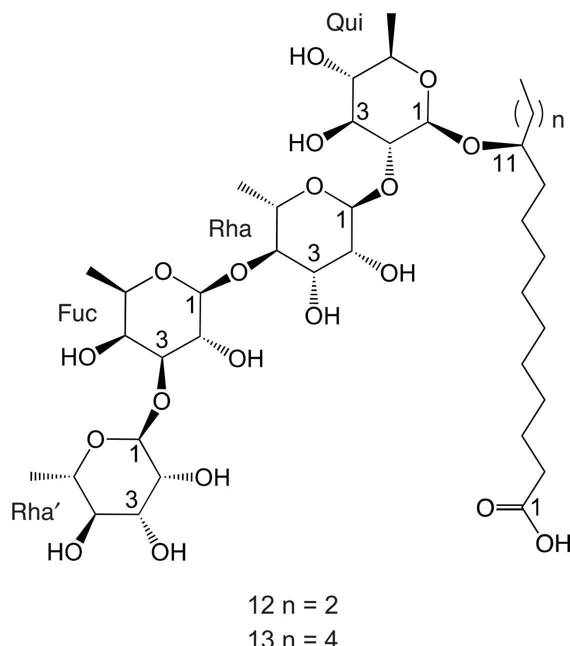
**Table 3**
Modulation of vinblastine cytotoxicity in drug sensitive MCF-7 and multidrug-resistant MCF-7/Vin by 1 and 2.

| Compound[a] | $IC_{50}$ (μg/ml) | | | Reversal fold[c] | | |
|---|---|---|---|---|---|---|
| | MCF-7/Vin⁻ | MCF-7/Vin⁺ | MCF-7 sens | $RF_{MCF\text{-}7/Vin^-}$ | $RF_{MCF\text{-}7/Vin^+}$ | $RF_{MCF\text{-}7\ sens}$ |
| Vinblastine | 1.08 ± 0.06 | 1.37 ± 0.23 | 0.047 ± 0.01 | | | |
| **1** | 0.98 ± 0.16 | 0.79 ± 0.098 | 0.015 ± 0.02 | 1.1 | 1.7 | 3.1 |
| **2** | <0.00064 | <0.00064 | <0.00064 | >1687.5 | >2140.6 | >73.4 |
| Reserpine[b] | 0.037 ± 0.01 | 0.31 ± 0.19 | 0.003 ± 0.001 | 29.2 | 4.4 | 15.7 |

[a] Serial dilutions from 0.00064 to 10 μg/ml of vinblastine in the presence or absence of glycolipid (25 μg/ml).
[b] Reserpine = 5 μg/ml as positive control.
[c] RF = $IC_{50}$ Vinblastine/$IC_{50}$ Vinblastine in the presence of glycolipid. Each value represents the mean ± SD from three independent experiments.

of one tigloyl unit (tga) and H-4 (**1**: $\delta_H$ 5.63; **2**: $\delta_H$ 5.64) of Fuc correlated with C-1 ($\delta_C$ 169.0) of the second tigloyl group (tga′).




*Modulation of multidrug resistance*

Based on our previous results (Cruz-Morales et al., 2012, 2016), a modulation assay was used to identify chemosensitizers through the potentiation of vinblastine susceptibility by compounds **1** and **2** in MDR cells (MCF-7/Vin$^+$ cells), using the cytotoxicity screening with SRB. The reversal fold values ($RF_{MCF\text{-}7/Vin+}$) are included in Table 3. A moderate activity was displayed by the non-cytotoxic albinoside X (**1**) (RF 2.0) which was similar to that reported for albinosides I (**3**, RF 3.1), II (**4**, RF 2.6), V (**7**, RF 2.3), and VI (**8**, RF 2.1) (Cruz-Morales et al., 2012, 2016). Albinoside XI (**2**) was moderately active against various carcinoma cell lines, including the MCF-7/Vin$^+$ cells ($IC_{50}$ 4–9 μM). Consequently, its strong reversal activity (RF >2140.6) could be the result of an additive synergism between cytotoxicity and modulation of efflux pumps.

## Conclusion

For the HPLC-MS profiling, low resolution FABMS in negative mode was used for recording the fragmentation pattern of each eluted fraction and pure compounds, since this is the only procedure among the soft ionization techniques that provides a clear sequence for the fragmentation along the oligosaccharide core. Peaks resulting from the anomeric cleavages in conjunction with those produced by eliminations of the esterifying residues are abundant. In consequence, the intensity for some of the deprotonated molecule ions [M–H]$^-$ was low. However, ESIMS provided highly abundant [M–H]$^-$ ions in negative mode, as well as adduct ion [M+Na]$^+$ detected in positive mode. Thus, this profiling based on HPLC-MS allowed the identification of novel compounds **1** and **2** as well as known compounds **3**–**11**.

The unexpected susceptibility of the MDR MCF-7/Vin$^+$ cells to compound **2** could represent an example of collateral hypersensitivity, *i.e.*, the MCF-7 strain, in adapting to vinblastine also acquired hypersensitivity to alternative cytotoxins as albinosides. Therefore, the additive synergism expressed in the reversal activity of **2** could be significant in therapy regimes with combination of anticancer drugs and deserves further studies. These results support the potential of resin glycosides as inhibitors of multidrug efflux pumps in mammalian cancer cells, where glycoprotein-P is the prevailing translocase responsible for the resistant phenotype.

## Authors' contributions

JCG and SCM performed the isolation of novel compounds and completed their structure elucidation. DRR prepared derivatives and accomplished HPLC-MS dereplication. MFS performed the bioassays and supervised HPLC procedures. RPM planned the study and contributed to the preparation of the document final version.

## Ethical disclosures

**Protection of human and animal subjects.** The authors declare that no experiments were performed on humans or animals for this study.

**Confidentiality of data.** The authors declare that no patient data appear in this article.

**Right to privacy and informed consent.** The authors declare that no patient data appear in this article.

## Conflicts of interest

The authors declare no conflicts of interest.

## Acknowledgments

This research was supported by grants from Dirección General de Asuntos del Personal Académico (UNAM, IN215016) and Consejo Nacional de Ciencia y Tecnología (CB220535). Thanks are due to Georgina Duarte (Facultad de Química, UNAM) for FABMS recordings.

## References

Corona-Castañeda, B., Pereda-Miranda, R., 2012. Morning glory resin glycosides as modulators of antibiotic activity in multidrug-resistant Gram-negative bacteria. Planta Med. 78, 128–131.

Cruz-Morales, S., Castañeda-Gómez, J., Figueroa-González, G., Mendoza-García, A.D., Lorence, A., Pereda-Miranda, R., 2012. Mammalian multidrug resistance lipopentasaccharide inhibitors from *Ipomoea alba* seeds. J. Nat. Prod. 75, 1603–1611.

Cruz-Morales, S., Castañeda-Gómez, J., Rosas-Ramírez, D., Fragoso-Serrano, M., Figueroa-González, G., Lorence, A., Pereda-Miranda, R., 2016. Resin glycosides from *Ipomoea alba* seeds as potential chemosensitizers in breast carcinoma cells. J. Nat. Prod. 79, 3093–3104.

Fang, Y., Chai, W., Chen, S., He, Y., Zhao, L., Peng, J., Huang, H., Xin, B., 1993. On the structure of calonyctin A, a plant growth regulator. Carbohydr. Res. 245, 259–270.

Figueroa-González, G., Jacobo-Herrera, N., Zentella-Dehesa, A., Pereda-Miranda, R., 2012. Reversal of multidrug resistance by morning glory resin glycosides in human breast cancer cells. J. Nat. Prod. 75, 93–97.

Foxcroft, L., Richardson, D., Wilson, J., 2008. Ornamental plants as invasive aliens: problems and solutions in Kruger National Park, South Africa. Environ. Manage. 41, 32–51.

Lim, T.K., 2014. Edible medicinal and non-medicinal plants. Flowers 7, 710.

McDonald, A., 1994. Flora de Veracruz. Instituto de Ecología A.C. Xalapa, University of California, Riverside, CA, pp. 13–18.

Pereda-Miranda, R., Hernández-Carlos, B., 2002. HPLC isolation and structural elucidation of diastereomeric niloyl ester tetrasaccharides from Mexican scammony root. Tetrahedron 58, 3145–3154.

Pereda-Miranda, R., Kaatz, G.W., Gibbons, S., 2006. Polyacylated oligosaccharides from medicinal Mexican morning glory species as antibacterials and inhibitors of multidrug resistance in *Staphylococcus aureus*. J. Nat. Prod. 69, 406–409.

Pereda-Miranda, R., Rosas-Ramírez, D., Castañeda-Gómez, J., 2010. Resin glycosides from the morning glory family. In: Kinghorn, A., Falk, H., Kobayashi, J. (Eds.), Progress in the Chemistry of Organic Natural Products, vol. 92. Springer Verlag, New York, pp. 77–153.

Potterat, O., Hamburger, M., 2013. Concepts and technologies for tracking bioactive compounds in natural product extracts: generation of libraries and hyphenation of analytical processes with bioassays. Nat. Prod. Rep. 30, 546–564.

Smyth, W.F., Smyth, T.J.P., Ramachandran, V.N., O'Donnell, F., Brooks, P., 2012. Dereplication of phytochemicals in plants by LC–ESI–MS and ESI–MS. Trends Anal. Chem. 33, 46–54.