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Jalapinoside II, a bisdesmoside resin glycoside, and related glycosidic acids from the officinal jalap root (*Ipomoea purga*)

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ABSTRACT

Jalapinoside II, a macrocyclic bidesmoside resin glycoside with purgic acid C as its oligosaccharide core, was isolated by recycle preparative-scale HPLC from the MeOH-soluble extract of *Ipomoea purga* (Convolvulaceae), the officinal jalap root ("Rhizoma Jalapae"). This study also reports the complete NMR data assignment for purgic acid C and the structural elucidation of purgic acid D, both glycosidic acids were isolated, in conjunction with previously known purgic acids A and B, under saponification of the MeOH-soluble resin glycosides. HPLC and ^{13}C NMR profiles were used as analytical tools for the authentication of the commercialized Mexican scammony resin. Reversal factor of multidrug resistance by the non-cytotoxic jalapinoside II was evaluated in vinblastine-resistant human breast carcinoma cells ($RF_{MCF\text{-}7/Vin+} > 1906$).

1. Introduction

Historically, plant natural products have contributed to provide highly polyfunctionalized biologically active compounds or structural scaffolds for the development of anticancer drugs such as podophyllotoxin, taxol, and vinblastine (Cragg et al., 2014). However, the gradual loss of hypersensitivity by malignant cells to anticancer drugs has derived in multidrug resistance (MDR), which is one of the main causes limiting the success of cancer chemotherapy. An increasing number of mechanisms of multidrug resistance in cancer cells have been described. Nevertheless, the most commonly found is the efflux of a broad range of cytotoxic compounds mediated by ATP-binding cassette (ABC) transporters (Szakács et al., 2014), such as P-glycoprotein (MDR protein1/P-gp, ABCB1) and the breast cancer resistance protein (BCRP/ABCG2), which have the task of detoxifying cells by detecting and expelling hydrophobic toxins of innumerable structural types, including anticancer drug (Roundhill et al., 2015).

Resin glycosides are complex mixtures of glycolipids present in some members of the morning glory family (Convolvulaceae), many of which belong to the genera *Ipomoea*, *Merremia*, and *Operculina* (Pereda-Miranda et al., 2010). Recently, members of the *Ipomoea* genus were described as sources of resin glycosides which functioned as modulators of efflux pumps that produce the multidrug-resistant phenotype in Gram-positive (Pereda-Miranda et al., 2006b) and Gram-negative bacteria (Corona-Castañeda and Pereda-Miranda, 2012), as well as in mammalian cancer cells (Bautista et al., 2015; Castañeda-Gómez et al., 2013; Cruz-Morales et al., 2012; Figueroa-González et al., 2012). These glycolipids significantly lowered the efflux rate of rhodamine 123, a fluorescent efflux pump substrate, used to determine its accumulation in efflux assays with MDR MCF-7/Vin cells. Immunofluorescence flow cytometry was used to detect the decreased expression of P-gp by resin glycosides after incubation of MDR MCF-7/Vin cells with an anti-P-gp monoclonal antibody (Figueroa-González et al., 2012). Two examples of these potent MDR modulatory glycolipids are purgin II and jalapinoside I (**1**) from *I. purga.* Reversal fold ($RF_{MCF\text{-}7/Vin} > 1906$) indicated that both natural products are extremely potent as vinblastine chemosensitizer at 25 μg/mL in modulation assays (Castañeda-Gómez et al., 2013; Bautista et al., 2015).

For these reasons, our efforts have been oriented to investigate the chemical diversity of this class of MDR reversal agents from the convolvulin fraction (ether insoluble or methanol-soluble resin glycosides) of *I. purga*. In addition to its use as a purgative, this member of the Mexican jalap root complex has been employed as an anthelmintic and galactogogue, as well as in the treatment of tumors. A 2 cm section of root boiled in a liter of water produces a

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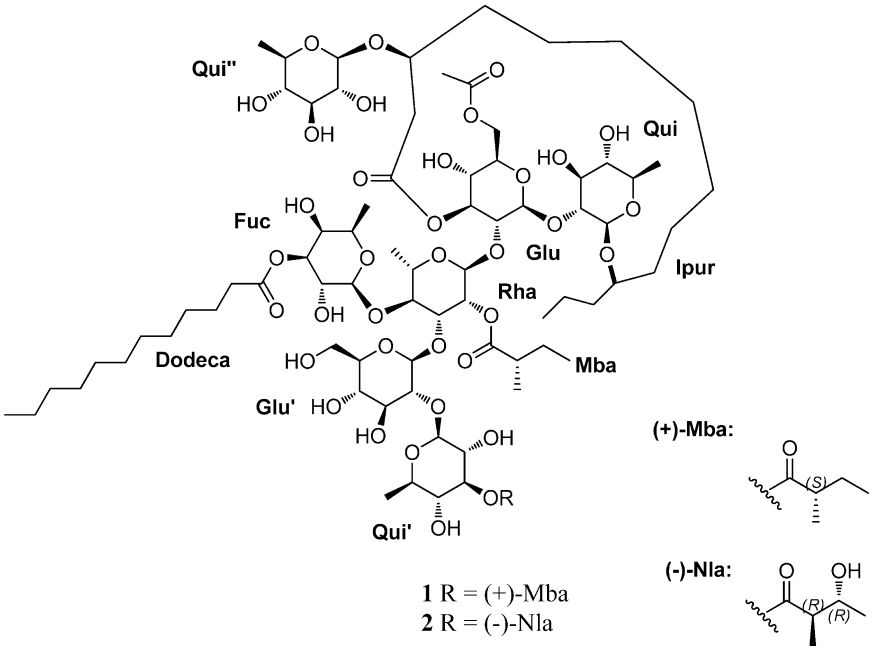

Fig. 1. Chemical structures of jalapinosides I (**1**) and II (**2**).

decoction, a cup of which is taken cold before bedtime (Pereda-Miranda et al., 2006a). In this work, we describe the isolation, structural elucidation, and the modulatory activity of multidrug-resistance in MCF-7 cancer cells of jalapinoside II (**2**), a novel bidesmoside resin glycoside from the MeOH-soluble extract prepared with the roots of *I. purga*. Also, it is described the complete NMR data assignment for purgic acid C (**3**) as well as the structural elucidation of a new glycosidic acid, named purgic acid D (**4**). These glycosidic acids were used to generate HPLC and ^{13}C NMR profiles both used as analytical tools for authentication of the commercialized Mexican scammony resin.

2. Results and discussion

From the soluble MeOH extract of *I. purga*, a bidesmoside resin glycoside, which was named as jalapinoside II (**2**, Fig. 1), was isolated by recycling preparative-scale HPLC. This compound represents the second intact bidesmosidic macrolactone resin glycoside isolated from the convolvulin fraction of the jalap root. It is important to emphasize the difficulties associated with the isolation and purification of intact highly polar resin glycosides, since all previous work on the chemical constituents of methanol-soluble extracts from members of the morning glory family have been limited to the isolation of their glycosidic acids after chemical degradation (Ono et al., 1990; 2010).

The saponification of the soluble MeOH crude extract yielded two fractions: a non-polar portion extracted with $CHCl_3$ and an H_2O-soluble fraction; the non-polar $CHCl_3$-soluble fraction was analyzed by GC–MS to allow the identification of acetic, (2*S*)-2-methyl-butanoic, (2*S*,3*S*)-3-hydroxy-2-methylbutanoic (nilic), and dodecanoic acids, as the acylating residues; the H_2O-soluble fraction was partitioned with *n*-BuOH, further peracetylated, and subsequently analyzed by recycling HPLC (Pereda-Miranda and Hernández-Carlos, 2002), allowing the isolation of four peaks. The first two peaks (t_R 7.8 and 11.5 min) were identified as the peracetates of purgic acids A (**5a**) and B (**6a**) (Fig. 2) through the comparison of their 1D NMR data, HPLC retention time, and melting points with those previously described (Pereda-Miranda et al., 2006a). Two additional peracetylated peaks (t_R values of 14.2 and 40.5 min) were subsequently saponificated and identified as new glycosidic acids on the basis of their MS and NMR data, and named as purgic acids C (**3**) and D (**4**) (Fig. 2). These glycosidic acids

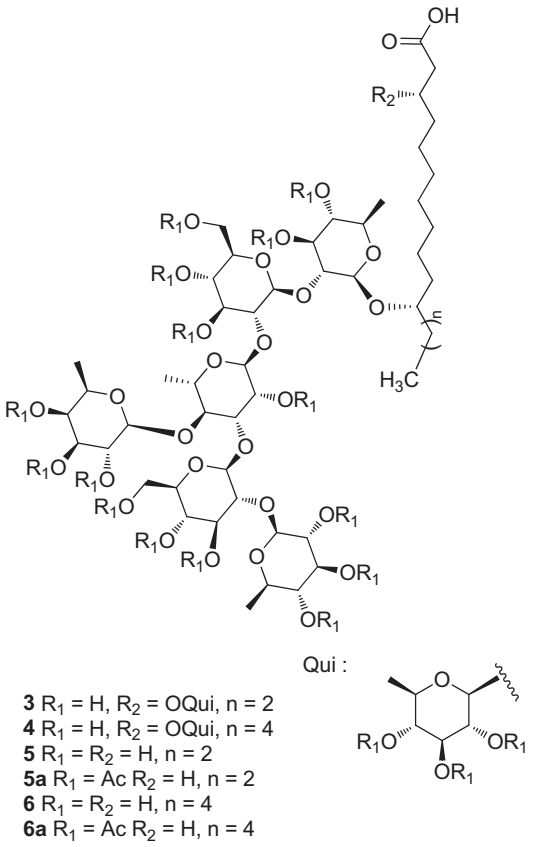

Fig. 2. Structure of purgic acids A-D.

possess the same glycosylation sequence in the oligosaccharide core as purgic acids A (**5**) and B (**6**) (Fig. 2); although both differing from each other in their aglycone: ipurolic acid, (3*S*,11*S*)-dihydroxytetradecanoic acid (Ono et al., 2010) in purgic acid C (**3**), and ipolearic acid, (3*S*,11*S*)-dihydroxyhexadecanoic acid (Ono et al., 1990) in purgic acid D (**4**). In addition, they presented a quinovose unit linked at the hydroxyl group at C-3 of the aglycone (Fig. 2) as do most of the glycosidic acids isolated from ether-insoluble resin glycosides (Ono et al., 1990; 2010).

These structural features for compound **3** were supported by its acid hydrolysis products which included ipurolic acid and the following monosacharides: D-quinovose, D-glucose, D-fucose, and L-ramnose in a ratio of 3:2:1:1 (Bautista et al., 2015). In addition, the ESIMS data for **3** gave quasi-molecular ions at *m/z* 1313 $[M-H]^-$ and 1337 $[M+Na]^+$ as well as readily detectable negative ions by FABMS resulting from glycosidic cleavage of the sugar moieties at *m/z* 1167 $[1313-146]^-$, 1021 $[1167-146]^-$, 859 $[1021-162]^-$, 713 $[859-146]^-$, 567 $[713-146]^-$, and 405 $[567-162]^-$. All of these ions are consistent with previously described spectra (Bautista et al., 2015). The micro-scale saponification originally performed with jalapinoside I (**1**) afforded purgic acid C (**3**) in a very small quantity to complete its spectroscopic characterization by NMR (Bautista et al., 2015). Thus, the scale-up of this reaction allowed the purification of a sufficient amount of compound **3** to complete the analysis of its 1D and 2D NMR spectra (Tables 1 and 2). Seven spin systems were observed in the TOCSY experiment. The signals for each one were assigned by the correlations showed in the COSY spectrum, starting from the anomeric signal for each system: δ_H 4.71, d, *J* = 7.6 Hz, Qui-1; 5.64, d, *J* = 7.6 Hz; Glc-1; 6.28, br s, Rha-1; 6.01, d, *J* = 7.8 Hz, Glc′-1; 5.20, d, *J* = 7.6 Hz, Qui′-1; 5.62, d, *J* = 7.5 Hz, Fuc-1; 4.75, d, *J* = 7.6 Hz, Qui″-1. In the ^{13}C NMR spectrum, the same number of anomeric signals was observed at δ_C 102.7 (Qui-1), 102.8 (Glc-1), 101.0 (Rha-1), 101.6 (Glc′-1), 105.0 (Qui′-1), 103.6 (Fuc-1), and 104.0 (Qui″-1). The HSQC and HMBC experiments allowed the assignments of the additional ^{13}C NMR signals. The glycosylation pattern of **3** was established as the same for purgic acids A (**5**) and B (**6**) on the basis of the observed following HMBC $^3J_{CH}$ cross peaks: H-1 (4.71, Qui-1) with C-11 (78.8, Ipur-11); H-1 (5.64, Glc-1) with C-2 (80.1, Qui-2); H-1 (6.28, Rha-1) with C-2 (74.8, Glc-2); H-3 (5.16, Rha-3) with C-1 (101.6, Glc′-1); H-2 (4.07-3.97, Glc′-2) with C-1 (105.0, Qui′-1); and from H-1 (5.62, Fuc-1) with C-4 (79.3, Rha-4). Additionally, the HMBC cross peak of H-1 (4.75, Qui″-1) with C-3 (63.8, Ipur-3) confirmed the position for the bidesmosidic portion.

Acid hydrolysis of **4** afforded ipolearic acid as well as D-quinovose, D-glucose, D-fucose, and L-ramnose in a ratio of 3:2:1:1. The ESIMS/MS data in the positive mode for purgic acid D (**4**), showed the quasi-molecular ion at *m/z* 1343 $[M+H]^+$. The following ion peaks resulted from the glycosidic cleavage common to bidesmosidic MeOH-soluble resin glycosides (Bautista et al., 2015; Ono et al., 2010): 1197 [M + H − 146 (methylpentose unit)]$^+$, 1051 $[1197-146]^+$, 889 (1051–162 (hexose unit)]$^+$, 743 $[889-146]^+$, 597

Table 1
^1H (400 MHz) NMR Data of Purgic acids C (**3**) and D (**4**), as well as their Peracetates (**3a** and **4a**) in Piridine-d_5 (δ in ppm, *J* in Hertz).

position	3	3a	4	4a	position	3	3a	4	4a
Qui-1	4.71 d (7.6)	4.87 d (7.9)	4.77 d (7.6)	4.86 d (7.9)	Qui'-1	5.20 d (7.6)	5.16 d (7.6)	5.10 d (7.6)	5.16 d (8.0)[a]
2	4.17–4.10[a]	5.42 dd (8.9, 7.9)	4.16 dd (8.6, 7.6)[a]	5.42 dd (8.6, 7.9)	2	4.08–4.01[a]	5.62–5.55[a]	4.08–4.01[a]	5.72–5.63[a]
3	4.43 dd (8.4, 8.3)	5.72–5.66[a]	4.63 dd (8.9, 8.6)	5.72–5.66[a]	3	4.03–3.97[a]	5.62–5.55[a]	4.03–3.97[a]	5.62–5.55[a]
4	3.45 dd (9.0, 8.4)	5.19 dd (9.4, 9.2)[a]	3.53 dd (9.0, 8.9)	5.17 dd (9.6, 9.1)[a]	4	3.63 dd (9.0, 8.8)	5.38 dd (9.4,8.9)	3.63 dd (9.0, 8.8)	5.37 dd (9.6, 9.0)
5	3.71–3.62[a]	3.98 dq (9.4, 5.8)	3.71–3.62[a]	4.00 dq (9.6, 6.0)	5	3.78–3.73[a]	3.73 m[a]	3.77 dd (8.8, 5.9)[a]	3.76 m[a]
6	1.45 d (6.0)	1.46 d (5.7)[a]	1.45 d (6.0)	1.46 d (6.0)[a]	6	1.64 d (5.4)	147 d (5.7)[a]	1.64 d (5.9)	1.48 d (6.0)[a]
Glc-1	5.64 d (7.6)	4.75 d (7.5)	5.74 d (7.6)	4.75d (7.3)	Fuc-1	5.62 d (7.5)	5.62 d (7.2)	5.79 d (7.5)	5.62 d (7.5)
2	4.29–4.22[a]	4.22 dd (7.8, 7.5)[a]	4.28 dd (9.6, 7.6)[a]	4.26 dd (8.1, 7.3)[a]	2	4.15–4.05[a]	5.73 dd (10.0, 8.3)	4.34 dd (9.3, 7.5)[a]	5.84 dd (9.8, 7.6)
3	4.21–4.10[a]	5.62 dd (9.0, 7.8)[a]	4.19–4.12[a]	5.63 dd (9.0, 7.9)[a]	3	4.10–4.04[a]	5.65–5.53[a]	4.35–4.24[a]	5.68–5.62[a]
4	4.07–4.01[a]	5.10 dd (9.4, 9.0)	4.07–4.01[a]	5.13 dd (9.5, 9.0)	4	4.05–3.97[a]	5.75–5.70[a]	4.05–3.97[a]	5.75–5.70[a]
5	3.81–3.75[a]	4.25–4.18[a]	3.81–3.75[a]	4.25–4.15[a]	5	3.76–3.72[a]	4.30–4.15[a]	3.76–3.72[a]	4.30–4.15[a]
6a	4.37 dd (9.1, 3.0)	4.60–4.55[a]	4.43 dd (10.5, 3.0)	4.60–4.55	6	1.59 d (6.3)	1.34 d (5.9)	1.53 d (6.3)	1.34 d (6.0)
6b	4.36–4.29[a]	4.25–4.21[a]	4.36–4.29[a]	4.28–4.23[a]	Qui"-1	4.75 d (7.6)	5.26 d (8.2)	4.83 d (7.6)	5.27 d (8.1)
Rha-1	6.28 br s	5.50 br s[a]	6.45 br s	5.51 br s[a]	2	4.01–3.89[a]	5.57–5.48[a]	4.05–3.97[a]	5.56–5.45[a]
2	4.86 dd (3.0, 1.5)[a]	5.70–5.65[a]	4.85 dd (3.0, 1.5)[a]	5.70–5.65[a]	3	4.20–4.09[a]	5.68 dd (9.0, 8.8)[a]	4.20–4.13[a]	5.68 dd (9.6, 9.0)
3	5.16 dd (9.1, 3.0)	4.70–4.50[a]	5.31 dd (9.1, 3.0)	4.66–4.60[a]	4	3.66–3.62[a]	5.52 dd (9.4, 8.8)[a]	3.80–3.72[a]	5.52 dd (9.4, 9.0)
4	4.86–4.76[a]	4.30–4.21[a]	4.80–4.76[a]	4.33–4.25[a]	5	3.73–3.68[a]	3.87 dq (9.4, 5.7)	3.83–3.78[a]	3.84 dq (9.4, 5.9)
5	5.06 dd (9.0, 6.0)	4.55–4.45[a]	5.20 dd (9.0, 6.1)	4.60–4.50[a]	6	1.47 d (6.2)	1.29 d (5.7)	1.67 d (6.2)	1.29 d (5.9)
6	1.83 d (6.0)	1.88 d (6.4)	1.95 d (6.1)	1.87 d (6.0)	Ipur−1 or Ipol−1[b]				
Glc'-1	6.01 d (7.8)	5.49 d (7.3)[a]	6.25 d (7.8)	5.49 d (7.5)[a]	2a	2.57–2.51[a]	2.62–2.56[a]	2.55–2.51[a]	2.65–2.57[a]
2	4.07–3.97[a]	4.25–4.19[a]	4.07–3.97[a]	4.32–4.28[a]	2b	2.51–2.46[a]	2.56–2.50[a]	2.50–2.46[a]	2.57–2.51[a]
3	4.56–4.48[a]	5.64–5.57[a]	4.56–4.48[a]	5.68–5.60[a]	3	4.30–4.25[a]	4.35–4.30[a]	4.30–4.25[a]	4.35–4.30[a]
4	4.08–4.02[a]	5.30 dd (10.4, 9.4)	4.08–4.02[a]	5.30 dd (10.6, 9.5)	11	3.85 m	3.70 m[a]	3.91 m	3.74 m[a]
5	4.20–4.13[a]	4.34–4.28[a]	4.20–4.13[a]	4.36–4.27[a]	14 or 16	0.90 t (7.1)	0.89 t (6.6)	0.95 t (7.1)[a]	0.90 t (7.0)
6a	4.47–4.41[a]	4.58–4.52[a]	4.55 dd (11.5, 5.6)[a]	4.60–4.53[a]					
6b	4.20–4.14[a]	4.39–4.30[a]	4.20–4.14[a]	4.36–4.30					

[a] Overlaped signa.
[b] Ipur: Ipurolic acid; Ipor: Ipolearic acid.

Table 2
^{13}C (100 MHz) NMR Data of Purgic acids C (**3**) and D (**4**), as well as their Peracetates (**3a** and **4a**) in Piridine-d_5 (δ in ppm).

position	3	3a	4	4a	position	3	3a	4	4a
Qui-1	102.7	100.8	102.5	100.4	Qui'-1	105.0	101.6	105.2	101.3
2	80.1	75.9	80.9	72.8	2	77.4	72.6	77.4	72.6
3	78.5	75.8	78.8	76.0	3	77.7	73.5	77.7	73.6
4	76.6	74.3	77.1	74.3	4	76.7	72.9	76.9	72.8
5	70.5	69.7	72.9	69.9	5	77.6	70.3	77.2	69.9
6	18.3	18.0	18.4	17.5	6	18.5	17.9	18.8	17.9
Glc-1	102.8	101.2	102.6	101.8	Fuc-1	103.6	101.4	103.2	101.0
2	74.8	72.8	78.1	72.7	2	73.1	69.4	73.6	69.6
3	77.4	76.0	73.8	75.9	3	75.7	73.9	74.9	73.9
4	71.0	69.8	72.9	69.8	4	71.0	71.2	72.8	71.2
5	77.6	72.6	77.1	72.6	5	71.2	69.5	71.2	69.3
6	62.2	63.3	62.0	63.4	6	18.8	16.4	17.1	16.4
Rha-1	101.0	97.7	100.4	97.3	Qui"-1	104.0	102.0	103.7	101.6
2	71.5	73.6	71.9	73.7	2	75.8	72.1	75.8	72.1
3	78.7	78.5	78.1	78.5	3	78.9	75.9	78.4	75.7
4	79.3	76.9	79.3	77.0	4	72.2	76.2	73.9	76.2
5	67.9	67.7	68.0	67.6	5	77.3	71.2	77.3	70.1
6	18.9	19.0	19.0	19.0	6	16.9	18.2	18.7	18.2
Glc'-1	101.6	102.8	100.9	102.4	Ipur–1 or Ipol–1[a]	180.7	176.8	176.6	176.2
2	83.7	78.4	84.9	78.3	2	38.0	35.6	34.8	34.9
3	76.8	75.9	76.8	75.9	3	63.8	63.0	63.6	62.8
4	72.7	70.0	72.7	69.8	11	78.8	80.5	78.6	80.0
5	77.8	69.6	78.2	69.5	14 or 16	14.2	14.7	14.4	14.3
6	63.1	62.6	63.1	62.4					

[a] Ipur: Ipurolic acid; Ipor: Ipolearic acid.

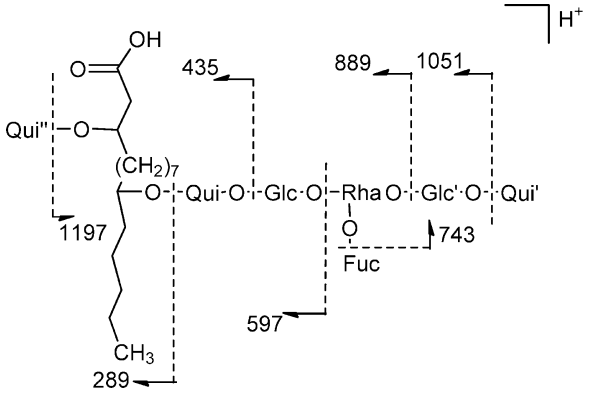

Fig. 3. Positive ESIMS fragmentation of compound **4**.

[1197–146–162–146–146]$^+$, 435 [597–162]$^+$, and 289 [435–146]$^+$ (Fig. 3). The difference of 28 mass units between compounds **3** and **4** as well as the production of the same general fragmentation pattern established that both glycosidic acids differed in the chain length of their aglycone and indicated the presence of ipolearic acid, (3*S*,11*S*)-dihydroxyhexadecanoic acid, in purgic acid D (**4**). Fig. 3 illustrates the fragmentation pattern in positive mode ESIMS for compound **4**. The 1D and 2D NMR data of compound **4** (Tables 1 and 2) were equivalent to purgic acid C (**3**) since both have the same oligosacharide core. Thus, the signals assignment for **4** was accompanied by comparison with the NMR data of compound **3**.

It has been reported that these saponification products are distinctive enough for HPLC and NMR differentiation of *I. purga* from other members of the Mexican jalap root complex (Pereda-Miranda et al., 2006b). Thus, these profiles were used as analytical tools for the authentication of the commercialized Mexican scammony resin, which is used as a purgative remedy, sold in Mexico and exported worldwide by Mixim Laboratories, manufactures of botanical extracts. A MeOH-soluble extract was prepared with this resin, after sonication with H_2O. Then, it was submitted to saponification. The dried neutral aqueous phase from

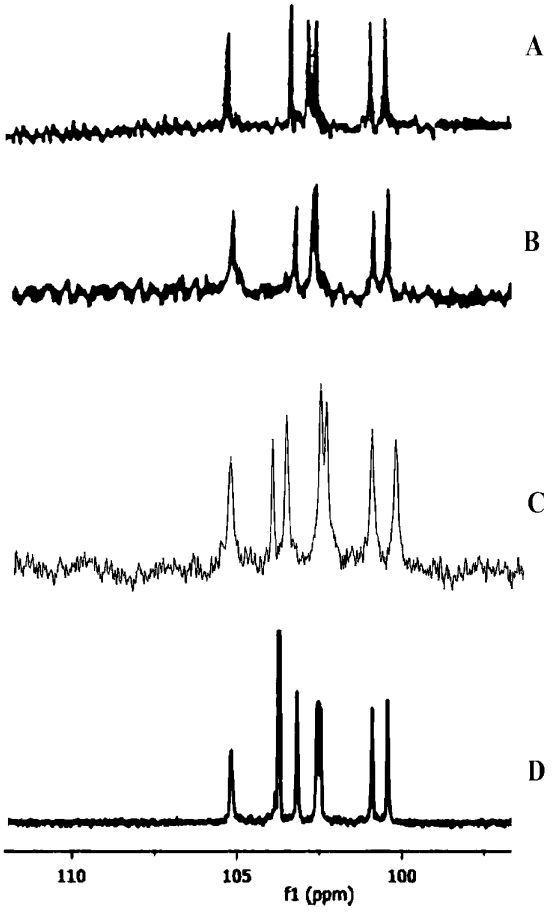

Fig. 4. Expansion at 100–110 ppm of ^{13}C NMR spectra of glycosidic acids A-D liberated by saponification of crude MeOH-soluble extracts from *Ipomoea purga* ("Rhizoma Jalapae").

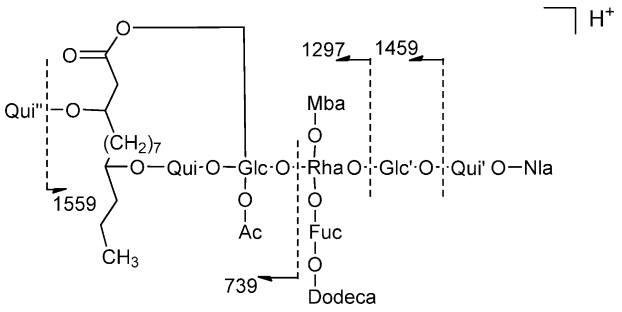

Fig. 5. Positive ESIMS fragmentation of compound **2**.

this alkaline hydrolysis was acetylated and directly analyzed by HPLC to afford the same four major peaks isolated from the convolvulin fraction prepared with the crude drug. These derivatives corresponded to peracetylated purgic acids A (**5a**, t_R 7.8 min), B (**6a**, t_R 11.5 min), C (**3a**, t_R 14.2 min), and D (**4a**, t_R 40.5 min) and were further submitted to an alkaline hydrolysis to obtain the glycosidic acids for the acquisition of their ^{13}C NMR profiles (Fig. 4). Their anomeric signals are easily distinguishable and used as a fingerprint for pattern recognition, as well as structural dereplication (Bautista et al., 2015; Pereda-Miranda et al., 2006a), and constitute a good starting point for structure elucidation as described above for purgic acids C (**3**) and D (**4**). The presence of these oligosaccharide cores suggests that the plant material used by the manufactures of the commercial Mexican scammony resin is the officinal jalap root and does not represent the species *I. orizabensis* (Mexican scammony) since the glycosidic core for this false jalap root should correspond to a tetrasaccharide, scammonic acid A: ^{13}C NMR anomeric signals δ 104.9, 102.1, 101.5, and 101.1; and not the purgic acids (Pereda-Miranda and Hernández-Carlos, 2002; Pereda-Miranda et al., 2006a).

Jalapinoside II (**2**) was isolated as a colorless solid. Its HRESIMS showed the quase-molecular ion peak at *m/z* 1705.8402 $[M+H]^+$ indicating a molecular formula $C_{80}H_{137}O_{38}$ (calcd 1705.8782). The analysis by HPLC-ESIMS/MS showed the presence of ion peaks produced through the glycosidic cleavage (Pereda-Miranda et al., 2006a) and the elimination of acylation residues (Cruz-Morales et al., 2012; Castañeda-Gómez et al., 2013) at *m/z* 1559 $[M+H-146$ (methylpentose unit)$]^+$, 1459 $[M+H-146–100$ (nylic acid)$]^+$, 1297 $[1459–162$ (hexose unit)$]^+$, and 739 $[1297–146–182–146–84]^+$ (Fig. 5). Jalapinosides I (**1**) and II (**2**) presented a common ion peak at *m/z* 1459 which was generated by the loss of a fragment of 246 uma [146–100 (nylic acid)]$^+$ in compound **2** and of 230 uma [146–84 (methylbutiric acid)]$^+$ in compound **1**. This difference of 16 uma indicated that compound **2** possess nylic acid as the acylating residue at the terminal quinovose unit in constrast to **1** with a residue of methylbutiric acid (Bautista et al., 2015; Hernández-Carlos et al., 1999). In the ^{13}C NMR spectrum, seven signals for anomeric carbons were observed in the region comprised between δ_C 95–110 ppm (Table 3). Each signal allowed the identification of the respective anomeric protons through their correlations in the HSQC spectrum, which established the spin systems of each monosaccharide moieties. The observed cross peaks in the COSY spectrum allowed completing the ^1H NMR signal assignment. The acylation positions as well as lactonization site were determined by the observed HMBC correlations: H-3 (5.98, Glc-3) with C-1 (173.1, Ipur-1), H-6 (5.66 and 5.70, Glc-6) with C-1 (168.2, Ac-1), H-2 (5.91, Rha-2) with C-1 (176.6, Mba-1), H-3 (5.38, Qui′-3) with C-1 (175.2, Nla-1), and H-3 (5.96, Fuc-3) with C-1 (173.8, Dodeca-1). The preparation and identification of 4-bromophenyacyl derivatives of (2*S*)-2-methylbutyric and (2*R*,3*R*)-3-hydroxy-2-

Table 3
^1H (700 MHz) and ^{13}C (175 MHz) NMR Data of Compound **2** in Piridine-d_5 (δ in ppm, *J* in Hertz).

position	δ_H	δ_C	position	δ_H	δ_C
Qui-1	4.90 d (7.6)	103.0	Fuc-1	5.78 d (7.8)	102.1
2	4.27–4.22[a]	80.4	2	4.27–4.23[a]	74.3
3	4.79 dd (8.8, 7.6)	78.5	3	5.96 d (7.8)	74.1
4	3.55 dd (9.9, 7.6)	77.2	4	4.33–4.27[a]	73.6
5	3.92 dd (9.9, 6.2)[a]	72.6	5	3.68 m	77.7
6	1.55 d (6.2)	18.3	6	1.66 d (6.0)	18.8
Glc-1	5.86 d (7.8)	101.7	Qui”-1	4.82 d (7.7)	103.5
2	4.23–4.27[a]	75.6	2	4.05–3.99[a]	75.6
3	5.98 dd (7.7, 7.7)[a]	74.1	3	4.17 dd (8.8, 8.0)	78.2
4	4.33–4.28[a]	69.4	4	4.50–4.47[a]	72.7
5	4.42 dd (8.0, 5.5)	63.1	5	3.75 dd (9.1, 6.2)	77.1
6a	5.70 dd (10.3, 3.2)	74.4	6	1.44 d (6.2)	16.4
6b	5.66 dd (10.3,5.5)		Ipur-1		173.1
Rha-1	6.46 d (1.8)	97.0	2a	2.71 ddd (14.2, 8.8, 3.3)	43.4
2	5.91 dd (3.6, 1.8)	74.1	2b	2.73 ddd (14.2, 8.8, 3.3)	
3	5.50 dd (9.2, 3.6)	74.0	3	4.42–4.38[a]	68.3
4	4.61 dd (9.5, 9.2)	80.1	11	3.92–3.88[a]	81.1
5	5.20 dd (9.5, 6.2)	67.5	14	1.01 t (7.4)	11.9
6	1.96 d (6.2)	19.2	Ac-1		168.2
Glc'-1	6.37 d (7.8)	101.5	2	2.04 s	12.3
2	3.99–4-04[a]	86.1	Mba-1		176.6
3	4.53 dd (9.0, 7.8)	72.7	2	2.64 ddd (7.0, 6.8, 2.5)	41.5
4	3.85 dd (9.5, 9.0)	72.4	2-Me	1.22 d (7.0)	16.9
5	4.34 dd (9.5, 7.2)	78.1	3-Me	0.96 t (7.1)	14.4
6a	4.55–4.49[a]	62.9	Nla-1		175.2
6b	4.05–3.98[a]		2	2.91 ddd (7.1, 7.1, 2.6)	48.9
Qui'-1	5.14 d (7.8)	107.1	2-Me	1.35 t (7.0)	21.1
2	4.10–4.03[a]	75.9	3	4.38–4.30[a]	69.3
3	5.38 dd (9.5, 9.5)	76.7	3-Me	1.22 d (7.0)	16.9
4	4.27–4.22[a]	76.4	Dodeca-1		173.8
5	3.75 dd (9.1, 6.2)	77.1	2a	2.35 dd (15.6, 7.0)	34.3
6	1.74 d (6.2)	18.4	2b	2.27 dd (15.6, 6.8)	

[a] Overlaped signal.

methylbutyric acids from the saponification of the crude resin glycosides was used to confirmed the absolute configuration for these chiral esterifying residues (Castañeda-Gómez et al., 2013; Cruz-Morales et al., 2012; Pereda-Miranda and Hernández-Carlos, 2002).

Compound **2** was tested for its cytotoxic activity against a panel of human cancer cell lines: colon (HCT-15), cervix (HeLa), and breast (MDA-MB-231, MCF-7sens, MCF-7/Vin$^-$ and MCF-7/Vin$^+$). No cytotoxicity was detected for all the cell lines (IC_{50} > 20 μg/mL). Based on our previous results of resin glycoside drug uptake inhibition with an anti-P-gp monoclonal antibody and rhodamine 123 (Figueroa-González et al., 2012), the capability of compound **2** to modulate the multidrug resistance in the MDR MCF-7/Vin cell line was carried out. The modulation assay employed parental or vinblastine-sensitive (MCF-7 sens) and vinblastine-resistant (MCF-7/Vin$^-$ and MCF-7/Vin$^+$) human breast cancer cells (Table 4). Reversal fold value for compound **2** ($RF_{MCF\text{-}7/Vin+}$ > 1906) showed that it was equipotent to jalapinoside I at a concentration of 25 μM (**1**, Bautista and Pereda-Miranda, 2015). Comparison of these data with those previously described for other convolvulaceous resin glycosides showed that compounds **1** and **2,** together with purgin II (Castañeda-Gómez et al., 2013) and albinoside III (Cruz-Morales et al., 2012), constitute the most potent glycolipid type chemosensitizers in modulation assays (Bautista et al., 2015). There are no clear evidences to establish structure-activity relationships since minor variations in the acylation pattern of the oligosaccharide cores could affect their MDR reversal activities (Corona-Castañeda and Pereda-Miranda, 2012; Corona-Castañeda et al., 2016; Figueroa-González et al., 2012). However, an essential structural requirement for the MDR modulatory activity is the presence of the macrolactone, since non-cyclic resin glycosides, as the glycosidic acids, displayed marginal activity (Song et al., 2015).

3. Conclusions

Due to the morphological resemblance among the false jalap roots, e.g., *I. orizabensis* and/or *I. stans*, with the officinal jalap (*I. purga*), adulteration occurs due to the negligence of suppliers and collectors. HPLC and ^{13}C NMR profiles can be employed to recognize the constant composition of the commercialized resins prepared from the officinal jalap root. Resin glycosides are a group of natural products with a high structural complexity that is responsible for its chemical diversity. Their glycolipid nature provides these amphipathic compounds with the ideal structural features for poly-specific drug binding to MDR efflux pumps of therapeutic interest. The results obtained for the vinblastine resensitization in MCF-7 cancer cell line by jalapinoside II (**2**) support the proposal for the development of potent chemosensitizers based on resin glycosides for cancer chemotherapy.

4. Experimental

4.1. General experimental procedures

Melting points (uncorrected) were determined on a Fisher-Johns apparatus. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. ^1H (700 MHz) and ^{13}C (175 MHz) NMR experiments were performed on a Bruker Avance III HD NMR spectrometer. Chemical shifts were referenced to TMS, and *J* values are given in Hz. ESIMS, HRESIMS and MALDI-TOFMS were recorded on a Bruker Daltonics Esquire 6000, Thermo LTQ Orbitrap XL, and Bruker MicrO-TOF-Q mass spectrometers, respectively. Waters HPLC equipment was composed of a 600E multisolvent delivery system with a refractive index detector (Waters 410). Control of the equipment, data acquisition, and processing of the chromatographic information were performed by the Empower 2 software (Waters). HPLC-ESIMS was carried out on an Agilent 1200 Rapid Resolution Liquid Chromatograph coupled to a Bruker Daltonics Esquire 6000 mass spectrometer. GC–MS was performed on a Thermo-Electron instrument coupled to a Thermo-Electron spectrometer. GC conditions: DB-5MS (5% phenyl)-methylpolysiloxane column (30 m × 0.25 mm, film thickness 0.18 μm); He, linear velocity 30 cm/s; 2 mL/min; 50 °C isothermal for 4 min, linear gradient to 300 °C at 40 °C/min; final temperature hold, 20 min. MS conditions: ionization energy, 70 eV; ion source temperature, 250 °C; interface temperature, 270 °C; scan speed, 2 scans s^{-1}; mass range, 45–600 amu.

4.2. Chemicals, cell lines, and cell cultures

Colon (HCT-15), cervix (HeLa), and breast (MCF-7 and MDA-MB-231) carcinoma cell lines were obtained from the American Type Culture Collection. The resistant MCF-7/Vin was developed through continuous exposure to vinblastine during five consecutive years as previously reported (Figueroa-González et al., 2012). All cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and were cultured at 37 °C in an atmosphere of 5% CO_2 in air (100% humidity). To maintain drug resistance, MCF-7/Vin$^+$ cells were cultured in medium containing 0.192 μg/mL of vinblastine. At the same time, a stock of MCF-7/Vin$^-$ cells was maintained in vinblastine-free medium.

4.3. Plant material

The roots of *I. purga* (Wender) Hayne were collected in Coxmatla, Municipio de Xico, Veracruz, Mexico, in November 2010 and identified by Botanist Alberto Linajes. Three voucher specimens were deposited at the following herbaria: Instituto de Ecología, Veracruz, México (XAL ID-365843); Departamento Farmacia, Facultad de Química, UNAM (J. Castañeda and R. Pereda RP-06); and the National Herbarium, Instituto de Biología, Universidad Nacional Autónoma de México (MEXU 426765).

Table 4
Modulation of Vinblastine Cytotoxicity in Drug Sensitive MCF-7 and Multidrug Resistant MCF-7/Vin by Jalapinoside II (**2**).

	IC_{50} (μg/mL)			reversal fold[c]		
compound[a]	MCF-7/Vin$^-$	MCF-7/Vin$^+$	MCF-7 sens	$RF_{MCF\text{-}7/Vin^-}$	$RF_{MCF\text{-}7/Vin^+}$	$RF_{MCF\text{-}7sens}$
vinblastine	1.02 ± 0.18	1.22 ± 0.14	0.047 ± 0.01			
Jalapinoside II (**2**)	<0.00064	<0.00064	<0.00064	>1593.8	>1906.3	73.4
reserpine[b]	0.037 ± 0.01	0.31 ± 0.19	0.003 ± 0.001	27.6	3.9	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.

4.4. Extraction and isolation

The powdered roots (3.8 kg) were extracted exhaustively by maceration with MeOH to obtain a dried extract (202.8 g), which was suspended (25 g) in deionized water (3 × 100 mL) and subjected to sonication for 30 min to afford H_2O-soluble and −insoluble fractions (9.4 and 13.3 g, respectively). The H_2O-insoluble fraction (12.0 g) was submitted to HPLC on a Symetry C_{18} column (Waters, 7 μm, 19 × 150 mm) with an isocratic elution of CH_3CN—H_2O (3:1), a flow of 8.2 mL/min and a sample concentration of 200 mg/mL, injecting 500 μL each time. The peak with t_R values of 5.3 min was collected by the techniques of heart cutting and peak shaving, and purified by recycling over 8–10 times at the same column, using a flow of 4.1 mL/min to afford compound 2 (5.3 mg).

4.4.1. Jalapinoside II (**2**)

White powder: mp 180–182 °C; $[\alpha]_{589}$ −51.4, $[\alpha]_{578}$ −55.7, $[\alpha]_{546}$ −61.4, $[\alpha]_{436}$ −95.7, $[\alpha]_{365}$ −145.7 (*c* 0.07, MeOH); ^1H and ^{13}C NMR, see Table 1; positive MALDI-TOFMS *m/z* 1705 $[M+H]^+$; positive ESIMS/MS *m/z* 1705 $[M+H]^+$, 1559 [M + H − 146 (methylpentose unit)]$^+$, 1459 [M + H − 146–100 (nylic acid)]$^+$, 1297 [1459–162 (hexose unit)]$^+$, and 739 [1297–146–182–146–84]$^+$; HRESIMS *m/z* 1705.8402 $[M+H]^+$ (calcd for $C_{80}H_{136}O_{38}+H^+$ requires 1705.8782);

4.5. Alkaline hydrolysis of the MeOH extract

A solution containing 1 g of the MeOH extract in 30 mL of KOH aqueous 5% was submitted to reflux at 95 °C for 3 h. Then, the mixture was acidified to pH 5.0 and extracted with $CHCl_3$ (3 × 30 mL). This organic layer was washed with H_2O, dried with Na_2SO_4 and the solvent was removed under reduced pressure and directly analyzed by GC–MS to displayed four peaks, identified as acetic acid, *m/z* $[M]^+$ 60 (65), 45 (80), 43 (100), 29 (19), 15 (25); 2-methylbutanoic acid, *m/z* [M]+ 102 (2), 87 (25), 74 (100), 57 (64), 41 (30); 3-hydroxy-2-methylbutanoic acid, *m/z* $[M]^+$ 118 (2), 115 (10), 101 (20), 84 (12), 73 (70), 60 (100); and dodecanoic acid, *m/z* $[M]^+$ 200 (16), 171 (9), 157 (22), 143 (5), 129 (34), 115 (18), 101 (12), 85 (28), 73 (100), 60 (95), 43 (62).

4.6. Preparation of 4-Bromophenyacyl derivatives of esterifying residues

The saponification organic layer residue (30 mg) was treated with triethylamine (three drops) and 4-bromo-phenacyl bromide (20 mg) in dried acetone (5 mL) for 2 h at room temperature. The reaction mixture was evaporated to dryness, resuspended in H_2O (10 mL) and extracted with Et_2O (20 mL). The resulting organic phase was concentrated and its residue was fractionated by normal phase HPLC on an ISCO column (150 × 19 mm, μporasil, 10 mm), using hexane-AcOEt (7:3; flow rate 2.0 mL/min) to afford (−)-4-bromophenacyl (2*R*,3*R*)-3-hydroxy-2-methylbutyrate (t_R 12.8 min) and (+)- 4-bromophenacyl (2*S*)-2-methylbutate (t_R 6.4 min). Also, 4-bromophenacylester derivatives of acetic and dodecanoic acids were collected (t_R 1.7 and 14.0 min, respectively). This procedure has been used to confirmed the absolute configuration for these chiral esterifying residues (Pereda-Miranda and Hernández-Carlos, 2002): 4-bromophenyacyl (2*S*)-2-methylbutyrate: mp 40–42 °C; $[\alpha]_D$ +18 (c 1.0, MeOH); GC–MS *m/z* 272 (6.8), 270 (7.3), 254 (3.8), 252 (3.8), 186 (2.1), 172 (8.6), 171 (100), 70 (9.7), 169 (88.7), 90 (13.9), 89 (23.4), 85 (11.5), 63 (5.3) 57 (19), 51 (2.3), 50 (2.9), 41 (8.5), 39 (9.4). 4-bromophenacyl (2*R*,3*R*)-3-hydroxy-2-methylbutyrate: mp 56−59 °C; $[\alpha]_D$ −6.0 (*c* 1.0 $CHCl_3$); GC–MS *m/z* 118 (2.0), 115 (10), 101 (20), 84 (12), 73 (70), 60 (100).

4.7. Acetylation of n-BuOH fraction

The saponification H_2O-soluble residue obtained from the *n*-BuOH fraction (0.712 g) was dissolved in pyridine (13 mL) and acetic anhydride (23 mL) was added by dropping. The resulting mixture was stirred for 24 h at room temperature. Then, the reaction mixture was worked up as usual to obtain a residue (0.726 g). This residue was submitted to HPLC on a NH_2 column (YMC, 5 μm, 10 × 150 mm) with an isocratic elution of CH_3CN:H_2O (3:1), a flow of 1.4 mL/min, and a sample concentration of 100 mg/mL, injecting 200 μL each time. The peaks with retention time of 7.8 min (**5a**, 106.1 mg), 11.5 min (**6a**, 52.1 mg), 14.2 min (**3a**, 179.7 mg), and 40.5 min (**4a**, 178.0 mg) were collected and further submitted to recycling HPLC to achieve chromatographic homogeneity by using the above-mentioned instrumental conditions. The peaks with retention time of 7.8 min and 11.5 min were identified as the peracetates of purgic acids A (**5**) and B (**6**) respectively, by comparison of their spectroscopic NMR and MS data with values previously described (Pereda-Miranda et. al., 2006). The peaks with retention time of 14.2 min and 40.5 min corresponded to compounds **3a** and **4a**.

4.7.1. Spectroscopic characterization of ***3a***

White powder: mp 92–95 °C; $[\alpha]_{589}$ −17.9, $[\alpha]_{578}$ −18.5, $[\alpha]_{546}$ −20.8, $[\alpha]_{436}$ −34.4, $[\alpha]_{365}$ −52.0 (*c* 12.6, MeOH); For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; MALDITOF *m/z* 2071.7985 $[M+H]^+$ (calcd for $C_{92}H_{134}O_{52}+H^+$ requires 2071.7914).

4.7.2. Spectroscopic characterization of ***4a***

White powder: mp 89–90 °C; $[\alpha]_{589}$ −13.3, $[\alpha]_{578}$ −14.4, $[\alpha]_{546}$ −15.6, $[\alpha]_{436}$ −26.7, $[\alpha]_{365}$ −40.0 (*c* 0.9, MeOH); For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; MALDITOF *m/z* 2099.8285 $[M+H]^+$ (calcd for $C_{94}H_{138}O_{52}+H^+$ requires 2099.8227).

4.8. Alkaline hydrolysis of compounds ***3a*** and ***4a***

In a ball flask containing 150 mg of compound **3**a were added 10 mL of a solution of 5% KOH—H_2O and the mixture was stirred under reflux conditions at 95 °C for 4 h. The mixture reaction was acidified to pH 5.0 and extracted with EtOAc (3 × 30 mL). Then, it was further extracted with *n*-BuOH and concentrated under vacuum to obtain 80 mg of compound **3**. The same procedure was repeated with derivative **4a** (130 mg) to obtain 65 mg of compound **4**.

4.8.1. Purgic acid C (***3***)

White powder: mp 124–126 °C; $[\alpha]_{589}$ −46.8, $[\alpha]_{578}$ −49.5, $[\alpha]_{546}$ −55.3, $[\alpha]_{436}$ −88.9, $[\alpha]_{365}$ −134.7 (*c* 0.19, MeOH); For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; positive ESIMS *m/z* 1337 $[M+Na]^+$, 1191 $[M+Na-146]^+$, 1045 $[1191-146]^+$, 883 $[1045-162]^+$, 737 $[883-146]^+$, 591 $[737-146]^+$; HRESIMS *m/z* 1337.5834 $[M+Na]^+$ (calcd for $C_{56}H_{98}O_{34}Na^+$ requires 1337.5832).

4.8.2. Purgic acid D (***4***)

White powder: mp 158–160 °C; $[\alpha]_{589}$ −19.3, $[\alpha]_{578}$ −20.7, $[\alpha]_{546}$ −22.9, $[\alpha]_{436}$ −38.6, $[\alpha]_{365}$ −57.1 (*c* 0.14, MeOH); For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; positive ESIMS *m/z* 1343 $[M+H]^+$, 1197 $[M-146]^+$, 1051 $[1197-146]^+$, 889 $[1051-162]^+$, 743 $[889-146]^+$, 597 $[743-146]^+$, 435 $[597-162]^+$, 289 [435 −146]$^+$; HRESIMS *m/z* 1343.6324 $[M+H]^+$ (calcd for $C_{58}H_{102}O_{34}+H^+$ requires 1343.6325).

4.9. Sugar analysis and identification of aglycones

20 mg of purgic acids C (**3**) and D (**4**) in 10 mL HCl 4 N were independently refluxed at 90 °C for 1 h. Then, the reaction mixtures

were diluted with 5 mL H_2O and extracted with ether (3 × 10 mL). The aqueous phases were neutralized with 1 N KOH and extracted with *n*-BuOH (2 × 5 mL), washed with H_2O (3 × 5 mL), and concentrated to give a colorless solid. Thiazolidine derivatives for the sugar constituents (2 mg) were prepared by reaction with L-cysteine (0.5 mg) in pyridine (0.1 mL) at 100 °C according to previously described procedures (Hara et al., 1987; Miyase et al., 1995). These reaction mixtures were converted into volatile derivatives by treatment with chlorotrimethylsilane, and analyzed by GC–MS: DB-5MS (10 m × 0.18 mm, film thickness 0.18 μm); He, 30 cm/s, 2 mL/min; 100 °C isothermal for 3 min, linear gradient to 300 at 20 °C/min. Retention times for TMS thiazolidine derivatives of common sugars were used as standards for GC identification: D-fucose, t_R 4.53; L-rhamnose, t_R 4.58 min; min; D-quinovose t_R 4.64 min; and D-glucose t_R 6.53 min. The organic layers were evaporated to dryness, dissolved in $CHCl_3$ (3 mL), and treated with CH_2N_2. The derivatized fractions were individually submitted to normal-phase HPLC (ISCO, 21.2 × 250 mm, 10 μm), using isocratic elution [*n*-hexane-$CHCl_3$-Me_2CO (5:4:1)] and a flow rate of 5 mL/min to give 6 mg of methyl ipurolate from compound **3** and 5.8 mg of methyl ipolearate from **4**.

4.9.1. Methyl ipurolate [methyl (3S,11S)-dihydroxytetradecanoate]

Colorless needles; mp 67–69 °C, $[\alpha]_D$ +12.9 (*c* 1.5, $CHCl_3$); positive ESIMS/MS *m/z* 275 $[M+H]^+$, 257 $[M+H-H_2O]^+$, 231 $[M-CH_3(CH_2)_2]^+$, 103 $[CH(OH)CH_2CO_2CH_3]^+$, 73 $[CH_3(CH_2)_2CHOH]^+$; ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 14.1 (C-14), 18.9, 25.6, 25.7, 29.6, 29.56, 29.7, 36.7, 37.6, 39.8, 41.4, 51.8 (OCH_3), 68.1 (C-3), 71.7 (C-11), 173.5 (C-1). This compound was identified by comparison of its physical and spectroscopic constants with published values (Ono et al., 2010).

4.9.2. Methyl ipolearate [methyl (3S,11S)-dihydroxyhexadecanoate]

Colorless needless 72–74 °C, $[\alpha]_D$ +10.5°(*c* 1.5, $CHCl_3$); positive ESIMS/MS *m/z* 303 $[M+H]^+$, 285 $[M+H-H_2O]^+$, 231 $[M-CH_3(CH_2)_4]^+$, 103 $[CH(OH)CH_2CO_2CH_3]^+$, 101 $[CH_3(CH_2)_4CHOH]^+$; ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 14.1 (C-16), 18.9, 22.8, 25.5, 25.6, 25.7, 29.7, 29.8, 32.0, 36.6, 37.5, 39.8, 41.3, 51.7 (OCH_3), 68.1 (C-3), 72.1 (C-11), 173.5 (C-1). This compound was identified by comparison of its physical and spectroscopic constants with published values (Ono et al., 1990).

4.10. Profiling of the mexican scammony resin

Mexican scammony resin was acquired from Mixim Laboratories (Laboratorios Mixim, S.A. de C.V.). A sample (0.5 g) was submitted to sonication with H_2O (2 mL) for 30 min. Then, the solid residue (0.45 g) was dissolved in 10 mL of a solution of aqueous KOH 5%, the resulting mixture was refluxed at 95 °C for 3 h. Workup of the reaction was carried out as previously described (Section 4.5) to obtain a dark orange-yellowish residue (310 mg). This residue was dissolved in pyridine (5 mL) and acetylated using acetic anhydride (5 mL) as described above (Section 4.7). The crude reaction mixture (290 mg) was analyzed by HPLC on a NH_2 column (YMC, 5 μm, 10 × 150 mm) with an isocratic elution of CH_3CN:H_2O (3:1), a flow of 1.4 mL/min, and a sample injection of 10 μL (concentration of 50 mg/mL) to generate the corresponding HPLC profile. For preparative scale, samples of 200 μL (concentration of 100 mg/mL) were injected for collecting the peaks with retention time of 7.8 min (**5a**, 42.4 mg), 11.5 min (**6a**, 21.0 mg), 14.2 min (**3a**, 71.8 mg), and 40.5 min (**4a**, 71.0 mg). The ^{13}C NMR fingerprint (Fig. 4) for the anomeric region (95–110 ppm) was obtained after the saponification of each isolated peak (Pereda-Miranda et al., 2006a).

4.11. Cytotoxicity and modulation of multidrug-Resistance assays

Cytotoxicity and MDR reversal fold of compound **2** were determined by using the SRB assay. The cells were harvested at log phase of their growth cycle and were treated in triplicate with various concentrations of the test samples (0.2–25 μg/mL) and incubated for 72 h at 37 °C in a humidified atmosphere of 5% CO_2. The assay was carried out under static conditions and the results are expressed as the concentration that inhibits 50% control growth (IC_{50}). The values were estimated from a semilog plot of the drug concentration (μg/mL) against the percentage of growth inhibition. Vinblastine was included as a positive control drug. The reversal effects as modulators were further investigated with the same method. Parental 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 (dissolved in H_2O/DMSO, 9:1) at 25 and 5 μg/mL for 72 h (Figueroa-González et al., 2012). Reserpine (5 μg/mL) was used as a positive control drug. The reversal fold (RF) value, as a parameter of potency, was calculated from dividing IC_{50} of vinblastine alone by IC_{50} of vinblastine in the presence of test compounds.

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