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Jalapinoside, a Macrocyclic Bisdesmoside from the Resin Glycosides of *Ipomea purga*, as a Modulator of Multidrug Resistance in Human Cancer Cells

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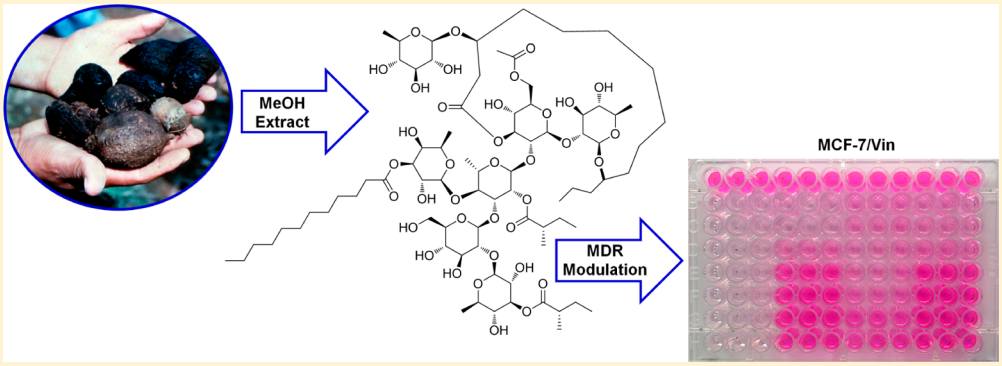
Supporting Information



ABSTRACT: The first macrocyclic bisdesmoside resin glycoside, jalapinoside (**4**), was purified by preparative-scale recycling HPLC from the MeOH-soluble extracts of *Ipomoea purga* roots, the officinal jalap. Purgic acid C (**3**), a new glycosidic acid of ipurolic acid, was identified as 3-*O*-*β*-D-quinovopyranoside, 11-*O*-*β*-D-quinovopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-[*β*-D-fucopyranosyl-(1→4)]-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-*β*-D-quinovopyranoside (3*S*,11*S*)-dihydroxytetradecanoic acid. The acylating residues of this core were acetic, (+)-(2*S*)-methylbutanoic, and dodecanoic acids. The site of lactonization was defined as C-3 of the second saccharide moiety. Reversal of multidrug resistance by this noncytotoxic compound was evaluated in vinblastine-resistant human breast carcinoma cells.

Resin glycosides are complex mixtures of glycolipids found in members of the morning glory family (Convolvulaceae).[1] Several biological effects of the crude extracts of the purgative Mexican jalaps (e.g., *Ipomea purga*, *I. orizabensis*, and *I. stans*) have been attributed to their resin glycosides.[1,2] These resin glycosides represent noncytotoxic inhibitors of multidrug efflux pumps in Gram-positive[3] and -negative[4] bacteria as well as in mammalian cancer cells.[5]

Phytochemical reports on the jalap root ("officinal jalap", *I. purga*), a drug used since pre-Columbian times in Mexico,[2] were published as early as the second half of the 19th century.[6] Most of the early and even recent botanical and chemical descriptions are confusing because of poor plant material identification. HPLC and ^{13}C NMR spectroscopic profiles of the glycosidic acids obtained through saponification of the resin glycoside contents were used as analytical tools for authentication and quality control of the Mexican jalaps.[2,7] The ratio of ether-soluble ("jalapin") to alcohol-soluble ("convolvulin") resin glycoside portions can also distinguish between authentic and false crude drugs; for example, the officinal jalap root yields the highest amount of MeOH-soluble resin glycosides (15−20% dried weight), in comparison to the yields from the Mexican scammony, or false jalap (*I. orizabensis*): 10−18% ether-soluble resins and <1% alcohol-soluble content.[2] According to Mannich and Schumann,[8] convolvulin resin glycosides are composed as large, high molecular weight polymers of oligosaccharides glycosidically linked to a hydroxylated fatty acid. Previously, the isolation of purgins I−III,[9] partially acylated ester-type dimers of branched pentasaccharides from the jalap root, permitted the characterization of these compounds as individual macrolactones of distinctive glycosidic acids. Therefore, the hypothesis of a high molecular weight polymeric structure for this class of compounds had to be revisited.

The structural complexity of the highly polar alcohol-soluble resin glycosides has seriously hampered the isolation of the individual glycolipids, limiting chemical studies to the characterization of their degradation products, i.e., esterifying organic acids and major glycosidic acids.[2,10] Saponification of the jalap root MeOH-soluble contents afforded purgic acids A and B (**1** and **2**) as their major glycosidic acids.[2] Compound **1** was identified as (11*S*)-hydroxytetradecanoic acid 11-*O*-*β*-D-quinovopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-[*β*-D-fucopyranosyl-(1→4)]-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-*β*-D-quinovopyranoside. Compound **2**

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was characterized as the (11*S*)-hydroxyhexadecanoic acid homologue of **1** (Figure 1).[2] In this context, the present

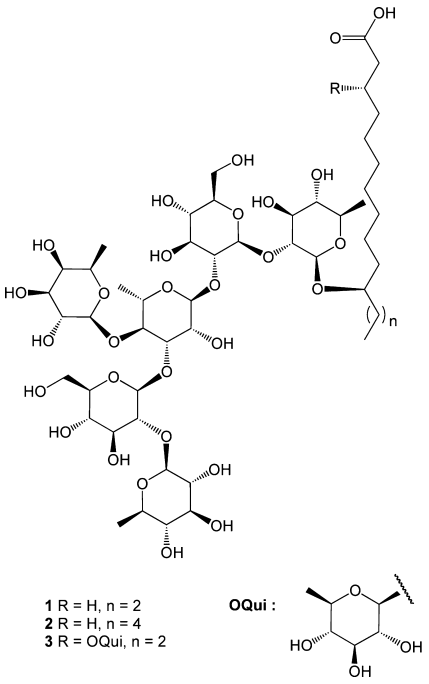
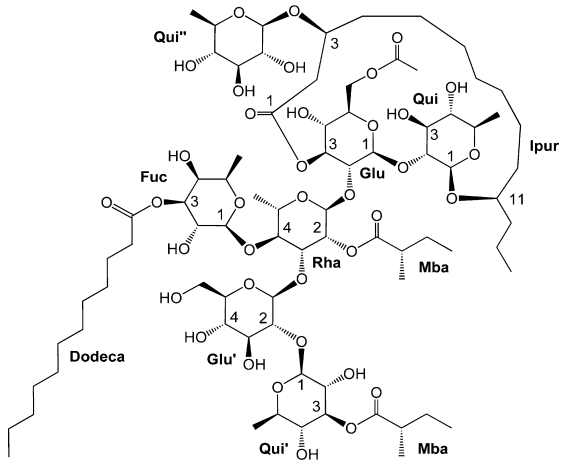


Figure 1. Chemical structures of purgic acids A–C (**1**–**3**).

investigation describes the structural elucidation of an intact partially acylated macrocyclic bisdesmoside resin glycoside, having ipurolic acid [(3*S*,11*S*)-dihydroxytetradecanoic acid] as the aglycone, while having the same glycosidation sequence in the oligosaccharide core as purgic acids A and B (**1** and **2**). The second sugar linkage of an additional quinovose moiety was established at C-3 of the aglycone. This new glycosidic acid obtained from the saponification of the isolated resin glycoside was named purgic acid C (**3**). Purification of the highly polar bisdesmoside, jalapinoside (**4**), found as a minor constituent in the H_2O-insoluble resin glycosides from the "officinal jalap" MeOH-soluble extract, was performed by preparative-scale HPLC through the application of column overload, peak shaving, heart cutting, and recycling techniques.[11] Finally, reversal of multidrug resistance (MDR) by compound **4** was evaluated in vinblastine-resistant human breast carcinoma cells.[5]

Alkaline hydrolysis of **4** produced a nonpolar fraction, which was analyzed by GC-MS to establish acetic, methylbutyric, and dodecanoic acids as the acylating residues. The water-soluble polar fraction extracted with *n*-BuOH afforded purgic acid C (**3**), which was hydrolyzed in acid, and their Et_2O-soluble extract was methylated. The product corresponded to methyl (3*S*,11*S*)-dihydroxytetradecanoate, which was identified by comparison of optical rotation and MS data with published values.[12a] The monosaccharide mixture was derivatized with L-cysteine to form thiazolidines,[13] identified by GC-MS as their TMS ethers. This sugar analysis also confirmed the absolute configuration of all the monosaccharides as the L-series for rhamnose and the D-series for fucose, quinovose, and glucose.

The MS analysis of compound **4** was conducted by HRESIMS and MALDI-TOFMS in the positive ion detection mode. HRESIMS identified the quasi-molecular ion at *m*/*z* 1689.8448 $[M + H]^+$ ($C_{80}H_{137}O_{37}$). LC-ESIMS/MS provided



4

readily detectable ions resulting from glycosidic cleavage[2] and eliminations of the esterifying groups[10,12] with observed ions at *m*/*z* 1543 [1689 (M + H) − 146 (methylpentose unit)]$^+$, 1459 [1689 (M + H) − 146 − 84 (methylbutyrate)]$^+$, 1297 [1459 − 162 (hexose unit)]$^+$, 739 [1297 − 146 − 182 − 146 − 84]$^+$ (Figures 2 and S1, Supporting Information). Purgic acid C (**3**)

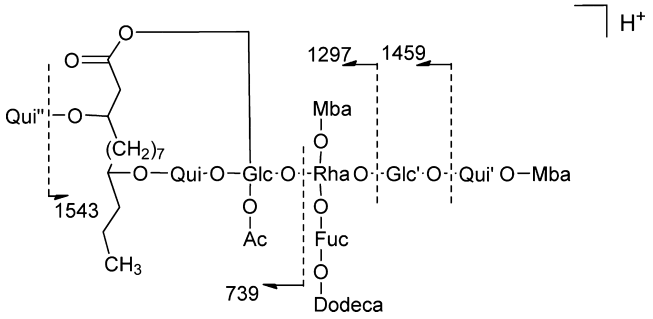


Figure 2. Positive ESIMS fragmentation for compound **4**.

showed a quasi-molecular ion of low abundance at *m*/*z* 1313 $[M - H]^-$, corresponding to the molecular formula $C_{56}H_{97}O_{34}$, and an intense ion at *m*/*z* 1167 $[M - H - 146]^-$, corresponding to the elimination of one methylpentose unit, which, in turn, produced the fragmentation pattern of a hexasaccharide having the same glycosidation sequence as purgic acid A[2] (Figure 1) but with a difference of 16 amu based upon a negative ion FAB with diagnostic ions at *m*/*z* 1021 $[1167 - 146]^-$, 859 $[1021 - 162]^-$, 713 $[859 - 146]^-$, 567 $[713 - 146]^-$, and 405 $[567 - 162]^-$, indicating the presence of ipurolic acid as the aglycone.[12]

Structure elucidation of jalapinoside (**4**) was accomplished by comparison of its ^1H and ^{13}C NMR data (Figures S2 and S3, Supporting Information) with reported data for the purgic acids A (**1**) and B (**2**) since all of them possess the same hexasaccharide unit (Figure 1).[2] The main differences were in the esterifying residues and macrolactonization in **4**, as well as the presence of ipurolic acid as the aglycone, containing an additional quinovose glycosidically linked at C-3 to constitute a macrocyclic resin glycoside bisdesmoside. In order to identify each constitutive monosaccharide moiety, COSY (Figure 3) and TOCSY experiments (Figures S4 and S5, Supporting Information) were used to assign chemical shift values, after identifying and differentiating each of the ^1H NMR signals

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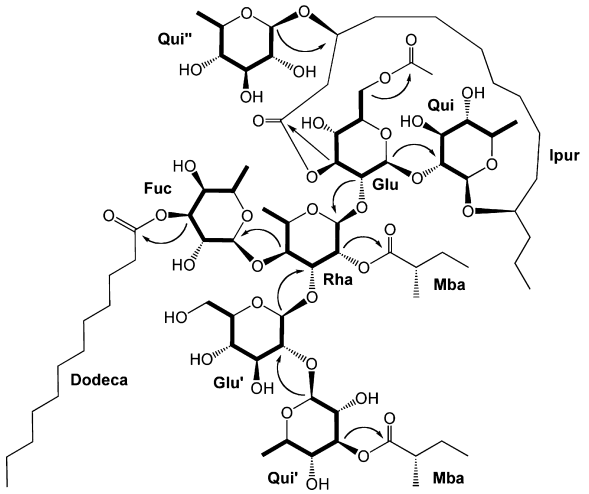

Figure 3. Key ^{1}H−^{1}H COSY and HMBC correlations of jalapinoside (**4**).

(Table 1). 2D ^{1}H−^{13}C HSQC NMR experiments (Figure S6, Supporting Information) were used for the assignments of the ^{13}C NMR signals (Table 1 and Figure 3). In the low-field region of the NMR spectra, seven anomeric signals were confirmed at δ_H 4.90 (1H, d, J = 7.6 Hz; δ_C 102.9, Qui-1); 5.86 (1H, d, J = 7.8 Hz; δ_C 101.6, Glc-1); 6.47 (1H, d, J = 1.3 Hz; δ_C 96.8, Rha-1); 6.42 (1H, d, J = 7.8 Hz; δ_C 101.6, Glc′-1); 5.15 (1H, d, J = 7.8 Hz; δ_C 106.0, Qui′-1); 5.78 (1H, d, J = 7.6 Hz; δ_C 101.8, Fuc-1); and 4.82 (1H, d, J = 7.8 Hz; δ_C 103.0, Qui″-1). Therefore, seven separate spin systems for sugar skeletons were readily distinguished in the ^{1}H−^{1}H COSY (Figure 3) and TOCSY spectra. The glycosylation sequence and the positions of esterification, lactonization, and ester-type linkage were established by the observed long-range correlations ($^3J_{CH}$) in the HMBC spectrum (Figures 3 and S7, Supporting Information). The following key correlations were observed: connectivities between H-1 (δ_H 4.90, Qui-1) and C-11 (δ_C 81.2, Ipur-11); H-1 (δ_H 4.82, Qui″-1) and C-3 (δ_C 68.6, Ipur-3); H-1 (δ_H 5.86, Glc-1) and C-2 (δ_C 80.4, Qui-2); H-2 (δ_H 4.28−4.26, Glc-2) and C-1 (δ_C 96.8, Rha-1); H-1 (δ_H 6.42, Glc′-1) and C-3 (δ_C 74.0, Rha-3); H-1 (δ_H 5.15, Qui′-1) and C-2 (δ_C 86.3, Glc′-2); and H-4 (δ_H 4.61, Rha-4) and C-1 (δ_C 101.8, Fuc-1). The locations of the acyl residues were established by the following HMBC correlations: H_2-6 (δ_H 5.69 and 5.66, Glc-6) and the carbonyl of an acetate group (δ_C 168.5); H-2 (δ_H 5.91, Rha-2) and C-1 of a 2-methylbutyrate residue (δ_C 176.6); H-3 (δ_H 5.35, Qui′-3) and C-1 of a second methylbutyrate moiety (δ_C 176.2); and H-3 (δ_H 5.99, Fuc-3) with the carbonyl of a dodecanoate group (δ_C 173.1). Therefore, the structure of jalapinoside was elucidated as (3*S*,11*S*)-dihydroxytetradecanoic acid 11-[3-*O*-(2*S*-methylbutyryl)]-*O*-6-deoxy-α-L-mannopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→3)-*O*-[6-deoxy-β-D-galactopyranosyl-(1→4)]-*O*-α-L-[2-*O*-(2*S*-methylbutyryl)]-6-deoxy-β-D-glucopyranosyl-(1→2)-*O*-β-D-[6-*O*-acetyl]-glucopyranosyl-(1→2)-*O*-6-deoxy-α-L-mannopyranosyloxy]-3-*O*-[6-deoxy-α-L-mannopyranosyloxy]-1,3″-lactone (**4**).

Prior to this investigation, only two examples of glycosidic acid bisdesmosides had been described, i.e., quamoclinic acids G and H, which were isolated from the seeds of *Quamoclit pennata* Bojer. These were purified from the H_2O-soluble fraction produced by alkaline hydrolysis of the ether-insoluble resin glycosides.[12b] Both compounds, as well as purgic acid C (**3**), are bidesmosides of distinctive glycosidic acids having an oligosaccharide core linked at C-11 of the aglycone (ipurolic acid) in addition to one monosaccharide moiety at C-3. Consequently, jalapinoside (**4**) represents the first intact macrocyclic resin glycoside isolated from a convolvulin fraction.

Compound **4** was not cytotoxic (IC_{50} > 10 μg/mL) when tested against colon (HCT-15), cervix (HeLa), and breast (MCF-7 and MDA-MB-231) carcinoma cell lines (Tables 2 and S1, Supporting Information).[15] Thus, its potential as an MDR modulator was explored. Results of the modulation assay are shown in Table 2, and this screening utilized both vinblastine-sensitive and vinblastine-resistant human breast carcinoma cells

Table 1. ^{1}H (700 MHz) and ^{13}C (175 MHz) NMR Data of Compound 4 in Pyridine-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)	102.9	Fuc-1	5.78 br d (7.6)	101.8
2	4.27−4.22[a]	80.4	2	4.26−4.23[a]	73.6
3	4.75 dd (8.8, 6.3)	78.6	3	5.99 d (7.8)	74.2
4	3.52 dd (9.0, 8.8)	76.8	4	4.29−4.26[a]	72.0
5	3.95 dd (9.0, 6.3)	75.5	5	3.68 m	77.7
6	1.55 d (6.3)	17.2	6	1.67 d (5.2)	17.0
Glc-1	5.86 d (7.8)	101.6	Qui″-1	4.82 d (7.8)	103.0
2	4.28−4.26[a]	75.9	2	4.03−3.97[a]	75.7
3	5.98 dd (7.6, 7.6)	74.2	3	4.15 dd (9.0, 9.0)	78.5
4	4.30−4.26[a]	69.3	4	4.50−4.47[a]	74.8
5	4.41 dd (8.7, 8.5)	62.8	5	3.79 br d (9.2, 6.0)	77.2
6a	5.69 dd (10.3, 3.5)	74.9	6	1.44 d (6.0)	14.6
6b	5.66 dd (10.3, 6.8)		Ipur-1		173.3
Rha-1	6.47 d (1.3)	96.8	2a	2.71 dd (14.7, 4.2)	43.8
2	5.91 dd (3.5, 1.3)	74.2	2b	2.72 dd (14.2, 3.8)	
3	5.50 dd (9.3, 3.5)	74.0	3	4.42−4.38[a]	68.6
4	4.61 dd (9.5, 9.3)	80.1	11	3.90 m	81.2
5	5.26 dq (9.5, 6.2)	67.6	14	1.01 t (7.4)	12.8
6	1.96 d (6.2)	19.3	Ac-1		168.5
Glc′-1	6.42 d (7.8)	101.6	2	2.04 s	12.4
2	4.05−3.99[a]	86.3	Mba-1		176.6
3	4.53 dd (9.0, 9.0)	72.7	2	2.63 t (7.3)	41.6
4	3.84 d (9.5, 9.0)	72.8	2-Me	1.24 d (7.3)	16.9
5	4.34 dd (9.0, 6.5)	78.1	3-Me	0.98 t (7.4)	14.7
6a	4.56−4.52[a]	63.1	Mba′-1		176.2
6b	4.06−3.99[a]		2	2.53 dq (7.2, 3.6)	42.0
Qui′-1	5.15 d (7.8)	106.0	2-Me	1.22 d (7.2)	17.1
2	4.10−4.06[a]	77.6	3-Me	0.97 t (7.0)	11.9
3	5.35 dd (9.5, 9.5)	76.7	Dodeca-1		173.1
4	4.25−4.21[a]	75.5	2	2.72 t (4.0)	43.4
5	3.74 br t (8.8, 6.2)	77.1			
6	1.71 d (6.2)	16.9			

[a]Overlapped signal.

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

	IC_{50} (μg/mL)			reversal fold[c]		
compound[a]	MCF-7/Vin$^-$	MCF-7/Vin$^+$	MCF-7 sens	$RF_{MCF\text{-}7/Vin^-}$	$RF_{MCF\text{-}7/Vin^+}$	$RF_{MCF\text{-}7\ sens}$
vinblastine	1.02 ± 0.18	1.22 ± 0.14	0.047 ± 0.01			
4	<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.

(MCF-7/Vin; Figure S8, Supporting Information).[5a] Reversal fold ($RF_{MCF\text{-}7/Vin+}$ >1906) indicated that jalapinoside (**4**) is extremely potent at 25 μg/mL, as previously observed for other morning glory resin glycosides, e.g., murucoidin V ($RF_{MCF\text{-}7/Vin+}$ 255)[5a] and purgin II ($RF_{MCF\text{-}7/Vin+}$ >2140),[10b] which are proven substrates of glycoprotein P. These results further support the potential of this family of compounds as inhibitors of multidrug efflux pumps in mammalian cancer cells, where glycoprotein-P is the chief plasma membrane-associated translocase responsible for the MDR phenotype,[5a] but also highlight their importance for overcoming MDR in cancer therapy.

EXPERIMENTAL SECTION

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 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 was 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. TLC was carried out on precoated Macherey−Nagel silica gel/UV254 plates of 0.25 thickness, and spots were visualized by spraying with 3% $CeSO_4$ in 2 N H_2SO_4 followed by heating.

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.[5a] 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.

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).

Extraction and Isolation. The dried, powdered roots (3.8 kg) were extracted exhaustively by maceration with MeOH to obtain a dried extract (202.8 g), which was suspended (22.8 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 a t_R value of 10.1 min was collected by the techniques of heart cutting and peak shaving and was purified by recycling[11] over 10 times at the same column, using a flow of 4.1 mL/min to afford compound **4** (6.2 mg).

Jalapinoside (4): colorless solid; mp 163−165 °C; $[\alpha]_{589}$ −33, $[\alpha]_{578}$ −35, $[\alpha]_{546}$ −39, $[\alpha]_{436}$ −63, $[\alpha]_{365}$ −99 (*c* 0.1, MeOH); ^1H and ^{13}C NMR, see Table 1; positive ESIMS *m/z* 1689 $[M + H]^+$; MALDI-TOFMS *m/z* 1689 $[M + H]^+$; HRESIMS *m/z* 1689.8448 $[M + H]^+$ (calcd for $C_{80}H_{137}O_{37}$ requires 1689.8760).

Alkaline Hydrolysis of Jalapinoside. A solution of compound **1** (4 mg) in 5% KOH−H_2O (0.75 mL) was subjected to reflux at 95 °C for 3 h. The mixture was acidified to pH 5.0 and extracted with $CHCl_3$ (3 × 5 mL), the organic layer was washed with H_2O and dried with Na_2SO_4, and the solvent was evaporated under reduced pressure. The aqueous phase was extracted with *n*-BuOH (3 × 10 mL) and concentrated to dryness. The organic phase was directly analyzed by CG-MS[2] to display three peaks, which were identified as acetic acid (t_R 3 min) [*m/z* $[M]^+$ 60 (65), 45 (80), 43 (100), 29 (19), 15 (25)]; 2-methylbutanoic acid (t_R 7 min) [*m/z* $[M]^+$ 102 (2), 87 (25), 74 (100), 57 (64), 41 (30)]; and *n*-dodecanoic acid (t_R 18 min) [*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)]. The residue from the *n*-BuOH fraction was directly analyzed by HPLC-ESIMS on a Synergi Polar column (Phenomenex, 7 μm, 2 × 150 mm) with a gradient elution of aqueous formic acid 0.1% and methanol, 6:4 (0 min) to 1:9 (40 min), a flow of 0.2 mL/min, and a sample injection of 500 μL (1 mg/mL). The peak with a t_R value of 21.5 min corresponded to purgic acid C (**3**) (1.7 mg).

Purgic acid C (3): colorless solid; negative ion FABMS *m/z* 1313 $[M - H]^-$, 1167 $[1313 - 146]^-$, 1021 $[1167 - 146]^-$, 859 $[1021 - 162]^-$, 713 $[859 - 146]^-$, 567 $[713 - 146]^-$, 405 $[567 - 162]^-$.

Acid Hydrolysis and Sugar Analysis. Glycosidic acid **3** (1.5 mg) in 4 N HCl (2 mL) was refluxed at 90 °C for 2 h. The reaction mixture was diluted with H_2O (5 mL) and extracted with ether (5 mL). The organic layer was evaporated to dryness, dissolved in $CHCl_3$ (2 mL), and treated with CH_2N_2. The aqueous phase was neutralized with 1 N KOH, extracted with *n*-BuOH (5 mL), washed with H_2O (5 mL), and concentrated to give a colorless solid. Thiazolidine derivatives for the sugar constituents were prepared according to previously described procedures[13] and analyzed by GC-MS after conversion into volatile derivatives by treatment with chlorotrimethylsilane (Sigma Sil-A)[14] by applying the following conditions: DB-5MS (10 m × 0.18 mm, film thickness 0.18 μm); He, 30 cm/s, 2 mL/min; 100 °C isothermal for 3

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min, linear gradient to 300 at 20 °C/min. Retention times for TMS derivatives of common sugars were used as standards for GC identification: D-fucose, t_R 4.53; L-rhamnose, t_R 4.58 min; D-quinovose, t_R 4.64 min; and D-glucose, t_R 6.53 min. The derivatized (CH_2N_2) organic layer residue obtained from acid-catalyzed hydrolysis was submitted to normal-phase HPLC (ISCO, 21.2 m × 250 mm, 10 μm) using an isocratic elution of hexanes−$CHCl_3$−acetone (6:3:1)[2] and a flow rate of 6 mL/min to give 0.3 mg of methyl (3*S*,11*S*)-dihydroxytetradecanoate (ipurolic acid methyl ester): t_R 20.8 min; colorless needles; mp 68−70 °C; $[\alpha]_D$ +13 (*c* 0.02, MeOH); HPLC-ESIMS *m*/*z* 273 $[M - H]^-$, 255 $[273 - H_2O]^-$, 231 $[M - CH_3(CH_2)_2]^-$, 103 $[CH(OH)CH_2COOCH_3]^-$, 73 $[CH_3(CH_2)_2CH(OH)]^-$; identified by comparison of melting point and optical rotation with published values.[12a]

The preparation and identification of 4-bromophenylacyl (2*S*)-2-methylbutyrate were performed according to reported procedures:[11] mp 40−41 °C; $[\alpha]_D$ +16 (*c* 0.03, MeOH); GC-MS (t_R 4.75 min) *m*/*z* $[M + 2]^+$ 272 (6.8), $[M]^+$ 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). This transesterification procedure has been used to confirm the absolute configuration for 2-methylbutyric acid.[16]

Cytotoxicity and Modulation of Multidrug-Resistance Assays. The cytotoxicity and MDR reversal of compound **4** were determined by using the SRB assay.[15] The cells were harvested at log phase of their growth cycle, 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 after the incubation period (IC_{50}). The values were estimated from a semilog plot of the drug concentration (μg/mL) against the percentage of growth inhibition.[15] Vinblastine was included as a positive control. The reversal effects as modulators were further investigated with the same method. MCF-7 and MDR MCF-7/Vin cells were seeded into 96-well plates and treated with various concentrations of vinblastine (0.000 64−10 μg/mL) in the presence or absence of glycolipids (dissolved in a mixture of water−DMSO, 9:1) at 25 and 5 μg/mL for 72 h as previously described.[5a] The ability of glycolipids to potentiate vinblastine cytotoxicity was measured by calculating the IC_{50} as described above. In these experiments, reserpine (5 μg/mL) was used as a positive control drug. The reversal fold 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.

■ ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR spectra as well as the ESIMS/MS data of compound **4** are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(7) The crude drug consists of the dried, usually fragmented, rhizomes. The ochre-yellow to dark brownish pieces often have a powdery surface, a characteristically faint smoky odor, and a somewhat acrid, sweetish taste. The Mexican jalaps have also been employed as an antihelmintic and galactogogue and in the treatment of abdominal fever, dysentery, epilepsy, hydrocephaly, skin spots, meningitis, and tumors. A decoction of the root of *I. purga* is normally prepared using a 2 cm section of root to 1 L of water. The usual recommendation is to drink one cup of the cold decoction before bedtime. The recommended dosages of jalap root (to 1 L of water) are 1−3 g if a powder, 0.2−0.4 g if an extract, 0.1−0.6 g if a resin, and 10 to 20 drops every 4 h if a tincture; if given in sugar or jelly, this remedy is a safe purge for children. A teaspoon of the root, cut small or granulated, to a cup of boiling water is suggested.

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