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Resin Glycosides from the Herbal Drug Jalap (*Ipomoea purga*)

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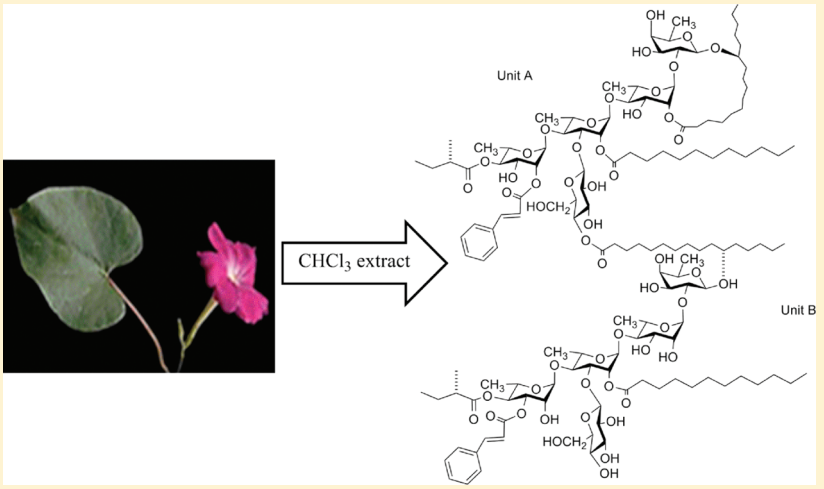
Supporting Information

ABSTRACT:



Three new resin glycosides, purginosides I and II (**1** and **2**) and purgin I (**3**), were isolated from the aerial parts of *Ipomoea purga* and purified by preparative-scale recycling HPLC from a chloroform-soluble extract. Their structures were established through NMR spectroscopy and mass spectrometry. Purginosides I and II (**1** and **2**) are partially acylated branched pentasaccharides derived from operculinic acid A, which is composed of one D-fucose, one D-glucose, and three L-rhamnose units. The site of the aglycon macrolactonization is at C-2 of the second saccharide (rhamnose). In both compounds **1** and **2**, three different esterifying residues were located at C-2 of the second rhamnose unit and at C-2 (or C-3) and C-4 on the third rhamnose moiety. The acylating residues were characterized as *trans*-cinnamic, *n*-decanoic, and either (+)-(2*S*)-2-methylbutanoic or *n*-hexanoic acid. Purgin I (**3**) was found to be an ester-type dimer of operculinic acid A, acylated by *n*-dodecanoic, (+)-(2*S*)-2-methylbutanoic, and *trans*-cinnamic acids at the same oligosaccharide core positions found in compounds **1** and **2**. The site of lactonization by the aglycon in unit A was placed at C-2 of the second saccharide. The position for the ester linkage for the monomeric unit B on the macrocyclic unit A was identified as C-4 of the terminal glucose. This is the first report on the isolation, purification, and structure elucidation of intact individual resin glycoside constituents from the herbal drug jalap.

Ipomoea purga[1] is an evergreen morning glory vine native to Mexico but found only on the eastern slopes of the Sierra Madre Oriental in the states of Hidalgo, Puebla, and Veracruz.[2] It is one of several well-known tuberous New World *Ipomoea* species that form the jalap root medicinal plant complex producing purgative principles, which also includes *I. jalapa*, *I. orizabensis*, and *I. simulans*, among others.[3] *I. purga* is the signature species ("Officinal Jalapae Radix") and has been used in traditional medicine as a mild cathartic since pre-Hispanic times.[4] These Mexican purgative roots were readily accepted as a New World succedaneum of scammony (*Convolvulus scammonia*)[5] since their introduction to Europe around 1610, resulting in a commercial enterprise that continues to this day.[6]

In Mexico, apart from its historical indigenous use, jalap root has been highly regarded and documented as a hydragogue purgative to expel parasitic worms from colonial times[7] through the 19th and 20th centuries.[8,9] The contemporary uses parallel those of the past, but also include additional applications. A decoction of the aerial parts of this medicinal species is recommended as a galactogogue. This herb is further employed in the treatment of gastritis and abdominal inflammation, bilious disorders, dysentery, entero-meningitis, hydrocephaly, and ulcerating skin. The aqueous extract is used as a diuretic. The aerial parts, roots, and seeds contain resin glycosides that have anti-inflammatory properties. Extracts from leaves exhibited antibacterial activity against methicyclin-resistant *Staphylococci* strains.[10] Despite various phytochemical reports on jalap roots published as early as the second half of the 19th century,[11] the structural complexity of their resin glycoside content has seriously

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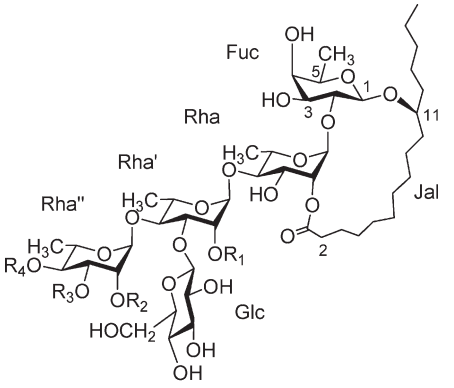
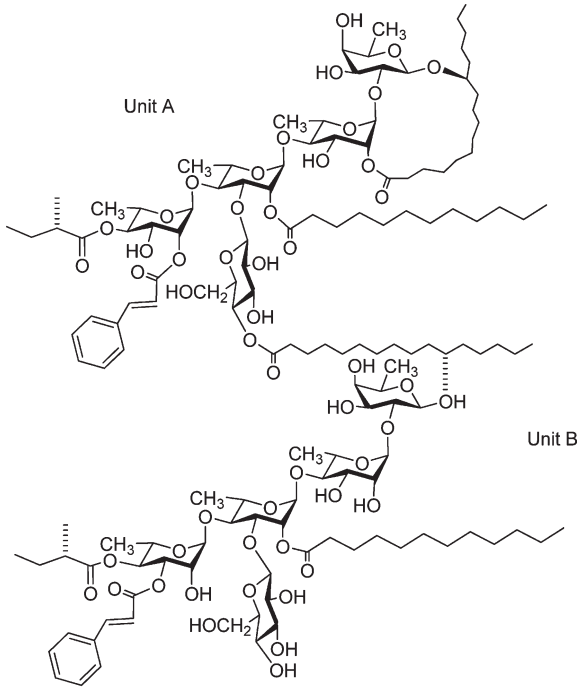
hampered the isolation of individual constituents, thereby limiting these studies to the characterization of only the products of chemical degradation.[3,4] The present study is the first report that describes the isolation, purification, and structural elucidation of three intact individual resin glycosides from the aerial parts of the herbal drug jalap (*I. purga*). Two pentasaccharides and one ester-type dimer of operculinic acid A were isolated from the chloroform-soluble extract and were named purginosides I (**1**) and II (**2**) and purgin I (**3**), respectively. Prior to this investigation, only 12 ester-type dimers from four different members of the morning glory family (Convolvulaceae) have been isolated.[12−16] Compound **3** is related to merremin, also an ester type of operculinic acid A obtained from the roots of *Merremia hungaiensis*.[16]



	R_1	R_2	R_3	R_4
1	deca	Cna	H	mba
2	deca	H	Cna	hexa



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RESULTS AND DISCUSSION

Aerial parts of *I. purga* were powdered and extracted with $CHCl_3$. The extract was fractionated by column chromatography on silica. The more polar fraction rich in resin glycosides was saponified to give a water-soluble glycosidic acid and a mixture of organic solvent-soluble acids. GC-MS analysis permitted the identification of 2-methylbutyric (mba), *n*-hexanoic (hexa), *n*-decanoic (deca), *n*-dodecanoic (dodeca), and *trans*-cinnamic (cna) acids, as the esterifying moeties. The glycosidic acid was acetylated and methylated to give a residue that was purified by C_{18} reversed-phase HPLC, affording compound **4** as the major glycosidic acid, which was identified as the peracetylated methyl ester derivative of operculinic acid A (**5**), (11*S*)-jalapinolic acid 11-*O*-β-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-[α-L-rhamnopyranosyl-(1→2)]-β-D-fucopyranoside, previously obtained from *I. operculata*,[17] *I. leptophylla*,[18] and *I. murucoides*.[19]

The resin glycosidic fraction was submitted to purification of the individual constituents by preparative reversed-phase HPLC using the recycling technique.[20] This procedure allowed the purification of three compounds named purginosides I and II (**1** and **2**) and purgin I (**3**). Purginosides I (**1**) and II (**2**) showed the following features in FABMS. For **1**, a pseudomolecular $[M - H]^-$ ion was detected at *m/z* 1367.7021 (Figure S1, Supporting Information), corresponding to the molecular formula $C_{70}H_{111}O_{26}$ (calcd error: δ = −24.6 ppm), and a peak at *m/z* 1381.7515 for **2** (Figure S8, Supporting Information) allowed the calculation of its molecular formula as $C_{71}H_{113}O_{26}$ (calcd error: δ = −12.7 ppm). Peaks from the consecutive elimination of one cinnamoyl and one decanoyl residue $[M - H - 130\ (C_9H_6O) - 154\ (C_{10}H_{18}O)]^-$ at *m/z* 1083 for **1** and at *m/z* 1097 for **2** and the observed difference of 230 mass units from *m/z* 1083 for **1** and 244 mass units from *m/z* 1097 for **2**, both of which generated the common peak at *m/z* 853, suggested the loss of a methylbutanoyl residue with a methylpentose unit for **1** $[1083 - C_5H_8O - C_6H_{10}O_4]^-$ and a hexanoyl residue with a methylpentose unit for **2** $[1097 - C_6H_{10}O - C_6H_{10}O_4]^-$. Other observed peaks were produced by glycosidic cleavage of the sugar moieties and are common to all resin glycosides.[21]

The ^1H (Table 1; Figures S2 and S9, Supporting Information) and ^{13}C (Table 1; Figures S3 and S10, Supporting Information) NMR spectra allowed the identification of five anomeric signals between δ_H 4.70 and 6.31 corresponding to the carbon signals at δ_C 98.0−106 ppm. Four doublet signals were observed between δ_H 1.4 and 1.7 (J = 6.0 Hz), which confirmed the presence of four methylpentose units in both compounds **1** and **2**. All protons of each saccharide unit were sequentially assigned by a combination of COSY (Figures S4 and S11, Supporting Information) and TOCSY (Figures S5 and S12, Supporting Information) NMR techniques, and all carbons were assigned by HSQC studies (Figures S6 and S13, Supporting Information).[4,22] These procedures allowed the identification of one glucopyranosyl, one fucopyranosyl, and three rhamnopyranosyl units in **1** and **2**, in accordance with the nature of their glycosidic acid (**4**). The interglycosidic connectivities were confirmed by HMBC experiments (Figures S7 and S14, Supporting Information). These studies also permitted to locate the sites for all ester linkages using long-range heteronuclear coupling correlations ($^3J_{CH}$).[4,22] Each of the paramagnetically shifted nonanomeric protons, which reflected the presence of four sites of esterification, correlated with a carbonyl carbon of an ester group, as follows: for purginoside I (**1**), H-2 (δ_H 6.26) of Rha′ with C-1 (δ_C 173.8) of the decanoyl group, H-2 (δ_H 6.28) of Rha″ with C-1 (δ_C 167) of the cinnamoyl substituent, H-4 (δ_H 5.80) of Rha″ with C-1 (δ_C 176.4) of the 2-methylbutanoyl moiety; for compound **2**, H-2 (δ_H 6.32) of Rha′ with C-1 (δ_C 173.5) of the decanoyl group, H-3 (δ_H 5.98) of Rha″ with C-1 (δ_C 166.5) of the cinnamoyl residue, and H-4

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds 1 and 2[a]

	1		2	
position[b]	δ_H	δ_C	δ_H	δ_C
fuc-1	4.70 d (7.0)	104.4	4.72 d (7.5)	104.3
2	4.12 dd (9.5, 7.0)	80.2	4.14 dd (9.5, 7.5)	80.0
3	4.03 dd (9.5, 3.5)	73.5	4.03 dd (9.5, 3.5)	73.3
4	3.94 brs	72.9	3.94 brs	72.9
5	3.72 q (6.5)	70.8	3.72 q (6.0)	70.8
6	1.49 d (6.0)	17.3	1.48 d (6.0)	17.3
rha-1	5.49 d (1.5)	98.6	5.49 d (1.5)	98.5
2	5.92 brs	73.8	5.91 dd (3.0, 1.5)	73.6
3	5.0 dd (9.5, 3.5)	70.0	5.01 dd (9.5, 3.0)	69.3
4	4.16 t (9.5, 9.5)	81.4	4.15 dd (9.5, 9.5)	82.0
5	4.46 dq (9.5, 6.0)	69.0	4.48 m*	69.0
6	1.62 d (6.0)	19.1	1.63 d (6.5)	19.1
rha′-1	5.92 brs	100.0	5.82 d (2.0)	100.3
2	6.26 dd (3.0, 1.0)	74.4	6.32 dd (3.0, 2.0)	73.4
3	4.81 dd (9.0, 3.0)	79.8	4.80 dd (10.0, 3.0)	80.0
4	4.42 m*	78.4	4.38 m*	78.9
5	4.46 dq (9.5, 6.0)	68.4	4.38 m*	68.5
6	1.51 d (6.0)	18.0	1.64 d (6.0)	19.0
rha″-1	6.31 d (1.5)	100.0	6.29 d (1.5)	103.3
2	6.28 dd (3.0, 1.5)	72.9	5.25 dd (3.0, 1.5)	69.9
3	4.77 ddd (9.5, 3.0)	68.3	5.98 dd (10.0, 3.0)	73.2
4	5.80 t (9.5)	74.8	6.08 t (10.0)	71.9
5	4.40 dq (9.5, 6.0)	68.6	4.48 m*	68.1
6	1.67 d (6.0)	19.1	1.43 d (6.0)	17.9
glc-1	5.08 d (7.5)	105.1	5.09 d (7.5)	105.5
2	3.97 t (9.5)	75.2	3.95 m*	75.2
3	4.13*	80.2	4.07 dd (9.0, 9.0)	78.4
4	3.95 t (9.5)	71.6	3.94 m*	71.5
5	3.78 ddd (9.0, 5.5, 2.5)	78.3	3.80 ddd (8.0, 5.5, 2.5)	78.1
6a	4.11 m*	63.2	4.1 m*	63.0
6b	4.42		4.39 m	
jal-1		173.2		173.1
2a	2.26 ddd (15.0, 8.0, 4.0)	34.4	2.26 ddd (15.0, 7.0, 4.5)	34.2
2b	2.44 ddd (14.5, 9.0, 4.0)		2.44 ddd (15.0, 8.5, 4.5)	
11	3.83 m	82.5	3.84 m	82.4
16	0.84 t (7.5)	14.3	0.85 t (7.0)	14.3
deca-1		173.8		173.5
2	2.34 t (7.5)	34.7	2.32 m	34.5
10	0.81 t (7.0)	14.3	0.83 t (7.0)	14.2
mba-1		176.4		
2	2.52 tq (7.5, 7.0)	41.6		
2-Me	1.20 d (7.0)	17.0		
3-Me	0.89 t (7.5)	11.7		
hexa-1				173.1
2			2.42 t (7.5)	34.5
6			0.66 t (7.0)	13.8
Cna-1		167.0		166.5
2	6.35 d (16)	118.9	6.59 d (16)	118.6
3	7.64 d (16)	145.3	7.85 d (16)	145.4

[a] 500 MHz for ^{1}H and 125.7 MHz for ^{13}C NMR in C_5D_5N. Chemical shifts (δ) are in ppm relative to TMS. The spin coupling (J) is given in parentheses (Hz). Chemical shifts marked with an asterisk (*) indicate overlapped signals. Spin-coupled patterns are designated as follows: s = singlet, brs = broad singlet, d = doublet, t = triplet, m = multiplet, q = quartet, sept = septet. All assignments are based on ^{1}H−^{1}H COSY and TOCSY experiments. [b] Abbreviations: fuc = fucose; rha = rhamnose; glc = glucose; jal = 11-hydroxyhexadecanoyl; dec = decanoyl; hex = hexanoyl; mba = 2-methylbutanoyl; Cna = *trans*-cinnamoyl.

(δ_H 6.08) of Rha″ with C-1 (δ_C 173.1) of the hexanoyl group. In compounds **1** and **2**, the carbonyl resonance for the lactone (δ_C 173) was identified through the correlations ($^2J_{CH}$) with the C-2 diastereotopic methylene protons at δ_H 2.26 and 2.44, while the site for the aglycon macrolactonization was placed at C-2 of the second saccharide (Rha), as confirmed by the observed correlation of H-2 (δ_H 5.9) of Rha with C-1 (δ_C 173) of jalapinolic acid.

A combination of mass spectrometric techniques (FABMS and ESIMS) and NMR methods was applied for the structural characterization of purgin I (**3**), allowing the identification of its dimeric nature.[12−16] The negative HRESIMS of **3** (Figure S15, Supporting Information) showed the pseudomolecular ion $[M + Cl]^-$ at m/z 2828.5374 (calcd error: δ = +5.9 ppm), corresponding to the molecular formula $C_{144}H_{232}O_{52}$ for the natural product. A high-mass fragment ion $[M/2 - H]^-$ resulting from the ester-type dimer cleavage[12,14] was observed at m/z 1395.7767 in addition to its corresponding chlorine adduct at m/z 1431.7543. Other peaks produced by cleavage of the lipophilic esters as well as the glycosidic linkages were similar to those observed for purginoside I (**1**), confirming the branched pentasaccharide core as operculinic acid A for both units A and B in the structure of compound **3**. Accordingly, the general structure for compound **1** was used as the starting point for the characterization of each unit forming the ester-type dimer in **3**. The difference of 182 mass units between the peaks at m/z 1265 $[M/2 - H - 130\ (C_9H_6O)]^-$ and 1083 $[M/2 - H - 130\ (C_9H_6O) - 182\ (C_{12}H_{22}O)]^-$ suggested the loss of a dodecanoyl residue instead of a decanoyl ester as observed for compound **1**. Ten signals were present in the anomeric region of the ^{1}H NMR (δ_H 4.5−6.3; Figure S16, Supporting Information) and ^{13}C NMR (δ_C 95−110; Figure S17, Supporting Information) spectra of **3**, which allowed the number of sugar units in each pentasaccharide unit to be confirmed (Table 2). The nonanomeric signals of each monosaccharide unit were assigned by a combination of COSY (Figure S18, Supporting Information) and TOCSY (Figure S19, Supporting Information) experiments. In cases where it was not possible to assign specific methine resonances due to signal overlap, spectroscopic simulation by means of nonlinear fit of the ^{1}H NMR spectrum through an iterative process using the MestRe-C program was used to determine the correct assignment for the coupling constants of all superimposed protons in each monosaccharide unit.[14,23] The HSQC spectrum permitted the assignment of all carbons via one-bond ^{13}C−^{1}H correlations (Figure S20, Supporting Information). Eight paramagnetically shifted nonanomeric protons were observed, indicating the presence of esterifying groups, and their location on each monomeric unit was identified by the HMBC technique (Figure S21, Supporting Information) as follows: (a) The unit A macrolactonization site was identified at C-2 of the second saccharide unit, due to the observed correlation between the lactone carbon (δ_C 173.1) and H-2 (δ_H 5.93) of Rha; (b) the ester-type linkage of the aglycone in unit A at the pentasaccharide core of unit B was placed at C-4 (δ_H 4.4) of the terminal glucose of unit A; (c) C-2 of the third saccharide (Rha′) in both pentasaccharide units A (δ_H 6.28) and B (δ_H 6.34) was esterified by a dodecanoyl ($\delta_{C\text{-}1}$ 173.5) residue; (d) each of the 2-methylbutyroyl groups ($\delta_{C\text{-}1}$ 176) showed a $^3J_{CH}$ cross-peak with H-4 of Rha″ at δ_H 5.81 (unit A) and 6.08 (unit B); (e) the cinnamoyl residue in unit A ($\delta_{C\text{-}1}$ 166.9) was located at C-2 of Rha″ (δ_H 6.31), while the same substituent in unit B ($\delta_{C\text{-}1}$ 166.4) was located at C-3 of Rha″ (δ_H 5.99).

Previous phytochemical studies on the resin glycoside content of the jalap root have suggested that these mixtures are composed of high molecular weight polymers of acylated oligosaccharides glycosidically linked to a hydroxylated fatty acid.[3,4] The isolation and identification of purgin I (**3**) confirmed this hypothesis and demonstrated the complexity of the chemical diversity of the resin glycosides present in the aerial parts of the herbal drug jalap.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer model 341 polarimeter. ^{1}H (500 MHz) and ^{13}C (125.7, and 75 MHz) NMR experiments were registered on a

Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data of Compound 3[a]

	unit A		unit B	
position[b]	δ_H	δ_C	δ_H	δ_C
fuc-1	4.72 d (7.0)	104.3	4.73 d (7.0)	104.3
2	4.14 dd (9.5, 7.5)	80.0	4.14 dd (9.5, 7.5)	80.0
3	4.04 dd (9.5, 3.5)	73.4	4.04 dd (9.5, 3.5)	73.4
4	3.94 d (3.5)	72.9	3.94 d (3.5)	72.9
5	3.73 q (6.0)	70.8	3.73 q (6.0)	70.8
6	1.49 d (6.0)	17.3	1.49 d (6.0)	17.3
rha-1	5.50 brs	98.5	5.50 brs	98.5
2	5.93*	73.6	4.5*	68.2
3	5.02 dd (9.0, 3.5)	69.3	5.02 dd (9.0, 3.5)	69.3
4	4.16 dd (9.5, 9.0)	82.0	4.16 dd (9.5, 9.0)	82.0
5	4.49 dq (9.5, 6.0)	68.9	4.49 dq (9.5, 6.0)	68.9
6	1.63 d (6.0)	19.1	1.63 d (6.0)	19.1
rha′-1	5.93 *	99.8	5.84 brs	100.3
2	6.28*	73.3	6.34 brs	73.3
3	4.79 m*	79.9	4.79 m*	79.9
4	4.37*	79.1	4.37*	79.1
5	4.37*	68.5	4.37*	68.5
6	1.52 d (6.5)	18.0	1.65 d (6.0)	19.0
rha′′-1	6.28*	99.8	6.28 brs	103.3
2	6.31*	73.4	5.27 brs	70.0
3	4.79 m*	68.2	5.99 dd (10.0, 3.0)	73.0
4	5.81 t (9.5)	75.1	6.08 t (9.5)	71.7
5	4.50 dq (9.5, 6.5)	68.1	4.50 dq (9.5, 6.0)	68.1
6	1.65 d (6.0)	19.0	1.43 d (6.0)	17.9
glc-1	5.09 d (8.0)	105.5	5.10 d (8.0)	105.5
2	3.95 dd (9.0, 7.5)	75.2	3.95 dd (9.0, 7.5)	75.2
3	4.07 dd (9.0, 9.0)	78.5	4.07 dd (9.0, 9.0)	78.5
4	4.4*	78.3	3.95 m*	71.6
5	3.82 m*	78.1	3.82 m*	78.1
6a	4.1*	62.9	4.1*	62.9
6b	4.41*		4.41*	
jal-1		173.1		173.1
2a	2.26 ddd (15.0, 7.0, 4.0)	34.2	2.26 ddd (15.0, 7.0, 4.0)	34.2
2b	2.47 m*	34.4	2.47 m*	34.4
11	3.84 m	82.4	3.84 m	82.4
16	0.85*	14.2	0.85*	14.2
dodeca-1		173.5		173.5
2	2.34 t (7.5)	34.6	2.33 t (7.5)	34.6
12	0.81 t (7.5)	14.2	0.81 t (7.5)	14.2
mba-1		176.4		175.9
2	2.52*	42.0	2.43 tq (7.5, 7.5)	42.0
2-Me	1.14 d (7.0)	17.0	1.14 d (7.0)	17.0
3-Me	0.90 t (7.0)	11.7	0.90 t (7.0)	11.6
Cna-1		166.9		166.4
2	6.35 d (15.5)	118.6	6.59 d (16.0)	118.6
3	7.65 d (16)	145.4	7.85 d (16)	145.4

[a] 500 MHz for ^{1}H and 125.7 MHz for ^{13}C NMR in C_5D_5N. Chemical shifts (δ) are in ppm relative to TMS. The spin coupling (J) is given in parentheses (Hz). Chemical shifts marked with an asterisk (*) indicate overlapped signals. Spin-coupled patterns are designated as follows: s = singlet, brs = broad single, d = doublet, t = triplet, m = multiplet, q = quartet, sept = septet. All assignments are based on ^{1}H−^{1}H COSY and TOCSY experiments. [b] Abbreviations: fuc = fucose; rha = rhamnose; glc = glucose; jal = 11-hydroxyhexadecanoyl; dodeca = dodecanoyl; mba = 2-methylbutanoyl; Cna = *trans*-cinnamoyl.

Varian Inova instrument. The NMR techniques were performed according to a previously described methodology.[15] Negative-ion LRFABMS were recorded using a matrix of triethanolamine on a Thermo DFS spectrometer. Negative-ion HRESIMS experiments were performed on a Bruker MicrOTOF-Q high-resolution quadruple-time-of-flight mass spectrometer. The samples were dissolved in HPLC grade MeOH (0.4 mg/mL) and infused directly to the ESI source using a syringe pump at a flow rate of 180 μL/h. The nebulizer and drying gas was nitrogen and set at 0.4 bar and 4.0 L/min, respectively, with the drying gas temperature being 180 °C. Capillary voltage was 3.2 kV. Mass spectra were acquired over the range 50−3500 Da. The samples were processed using Bruker Data Analysis software. Waters HPLC equipment (Millipore Corp., Waters Chromatography Division, Milford, MA) was composed of a 600E multisolvent delivery system equipped with a 996 photodiode array detector. Control of the equipment, data acquisition, processing, and management of the chromatographic information were performed by the Empower 2 software (Waters). 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 0.1 μm); He linear velocity, 30 cm/s; 50 °C isothermal for 4 min, linear gradient to 300 at 40 °C/min; final temperature hold, 20 min. MS conditions: ionization energy, 70 eV; ion source temperature, 250 °C; interface temperature, 270 °C; mass range, 45−600 amu.

Plant Material. Aerial parts of *Ipomoea purga* were collected in Coxmatla, Municipio de Xico, Veracruz, Mexico, in November 2008. The plant material was identified by botanist Alberto Linajes, and a voucher specimen (J. Castañeda and R. Pereda RP-05, XAL ID-364932) has been deposited in the herbarium of the Instituto de Ecología, Xalapa, Veracruz, Mexico (XAL). Also, voucher specimens were archived at the Departamento de Farmacia, Facultad de Química, UNAM (sample code: RP-05-XAL), and in the Ethnobotanical Collection of the National Herbarium (MEXU 425654), Instituto de Biologa, UNAM, and compared by one of us (R.P.-M.) with previously deposited vouchers housed in the Herbarium of the Instituto de Ecología, A.C. (Ortega 1488; XAL ID-I77366; Martínez-Vázquez 863; XAL ID-94389).

Extraction and Isolation. Aerial parts (623 g) was powdered and extracted exhaustively by maceration at room temperature with $CHCl_3$ to afford after removal of the solvent a dark brown syrup (21.5 g). The extract (20 g) was fractionated by open column chromatography over silica gel (380 g) using a gradient of $CHCl_3$ in hexane, followed by $CHCl_3$−Me_2CO and Me_2CO−MeOH in several proportions. Altogether, 89 eluates (200 mL each one) were collected and combined as 20 fractions (1−20). Fraction 12 (2.3 g), eluted with Me_2CO and containing a mixture of resin glycosides, was treated with activated charcoal to eliminate pigmented residues. This fraction was resolved by HPLC on a Symmetry C_{18} column (Waters; 7 μm, 19 × 300 mm) with an isocratic elution of methanol and a flow rate of 8 mL/min. Eluates across the peaks with t_R values of 19.8 min (peak I), 21.9 min (peak II), and 29.3 min (peak III) were collected by the technique of heart cutting and independently reinjected (sample injection, 500 μL; concentration, 0.1 mg/mL) in the apparatus operating in the recycle mode to achieve total homogeneity between 10 and 20 consecutive cycles employing a Symmetry C_{18} column (Waters; 7 μm, 19 × 300 mm), isocratic elution with methanol, and a flow rate of 8 mL/min. These techniques afforded pure compound **1** (13 mg) from peak I and **2** (9 mg) from peak II. The same elution system with a flow rate of 9 mL/min allowed the purification of **3** (20 mg) from peak III.

Purginoside I (**1**): white powder; mp 133−135 °C; $[\alpha]_{589}$ −16, $[\alpha]_{578}$ −16, $[\alpha]_{546}$ −18, $[\alpha]_{436}$ −26, $[\alpha]_{365}$ −33 (*c* 0.2, MeOH); ^{1}H and ^{13}C NMR, see Table 1; negative FABMS *m/z* 1367 $[M - H]^-$, 1237 $[M - H - C_9H_6O$ (cinnamoyl ester)$]^-$, 1083 $[1237 - C_{10}H_{18}O$ (decanoyl ester)$]^-$, 853 $[1083 - C_5H_8O$ (methylbutanoyl ester) $- C_6H_{10}O_4$ (methylpentose unit)$]^-$, 691 $[853 - C_6H_{10}O_5$ (hexose 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)$]^-$; HRFABMS *m/z* 1367.7021 $[M - H]^-$ (calcd for $C_{70}H_{111}O_{26}$ requires 1367.7358).

Purginoside II (**2**): white powder; mp 135−137 °C; $[\alpha]_{589}$ −16, $[\alpha]_{578}$ −16, $[\alpha]_{546}$ −18, $[\alpha]_{436}$ −29, $[\alpha]_{365}$ −41 (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR, see Table 1; negative FABMS *m/z* 1381 $[M - H]^-$, 1251 $[M - H - C_9H_6O$ (cinnamoyl ester)$]^-$, 1097 $[1251 - C_{10}H_{18}O$ (decanoyl ester)$]^-$, 853 $[1097 - C_6H_{10}O$ (hexanoyl ester) $- C_6H_{10}O_4$ (methylpentose unit)$]^-$, 691 $[853 - C_6H_{10}O_5$ (hexose 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)$]^-$; HRFABMS *m/z* 1381.7339 $[M - H]^-$ (calcd for $C_{71}H_{113}O_{26}$ requires 1381.7515).

Purgin I (**3**): white powder; mp 128−130 °C; $[\alpha]_{589}$ −16, $[\alpha]_{578}$ −17, $[\alpha]_{546}$ −19, $[\alpha]_{436}$ −29, $[\alpha]_{365}$ −38 (*c* 0.14, MeOH); ^{1}H and ^{13}C

NMR, see Table 2; negative HRESIMS m/z 2828.5374 $[M + Cl]^-$ (calcd for $C_{144}H_{232}O_{52}Cl$ requires 2828.5208, δ = +5.87 ppm), m/z 1395.7767 $[M/2 - H]^-$ (calcd for $C_{72}H_{115}O_{26}$ requires 1395.7676).

Alkaline Hydrolysis of the Resin Glycoside Fraction. A solution of the resin glycoside fraction (253 mg) in 5% KOH−H_2O (6.5 mL) was refluxed at 95 °C for 3 h. Then, the reaction mixture was acidified to pH 5.0 and extracted with $CHCl_3$ (2 × 20 mL) and Et_2O (2 × 20 mL). The organic layers were combined and washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was directly analyzed by GC-MS: 2-methylbutanoic acid (t_R 2.0 min): m/z $[M]^+$ 102 (8), 87 (2), 74 (12), 57 (100); *n*-hexanoic acid (t_R 6.5 min): m/z $[M]^+$ 116 (2), 99 (3), 87 (21), 73 (63), 60 (100), 41 (16), 39 (7); *n*-decanoic acid (t_R 7.4 min): m/z $[M]^+$ 172 (2), 155 (1), 143 (8), 129 (50), 115 (12), 112 (8), 87 (16), 73 (100), 60 (90), 57 (32), 55 (30); cinnamic acid (t_R 7.7 min): m/z $[M]^+$ 148 (84), 147 (100), 131 (22), 103 (42), 102 (21), 77 (28), 74 (5), 51 (12), 50 (5); *n*-dodecanoic acid (t_R 8.01 min): m/z $[M]^+$ 200 (9), 183 (2), 171 (15), 157 (38), 143 (9), 129 (45), 115 (22), 101 (15), 85 (30), 73 (100), 60 (68), 57 (30), 55 (30).

The aqueous phase was extracted with *n*-BuOH (40 mL) and concentrated to give a colorless solid (116 mg). The residue (54 mg) was further acetylated (Ac_2O−C_5H_5N, 2:1) and methylated with CH_2N_2 to give a residue (45 mg), which was subjected to preparative HPLC on a reversed-phase C_{18} column (7 μm, 19 × 300 mm). The elution was isocratic with CH_3CN−H_2O (95:5) using a flow rate of 8.0 mL/min. The eluate across the peak with a t_R value of 14.13 min was collected by heart cutting and independently reinjected in the apparatus operated in the recycle mode to achieve total homogeneity after 10 consecutive cycles employing the same isocratic elution. The physical and spectroscopic constants (^1H and ^{13}C NMR; Figures S23−S28, Supporting Information) allowed the identification of this derivative as peracetyl operculinic acid A methyl ester (**5**): white powder; mp 83−85 °C; $[\alpha]_D$ −40 (*c* 0.14, MeOH); MALDIMS m/z $[M + Na]^+$ 1559 (Figure S22, Supporting Information), which was identified by comparison with an authentic sample;[19] therefore, both aglycone and sugar configurations were confirmed.

The preparation and identification of 4-bromophenyacyl (2*S*)-2-methylbutyrate were performed according to a previously reported procedure:[20] mp 40−42 °C; $[\alpha]_D$ +18 (*c* 1.0, 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.[24]

■ ASSOCIATED CONTENT

Supporting Information. ^1H, ^{13}C, COSY, TOCSY, HMQC, and HMBC NMR spectra as well as FABMS and ESIMS of natural products **1**−**3** and derivative **5** (Figures S1−S28). HMBC correlations for **1**−**3** (Figures S29 and S30). Structure for compounds **4** and **5** (Figure S31). This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) The generic name *Ipomoea* is derived from the Greek words ιπς (*ips*) or ιπος (*ipos*), meaning "worm" or "bindweed," and όμοιος (*homoios*), meaning "resembling". It refers to the twining habit of most members of the morning glory family (Convolvulaceae). The adjective *purga* meaning "purgative" (to purge) was used for the species name *Ipomoea purga* (Wender) Hayne, which is considered the "Officinal Jalapae Radix". Several synonyms exist for this plant, appearing in old reports: *Exogonium purga* (Wender) Benth., *Convolvulus officinalis* Pelletan, *Convolvulus jalapa* Scheide, *Convolvulus purga* Wender, and *Ipomoea jalapa* Nutt. & Cox.

(2) McDonald, A. *Flora de Veracruz*; Instituto de Ecología, A.C. Xalapa, University of California: Riverside, CA, 1994; pp 98−100.

(3) Pereda-Miranda, R.; Fragoso-Serrano, M.; Escalante-Sánchez, E.; Hérnandez-Carlos, B.; Linares, E.; Bye, R. *J. Nat. Prod.* **2006**, *69*, 1460–1466.

(4) Pereda-Miranda, R.; Rosas-Ramírez, D.; Castañeda-Gómez, J. *Progress in the Chemistry of Organic Natural Products*; In Kinghorn, A. D., Falk, H., Kobayashi, J., Eds.; Springer-Verlag: New York, 2010; Vol. 92, Chapter 2, pp 77−152.

(5) The chemical diversity of the resin glycosides from scammony (*Convolvulus scammonia*) has been extensively investigated; see: (a) Shellard, E. J. *Planta Med.* **1961**, *9*, 146–152. (b) Noda, N.; Kogetsu, H.; Kawasaki, T.; Miyahara, K. *Phytochemistry* **1990**, *29*, 3565–3569. (c) Noda, N.; Kogetsu, H.; Kawasaki, T.; Miyahara, K. *Phytochemistry* **1992**, *31*, 2761–2766. (d) Kogetsu, H.; Noda, N.; Kawasaki, T.; Miyahara, K. *Phytochemistry* **1991**, *30*, 957–963.

(6) Between 1761 and 1851, the Xico region in Veracruz, Mexico, exported 1 674 033 tons of the root to Europe. In the 1940s, about 40 tons of dried jalap root (*I. purga*) were exported to the United States per year, with this level maintained up to the 1990s. The commercial demand for jalap roots has declined due to the use of other laxatives derived from psyllium fruits (*Plantago* spp.) and cassia leaves (*Senna* spp.) as well as the domination of the world market by Italian and German herbal drug traders who import resins of the Brazilian jalap (*I. operculata*). For the traditional production system of jalap root, see: Linajes, A.; Rico-Gray, V.; Carrión, G. *Econ. Bot.* **1994**, *48*, 84–89.

(7) (a) Emmart, E. W. *The Badianus Manuscript (Codex Barberini, Latin 241). An Aztec Herbal of 1552*; The Johns Hopkins Press: Baltimore, 1940; p 260. (b) Gates, W. *An Aztec Herbal. The Classic Codex of 1552*; Dover: New York, 2000; p 55. (c) Hernández, F. *The Mexican Treasury: the Writings of Dr. Francisco Hernández*; Varey, S., Ed.; Stanford University Press: Stanford, CA, 2000; pp 117−156.

(8) (a) Navarro, J. *Historia Natural o Jardín Americano [Manuscrito de 1801]*; Universidad Nacional Autónoma de México: Mexico City, 1992; p 133. (b) Anonymous. *Eclectic and General Dispensatory*; Toward and Hogan: Philadelphia, 1827; p 154

(9) (a) Noriega, J. M. *Curso de Historia de Drogas*; Instituto Médico Nacional: Mexico City, 1902; pp 372−373. (b) Herrera, A. L. *Farmacopea Latino-Americana*; Herrero Hermanos: Mexico City, 1921; pp 658−659. (c) Williams, L. O. *Econ. Bot.* **1970**, *24*, 399–401. (d) Martínez, M. *Las Plantas Medicinales de México*; Ediciones Botas: Mexico City, 1989; pp 276−279. (e) Cano, L. M. *Flora Medicinal de Veracruz, I. Inventario Etnobotánico*; Universidad Veracruzana: Xalapa, Mexico, 1997; p 185. (f) *Extrafarmacopea Herbolaria de los Estados Unidos Mexicanos*; Secretaría de Salud: Mexico City, 2001; pp 18−19.

(10) Mitscher, L. A.; Telikepalli, H. In *Advances in Natural Product Chemistry*; Atta-ur-Rahman, Ed.; Harwood Academic Publishers: Chur, Switzerland, 1992; pp 281−309.

(11) (a) Hoehnel, W. *Arch. Pharm. (Weinheim, Ger.)* **1896**, *234*, 647. (b) Votoček, E. *Ber. Deut. Chem. Ges.* **1910**, *43*, 476. (c) Power, F. B.;

Rogerson, H. *J. Am. Chem. Soc.* **1910**, *32*, 80. (d) Stacey, B. E.; Singh, S. *Phytochemistry* **1973**, *12*, 1701–1705.

(12) Rosas-Ramírez, D.; Escalante-Sánchez, E.; Pereda-Miranda, R. *Phytochemistry* **2011**, in press. DOI: 10.1016/j.phytochem.2011.03.002.

(13) León-Rivera, I.; Mirón-López, G.; Estrada-Soto, S.; Aguirre-Crespo, F.; Gutiérrez, M.; Molina-Salinas, G. M.; Hurtado, G.; Navarrete-Vásquez, G.; Montiel, E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4652–4656.

(14) Escalante-Sánchez, E.; Pereda-Miranda, R. *J. Nat. Prod.* **2007**, *70*, 1029–1034.

(15) Bah, M.; Pereda-Miranda, R. *Tetrahedron* **1997**, *53*, 9007–9022.

(16) Noda, N.; Tsuji, K.; Kawasaki, T.; Miyahara, K.; Hanazono, H.; Yang, C. R. *Chem. Pharm. Bull.* **1995**, *43*, 1061–1063.

(17) Ono, M.; Kawasaki, T.; Miyahara, K. *Chem. Pharm. Bull.* **1989**, *37*, 3209–3213.

(18) Barnes, C. C.; Smalley, M. K.; Manfredi, K. P.; Kindscher, K.; Loring, H.; Sheeley, D. M. *J. Nat. Prod.* **2003**, *66*, 1457–1462.

(19) Chérigo, L.; Pereda-Miranda, R. *J. Nat. Prod.* **2006**, *69*, 595–599.

(20) Pereda-Miranda, R.; Hérnandez-Carlos, B. *Tetrahedron* **2002**, *58*, 3145–3154.

(21) (a) Pereda-Miranda, R.; Escalante-Sánchez, E.; Escobedo-Martínez, C. *J. Nat. Prod.* **2005**, *68*, 226–230. (b) Escalante-Sánchez, E.; Rosas-Ramírez, D.; Linares, E.; Bye, R.; Pereda-Miranda, R. *J. Agric. Food Chem.* **2008**, *56*, 9423–9428.

(22) Pereda-Miranda, R.; Bah, M. *Curr. Top. Med. Chem.* **2003**, *3*, 111–131.

(23) Mendoza-Espinoza, J. A.; López-Vallejo, F.; Fragoso-Serrano, M.; Pereda-Miranda, R.; Cerda-García-Rojas, C. M. *J. Nat. Prod.* **2009**, *72*, 700–708.

(24) (a) Bah, M.; Chérigo, L.; Taketa, A. T. C.; Fragoso-Serrano, M.; Hammond, G. B.; Pereda-Miranda, R. *J. Nat. Prod.* **2007**, *70*, 1153–1157. (b) Escobedo-Martínez, C.; Cruz-Morales, S.; Fragoso-Serrano, M.; Rahman, M. M.; Gibbons, S.; Pereda-Miranda, R. *Phytochemistry* **2010**, *71*, 1796–1801.