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Resin Glycosides from the Yellow-Skinned Variety of Sweet Potato (*Ipomoea batatas*)

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Ⓢ *Supporting Information*

ABSTRACT: Native to tropical America, *Ipomoea batatas* has been cultivated for over 5000 years in Mexico. The yellow-skinned tuber crop variety, with an orange flesh, has a higher nutritional value than potato. Raw sweet potato can cause a purge due to its resin glycoside content. Purification of the chloroform-soluble resin glycosides from the roots of this variety was accomplished by preparative-scale HPLC, which allowed for the collection of six oligosaccharides, batatin VII (**1**) and batatinosides VII−IX (**2**−**4**), all of novel structure, together with the known resin glycosides pescaprein I and batatinoside IV. High-field NMR spectroscopy and FAB mass spectrometry were used to characterize each structure, identifying operculinic acid A for compounds **2** and **4**, and simonic acid B for **3**, as their pentasaccharide glycosidic cores. Batatin VII (**1**) represents a dimer of the know batatinoside IV, consisting of two units of simonic acid B.

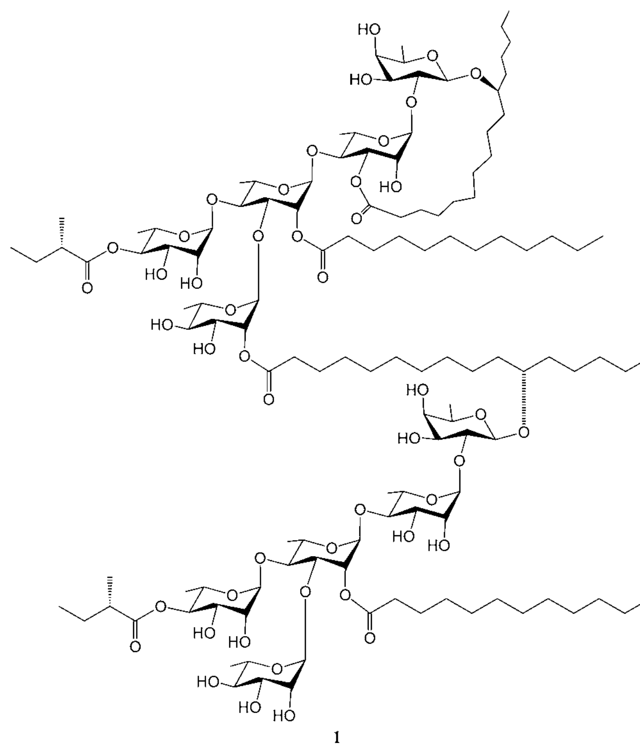
KEYWORDS: *Ipomoea batatas, sweet potato, morning-glory family, resin glycoside, glycolipid ester-type dimer, pentaoligosaccharide, batatin, batatinoside*

■ INTRODUCTION

Worldwide, sweet potatoes are one of the most important tuber crops, especially in developing countries.[1] Intensively cultivated for over 5000 years in Mexico and Central and South America, the Mexican varieties include white- and yellow-fleshed, with yellow, orange, red, or purple skin. All varieties are grown and consumed throughout the Mexican republic,[1] with preference for the white- and yellow-skinned varieties in central Mexico. *Ipomoea batatas* has edible tubers with a higher nutritional value than that of potatoes (*Solanum tuberosum*) and is a rich source of antioxidant flavonoids, vitamins, minerals, and dietary fiber essential for optimal health.[2] The yellow-skinned variety, with a pale copper skin and a deep orange flesh similar to that of pumpkin, has a β-carotene content greater than that of carrots.[1] In Mexico, sweet potatoes have been consumed roasted with other favorite root vegetables for a colorful side dish, but historically they were popularized as crystallized candies.

If eaten raw, the high content of resin glycosides is responsible for the purgative activity similar to that of such other species as *Ipomoea purga* and *Ipomoea orizabensis*, which are widely used in traditional medicine.[3,4] It is safe to eat a half cup without cooking, but eating more than that can cause flatulence and even a drastic purge. The resin glycosides isolated from this species have been found to produce various biological effects:[5−8] (a) cytotoxic activity of batatosides L and O in laryngeal carcinoma cell lines (Hep-2);[9] (b) reversal of multidrug resistance in vinblastine-resistant human breast carcinoma cells (MCF-7/Vin) by batatinoside IV and batatins I−II;[7] and (c) anti-inflammatory activity, as ipomotaoside A, which was evaluated for its inhibitory potential of COX-1 and COX-2 enzymes.[10]

Whereas our previous research was focused on the resin glycoside contents of both the white-[11,12] and purple-skinned staple-type tuber varieties,[13] this study investigated new lipophilic oligosaccharides of jalapinolic acid, batatin VII (**1**), and batatinosides VII−IX (**2**−**4**), isolated from the cultivated yellow-skinned variety of sweet potato.



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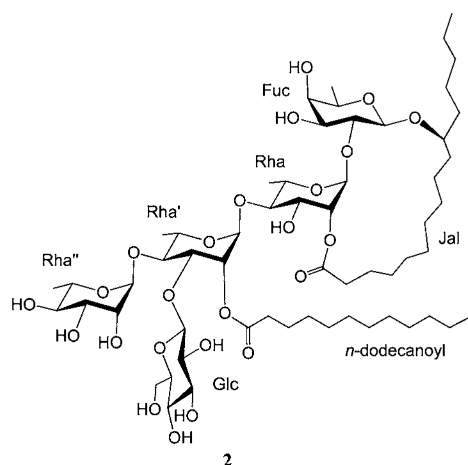

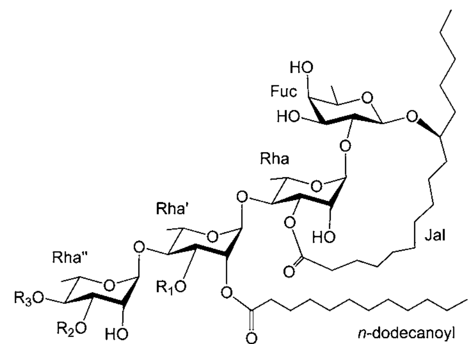

	R_1	R_2	R_3
3	α-L-rhamnopyranosyl = Rha	(2*S*)-methylbutanoyl	H
4	β-D-glucopiranosyl = Glc	H	H
5	Rha	H	H
6	Rha	H	(2*S*)-methylbutanoyl

■ MATERIALS AND METHODS

General Experimental Procedures. All melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer model 241 polarimeter. ^1H (500 MHz; TMS = 0 ppm) and ^{13}C (125.7 MHz; C_5D_5N = 123.5 ppm) NMR experiments were conducted on a Bruker DMX-500 instrument, whereas ^1H (400 MHz) measurements were carried out on a Varian VXL instrument. The instrumentation used for HPLC analysis consisted on a Waters (Millipore Corp., Waters Chromatography Division, Milford, MA, USA) 600 E multisolvent delivery system equipped with a Waters 410 refractive index detector. GC-MS was performed on a Hewlett-Packard 5890-II instrument coupled to a JEOL SX-102A spectrometer. GC conditions: HP-5MS (5% phenyl)-methylpolysiloxane column (30 m × 0.25 mm, Agilent Technologies, Santa Clara, CA, USA), film thickness = 0.25 μm; He, linear velocity = 30 cm/s; 50 °C isothermal for 3 min, linear gradient to 300 °C at 20 °C/min; final temperature hold = 10 min. MS conditions: ionization energy = 70 eV; ion source temperature = 280 °C; interface temperature = 300 °C; scan speed = 2 scans/s; mass range = 33−880 amu. Negative ion FAB/MS experiments were performed on a JEOL SX-102A spectrometer and recorded using a matrix of triethanolamine. High-resolution negative ion ESI/MS experiments were performed on a Bruker MicrOTOF-Q instrument. High-resolution MALDI/MS experiments were performed on a Waters MALDI Micro MX (MALDI-TOF) instrument equipped with a 20 Hz nitrogen laser (337 nm, 3 ns pulse) operating in positive reflectron mode. The matrix for laser desorption was α-cyano-4-hydroxycinnamic acid with 0.001 M sodium iodide in CH_3CN/H_2O (1:1).

Plant Material. The roots of the cultivated yellow-skinned variety (Heartgold) of *I. batatas* were collected from plantations in Salva Rita, Maravatio, Guanajuato, Mexico, in 2009. The plant material was identified by Dr. Robert Bye. A voucher specimen (R. Bye FB 1313) was deposited in the Ethnobotanical Collection of the National Herbarium (MEXUM), Instituto de Biología UNAM.

Extraction and Isolation. The powdered dry roots (1.2 kg) were extracted by maceration at room temperature with $CHCl_3$ (8 L) to give, after removal of the solvent, a dark brown syrup (7.15 g). The crude extract was subjected to column chromatography over silica gel (150 g) using gradients of CH_2Cl_2 in hexane and Me_2CO in CH_2Cl_2. A total of 45 eluates (100 mL each) were collected and combined in five fractions (I−V). Resin glycosides were concentrated in fraction II (670 mg; 5.5×10^{-2}% on a dry weight basis) that was partially purified by passage through activated charcoal to eliminate pigmented residues.

Recycling HPLC Separation. Control of the equipment, data acquisition, processing, and management of chromatographic information were performed by the Waters Millennium 2000 software program. The crude fraction II (500 mg) was independently subjected to preparative HPLC. The column used was a 300 × 19 mm i.d., 7 μm, Symmetry RP-18 (Waters). The elution was isocratic with MeOH/CH_3CN (3:2) using a flow rate of 8.16 mL/min. Eluates with t_R of 8.9 min (peak A), 9.6 min (peak B), 10.3 min (peak C), 10.9 min (peak D)/ and 21.2 min (peak E) were collected by the technique of heart cutting and independently reinjected in the apparatus operated in the recycle mode to achieve total homogeneity after 10−20 consecutive cycles employing the same isocratic elution.[14] These techniques afforded pure compound **2** (9.9 mg) and pescaprein I (16.3 mg) from peak A; **3** (11.8 mg) from peak B; **1** (10 mg) from peak C; batatinoside IV (38.5 mg) from peak D; and **4** (27.6 mg) from peak E. All pure compounds were recrystallized in MeOH. The total yield of pure resin glycosides from fraction II was 8.6×10^{-3}% on a dry weight basis.

*Batatin VII (**1**):* white solid; mp 124−125 °C; $[\alpha]_D$ −49.0 (*c* 0.16 EtOH); ^1H and ^{13}C NMR, see Table 1; negative FAB/MS *m/z* 2501 $[M-H]^-$, 2417 $[M-H-C_5H_8O]^-$, 1251 [unit B, $C_{63}H_{111}O_{24}]^-$, 1249 [unit A, $C_{63}H_{109}O_{24}]^-$, 1165 $[1249 - C_5H_8O]^-$, 1067 $[1249 - C_{12}H_{22}O]^-$; MALDI-TOF/MS *m/z* 1273.7953 [unit A, $C_{63}H_{109}O_{24}$ + Na]$^+$ (calcd for $C_{63}H_{109}O_{24}Na$, 1273.5290). ESI/MS *m/z* 2500.4516 $[M-H]^-$ (calcd for $C_{126}H_{219}O_{48}$, 2500.4695).

*Batatinoside VII (**2**):* white solid; mp 105−107 °C; $[\alpha]_D$ −53.1 (*c* 0.16 EtOH); ^1H and ^{13}C NMR, see Table 2; negative FAB/MS *m/z* 1181 $[M-H]^-$, 999 $[M-H-C_{12}H_{22}O]^-$, 837 $[999 - C_6H_{10}O_4]^-$, 545, 417, 271; MALDI-TOF/MS *m/z* 1205.7488 $[M+Na]^+$ (calcd for $C_{58}H_{102}O_{24}Na$, 1205.6658).

*Batatinoside VIII (**3**):* white solid; mp 118−120 °C; $[\alpha]_D$ −53.0 (*c* 0.1 EtOH); ^1H and ^{13}C NMR, see Table 2; negative FAB/MS *m/z* 1249 $[M-H]^-$, 1165 $[M-H-C_5H_8O]^-$, 1067 $[M-H-C_{12}H_{22}O]^-$, 1019 $[M-H-C_6H_{10}O_4-C_5H_8O]^-$, 983 $[1067 - C_5H_8O]^-$, 837 $[1067 - C_6H_{10}O_4]^-$, 545, 417, 271; MALDI-TOF/MS *m/z* 1273.7953 $[M+Na]^+$ (calcd for $C_{63}H_{110}O_{24}Na$, 1273.7284).

*Batatinoside IX (**4**):* white solid; mp 132−135 °C; $[\alpha]_D$ −74.6 (*c* 0.11 EtOH); ^1H and ^{13}C NMR, see Table 2; negative FAB/MS *m/z* 1181 $[M-H]^-$, 999 $[M-H-C_{12}H_{22}O]^-$, 837 $[999 - C_6H_{10}O_4]^-$, 545, 417, 271; MALDI-TOF/MS *m/z* 1205.7229 $[M+Na]^+$ (calcd for $C_{58}H_{102}O_{24}Na$, 1205.6658).

*Pescaprein I (**5**):* white solid; mp 133−135 °C; $[\alpha]_D$ −65 (*c* 0.1, EtOH); negative FAB/MS *m/z* 1165 $[M-H]^-$; MALDI-TOF/MS *m/z* 1189.7416 (calcd for $C_{58}H_{102}O_{23}Na$, 1189.6709); identified by comparison of NMR data with published values.

*Batatinoside IV (**6**):* white solid; mp 125−127 °C; $[\alpha]_D$ −71.1 (*c* 0.18 EtOH); negative FAB/MS *m/z* 1249 $[M-H]^-$; MALDI-TOF/MS *m/z* 1273.7705 $[M+Na]^+$ (calcd for $C_{63}H_{110}O_{24}Na$, 1273.7284); identified by comparison of NMR data with published values.

Alkaline Hydrolysis of Resin Glycoside Mixture. A solution of the crude fraction II (100 mg) in 5% KOH/H_2O (5 mL) was refluxed at 95 °C for 2 h. The reaction mixture was acidified to pH 4.0 and extracted with $CHCl_3$ (30 mL). The organic layer were washed with H_2O, dried over anhydrous Na_2SO_4, evaporated under reduced pressure, and directly analyzed by CG-MS and comparison of their spectra and retention times with those of authentic samples.[3,14] Two peaks were detected: 2-methylbutanoic acid (t_R 7 min) [*m/z* $[M]^+$ 102 (3), 87 (33), 74 (100), 57 (50), 41 (28), 39 (8)] and *n*-dodecanoic acid (t_R 18 min) [*m/z* $[M]^+$ 200 (15), 183 (2), 171 (18), 157 (40), 143 (20), 129 (48),

Table 1. ^{1}H and ^{13}C NMR Data of Batatin VII (1)^{a}

position^{d}	δ_H^{b} unit A	δ_H^{b} unit B	δ_C^{c} unit A	δ_C^{c} unit B
Fuc-1	4.83 d (7.5)	4.82 d (7.5)	101.0	101.0
Fuc-2	4.54 dd (8.5, 7.5)	4.15 dd (8.0, 7.5)	73.3	73.3
Fuc-3	4.17 dd (8.5, 2.5)	4.18 dd (8.0, 2.5)	76.3	76.3
Fuc-4	3.93 d (1.0)	3.93 d (1.0)	73.6	73.6
Fuc-5	3.82 dq (6.5, 2.0)	3.82 dq (6.5, 1.0)	71.4	71.4
Fuc-6	1.52 d (6.5)	1.52 d (6.5)	17.4	17.4
Rha-1	6.36 d (2.0)	6.36 d (1.0)	100.3	100.3
Rha-2	5.32 dd (3.0, 2.0)	4.81 bs	69.8	69.8
Rha-3	5.63 dd (9.4, 3.0)	4.48 dd (9.9, 3.2)	77.7	72.6
Rha-4	4.22 t (9.4)	4.22 t (9.9)	78.0	78.0
Rha-5	5.01 dq (9.4, 6.0)	5.01 dq (9.9, 6.5)	67.8	67.8
Rha-6	1.67 d (6.0)	1.66 d (6.5)	19.4	18.6
Rha′-1	5.69 d (2.0)	5.69 d (2.0)	99.1	99.1
Rha′-2	5.84 dd (3.0, 2.0)	5.68 dd (3.0, 2.0)	79.8	79.8
Rha′-3	4.49 dd (9.0, 3.0)	4.49 dd (9.5, 3.0)	72.8	72.8
Rha′-4	4.29 t (9.0)	4.29 t (9.5)	79.3	79.5
Rha′-5	4.32 dq (9.0, 6.0)	4.33 dq (9.5, 6.0)	68.4	70.8
Rha′-6	1.72 d (6.0)	1.72 d (6.0)	18.8	18.7
Rha″-1	5.65 d (1.0)	5.55 d (1.2)	104.3	104.3
Rha″-2	4.77 dd (3.0, 1.0)	4.77 dd (3.0, 1.2)	70.6	70.5
Rha″-3	4.48 dd (9.7, 3.0)	4.48 dd (9.5, 3.0)	71.3	71.3
Rha″-4	5.86 t (9.7)	5.93 t (9.5)	74.1	73.4
Rha″-5	4.37 dq (9.8, 6.5)	4.35 dq (9.5, 6.5)	68.2	71.0
Rha″-6	1.51 d (6.5)	1.51 d (6.5)	17.9	18.4
Rha‴-1	5.95 d (1.0)	5.59 d (1.0)	103.5	104.3
Rha‴-2	5.77 dd (3.0, 1.0)	4.81 bs	75.6	72.5
Rha‴-3	4.18 dd (9.3, 3.0)	4.18 dd (9.5, 3.0)	69.7	72.6
Rha‴-4	4.24 t (9.3)	4.21 t (9.5)	73.6	73.5
Rha‴-5	4.29 dq (9.3, 6.0)	4.44 dq (9.5, 6.0)	70.7	70.7
Rha‴-6	1.55 d (6.0)	1.55 d (6.0)	19.4	18.5
Jal-1			175.2	174.9
Jal-2a	2.26−2.30*	2.26−2.30*	33.6	33.6
Jal-2b	3.00 ddd (12.0, 8.0, 3.5)			
Jal-11	3.88 m	3.88 m	79.6	79.6
Jal-16	0.97 t (7.0)	0.97 t (7.0)	14.3	14.3
Mba-1			176.6	176.6
Mba-2	2.49 tq (7.0, 7.0)	2.50 tq (7.0, 7.0)	41.3	41.4
Mba-2-Me	1.20 d (7.0)	1.20 d (7.0)	16.6	16.6
Mba-3-Me	1.07 d (7.0)	0.93 t (7.5)	11.6	11.6
dodeca-1			173.4	173.4
dodeca-2	2.33−2.41*	2.33−2.41*	34.5	34.5
dodeca-12	0.88 t (7.0)	0.88 t (7.0)	14.3	14.3

^{a}Data recorded in C_5D_5N. ^{b}400 MHz for ^{1}H. ^{c}125 MHz for ^{13}C. 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, singulet; d, doublet; t, triplet; m, multiplet; q, quartet; bs, broad signal. All assignments are based on ^{1}H−^{1}H COSY and TOCSY experiments. All ^{13}C assignments are based on HMQC and HMBC experiments. ^{d}Abbreviations; Fuc, fucose; Rha, rhamnose; Jal, 11-hydroxyhexadecanoyl; Mba, 2-methylbutanoyl; dodeca, dodecanoyl.

115 (20), 101 (15), 85 (33), 73 (100), 60 (80), 57 (30), 55 (47), 43 (30)]. Saponification of all compounds yielded *n*-dodecanoic acid, whereas compounds **1**, **3**, and **6** also afforded (*S*)-(+)-2-methylbutyric acid, both of which were purified by HPLC according to previously described methologies.[14,15]

The residue (50 mg) extracted from the aqueous phase was subjected to preparative HPLC on a Waters μBondapak NH_2 column (300 × 7.8 mm id., 10 μm). The elution was isocratic with CH_3CN/H_2O (4:1), using a flow rate of 3 mL/min and a sample injection of 500 μL (50 mg/mL). This procedure yielded two glycosidic acids; simonic acid

Table 2. ^{1}H and ^{13}C NMR Data of Batatinosides VII−IX (2−4)[a]

	batatinoside VII[b]		batatinoside VIII[b]		batatinoside IX[c]	
position[d]	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
Fuc-1	4.81 d (8.0)	99.7	4.83 d (8.0)	101.8	4.81 d (8.0)	101.6
Fuc-2	4.51 dd (9.7, 8.0)	73.6	4.54 dd (9.5, 8.0)	72.5	4.51 dd (9.7, 8.0)	73.6
Fuc-3	4.16 dd (9.7, 3.5)	76.5	4.24 dd (9.5, 3.5)	76.6	4.19 dd (9.7, 3.5)	76.5
Fuc-4	4.02 d (3.5)	73.6	3.94	73.6	3.92 d (3.5)	73.6
Fuc-5	3.81 q (6.5)	71.3	3.83 q (6.0)	71.3	3.81 q (6.5)	71.3
Fuc-6	1.51 d (6.0)	17.2	1.54 d (6.0)	17.3	1.51 d (6.0)	16.9
Rha-1	6.26 d (1.5)	100.0	6.36 bs	100.3	6.33 d (1.5)	100.1
Rha-2	6.36 dd (9.5, 3.0)	68.0	5.33 bs	69.9	5.23 dd (3.5, 1.5)	68.0
Rha-3	4.68 dd (3.5, 1.5)	78.0	5.65 dd (9.6, 3.0)	77.9	5.65 dd (9.5, 3.0)	78.0
Rha-4	4.26 t (9.5)	76.3	4.66 dd (9.6, 9.6)	77.5	4.67 t (9.5)	76.3
Rha-5	4.32 dq (9.5, 6.0)	68.0	5.03 dq (9.6, 6.0)	68.0	4.97 dq (9.5, 6.0)	68.0
Rha-6	1.56 d (6.0)	19.2	1.57 d (6.0)	19.3	1.56 d (6.0)	18.9
Rha′-1	5.80 d (1.5)	98.4	5.67 bs	99.1	5.63 d (2.0)	99.2
Rha′-2	6.39 dd (3.5, 1.5)	72.4	5.85 bs	73.1	5.99 dd (3.0, 2.0)	72.4
Rha′-3	4.84 dd (9.5, 3.0)	80.3	4.61 dd (9.8, 3.0)	80.2	4.63 dd (9.0, 3.0)	80.3
Rha′-4	4.39 t (9.5)	77.9	4.31 dd (9.8, 9.8)	78.5	4.34 t (9.0)	77.9
Rha′-5	4.92 dq (9.5, 6.0)	68.3	4.32 dq (9.8, 6.3)	68.4	4.33 *	68.3
Rha′-6	1.60 d (6.0)	18.7	1.50 d (6.3)	18.7	1.60 d (6.0)	18.4
Rha″-1	6.28 d (1.5)	101.9	5.95 d (1.5)	103.6	6.23 d (1.5)	103.4
Rha″-2	4.92 dd (3.5, 1.5)	72.4	4.78 bs	72.7	4.90 dd (3.0, 1.5)	72.4
Rha″-3	4.25 dd (9.5, 3.0)	72.8	5.76 dd (9.5, 3.0)	75.5	4.42 dd (9.0, 3.0)	72.8
Rha″-4	4.26 t (9.5)	74.0	4.43 dd (9.5, 9.5)	71.3	4.26 t (9.0)	74.0
Rha″-5	4.32 dq (9.5, 6.0)	70.7	4.40 dq (9.5, 6.0)	71.0	4.32	70.7
Rha″-6	1.65 d (6.0)	18.5	1.67 d (6.0)	18.4	1.65 d (6.0)	18.2
Rha‴-1			5.65 d (1.0)	104.3		
Rha‴-2			4.65 dd (3.7, 1.0)	72.6		
Rha‴-3			4.35 dd (9.1, 3.7)	72.6		
Rha‴-4			4.19 dd (9.1, 9.1)	73.7		
Rha‴-5			4.29 dq (9.1, 6.1)	70.5		
Rha‴-6			1.73 d (6.1)	18.8		
Glc-1	5.18 d (7.5)	104.3			5.09 d (7.5)	104.9
Glc-2	4.03 dd (9.0, 8.5)	75.2			3.96 dd (9.0, 8.5)	75.2
Glc-3	4.16 *	78.3			4.14 *	78.3
Glc-4	4.18 *	70.7			4.18 *	70.7
Glc-5	3.87 m	78.0			3.87 m	78.0
Glc-6a	4.38 t (10.0)	62.5			4.38 t (10.0)	62.5
Glc-6b	4.47 dd (10.0, 8.0)				4.47 dd (10.0, 8.0)	
Jal-1		172.6		174.9		174.7
Jal-2a	2.27 ddd (14.5, 7.0, 2.0)	34.2	2.29 ddd (15.3, 7.1, 3.1)	33.8	2.28 ddd (14.5, 7.0, 2.0)	34.2
Jal-2b	2.41 ddd (14.5, 10.5, 3.0)		2.95 t (11.7)		2.67 ddd (14.5, 10.5, 3.0)	
Jal-11	3.81 m	79.6	3.89 m	79.6	3.87 m	79.6
Jal-16	0.86 t (7.0)	12.8	0.88 t (6.8)	14.3	0.87 t (7.0)	14.0
Mba-1				176.6		
Mba-2			2.42 tq (7.0, 6.7)	41.4		
Mba-2-Me			1.07 d (7.5)	16.6		
Mba-3-Me		172.1	0.91 t (7.5)	11.7		
dodeca-1		34.5		172.9		173.5
dodeca-2	2.40 ddd (16.0, 7.5, 2.0)	13.0	2.35 t (7.2)	34.4	2.40 ddd (16.0, 7.5, 2.0)	34.5
dodeca-12	0.93 t (7.0)		1.00 t	14.5	0.92 t (7.0)	14.2

[a]Data recorded in C_5D_5N. [b]400 MHz for ^{1}H; 125 MHz for ^{13}C. [c]500 MHz for ^{1}H; 125 MHz for ^{13}C. 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, singulet; d, doublet; t, triplet; m, multiplet; q, quartet; bs, broad signal. All assignments are based on $^{1}H-^{1}H$ COSY and TOCSY experiments. All ^{13}C assignments are based on HMQC and HMBC experiments. [d]Abbreviations: Fuc, fucose; Glc, glucose; Rha, rhamnose; Jal, 11-hydroxihexadecanoyl; Mba, 2-methylbutanoyl; dodeca, dodecanoyl.

B (t_R = 12.17 min) and operculinic acid A (t_R = 14.30 min), which were identified by comparison of physical constants and NMR data with published values.[16,17]

■ RESULTS AND DISCUSSION

The present investigation describes the isolation, purification, and structural characterization of glycolipids from the chloroform-soluble resin glycoside contents found in the tuberous roots of a cultivated yellow-skinned variety of sweet potato. The lipophilic crude extract was fractionated by silica gel column chromatography. The major fraction containing resin glycosides was purified by preparative-scale recycling HPLC, using the techniques of column overload as well as peak shaving and recycling.[14] This approach allowed for the collection of six macrocyclic oligosacharide derivatives of jalapinolic acid: batatin VII (**1**) and batatinosides VII–IX (**2**–**4**) of novel structure as well as the known pescaprein I[18] (**5**) and batatinoside IV (**6**).[12]

Characterization of the Oligosaccharide Cores. A small portion of the crude resin glycoside fraction II was saponified to liberate an H_2O-soluble mixture of oligosaccharides of jalapinolic acid. Two glycosidic acids were isolated: the first was characterized as the pentasaccharide operculinic acid A: (11*S*)-hydroxyhexadecanoate 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, which has been previously identified in the resin glycosides of sweet potato[9,12,19] and other morning glories of the genera *Ipomoea*[18,20−23] and *Merremia*.[24,25] The second glycosidic acid was also identified as the known pentasaccharide simonic acid B: (11*S*)-hydroxyhexadecanoate 11-*O*-*α*-L-rhamnopyranosyl-(1→3)-*O*-*α*-L-rhamnopyranosyl-(1→4)]-*O*-*α*-L-rhamnopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranoside, also previously found in *I. batatas*,[8,16,19,] *I. pes-caprae*,[18,20] *I. murucoides*,[21] and *I. stolonifera*.[22,23] The liberated organic acids were found to be 2-(*S*)-methylbutanoic and *n*-dodecanoic acids.

Interpretation of Mass Spectra of compounds 1–4. Batatin VII (**1**) is an oligosaccharide dimer composed of two units of the same glycosidic acid, arbitrarily designated units A and B.[11] The spectrometric analysis of compound **1** was conducted by ESI and FAB in the negative ion detection.[4,11] The ESI/MS of batatin VII (**1**) allowed for the detection of the quasi-molecular ion $[M - H]^-$, indicating a composistion of $C_{126}H_{219}O_{48}$. Fragment $[M/2 - H]^-$ was observed in both ionization techniques, which indicated the rupture of the ester type bond and represented the high-mass fragment ions for the two two monomeric units (*m*/*z* 1249 $[A - H]^-$ and *m*/*z* 1251 $[B - H]^-$) as previously reported for the batatin series.[11]

Negative ion FAB/MS of compounds **2**–**4** were also obtained. This technique provided an easily detectable and intense quasi-molecular ion $[M - H]^-$ as well as all of the characteristic ions resulting from glycosidic cleavage.[4] FAB/MS of batatinosides VII (**2**) and IX (**4**) showed a peak at *m*/*z* 1181 $[M - H]^-$, indicating a molecular formula of $C_{58}H_{102}O_{24}$, which was confirmed on the basis of the observed $[M + Na]^+$ ion by MALDI/MS. Thus, compounds **2** and **4** represent a pair of regioisomers. The fragmentation pattern was determined with the peaks observed at *m*/*z* 271, 417, 545, 837, and 999 that are common to resin glycosides containing operculinic acid A as their glycosidic acid.[8,15,20] The fragment at *m*/*z* 999 $[1181 - C_{12}H_{22}O]^-$ indicated the loss of 182 Da from the dodecanoyl residue, followed by the loss of glucose as identified by the fragment at *m*/*z* 837 $[999 - C_6H_{10}O_5]^-$. Batatinoside VIII (**3**) produced the quasi-molecular ion $[M - H]^-$ at *m*/*z* 1249, indicating a molecular formula of $C_{63}H_{110}O_{24}$, which was confirmed by the ion $[M + Na]^+$ as determined by MALDI/MS. The fragmentation pattern was determined with the observed peaks at *m*/*z* 271, 417, 545, 837, and 983 that are common to resin glycoside containing simonic acid B.[16] Other fragments were produced by the characteristic eliminations of the esterifying groups observed at *m*/*z* 1165 $[1249 - C_5H_8O]^-$ corresponding to the loss of 84 Da from the 2-methylbutanoyl residue and at *m*/*z* 1067 $[1249 - C_{12}H_{22}O]^-$ for the loss of one dodecanoyl residue.[15,18,20,21]

NMR Structure Elucidation of Compounds 1–4. NMR spectra of compounds **1**–**4** were recorded. ^1H and ^{13}C NMR spectra of batatin VII (**1**) are included in Table 1. The dimeric oligosaccharide structure of batatin VII (**1**) is constituted by two units of simonic acid B, and its structure elucidation was performed by comparison of its spectroscopic constants with those previously reported for batatins I (**7**) and II (**8**).[11] This dimer illustrates the four-step approach for identification of carbohydrate structural elements in the oligosacharaide core.[4] Estimations of the sugar units were done through the anomeric protons around 4.8–6.4 ppm, which were used as "structural reporter groups"[4,11] for the dimeric structure. To identify each constitutive monosaccharide, COSY and TOCSY experiments were simultaneously used to assign chemical shift values, after identifying and differentiating the ^1H NMR signals (Table 1). 2D $^1H-^{13}C$ NMR experiments using the HSQC technique provided assignments for the ^{13}C NMR data (Table 1). The glycosylation sequence and the positions of esterification and lactonization were verified by the observed long-range correlations ($^3J_{CH}$) in the HMBC spectrum.

The carbonyl resonances at δ_C 175.2 was assigned to the unit A lactone functionality due to its $^2J_{CH}$ coupling with the C-2 diastereotopic methylene protons centered at δ_H 2.26–2.30. The macrolactonization site at C-3 of the first rhamnose unit was established by the observed $^3J_{CH}$ correlation between this carbonyl carbon and H-3 of rhamnose (δ_H 5.63). The ester-type linkage established by the acyclic unit B at the macrocyclic unit A was identified by $^3J_{CH}$ correlation between the carbonyl group for the ester (δ_C 174.9; unit B) and H-2 of the terminal rhamnose (Rha‴, δ_H 5.77) on unit A. The dodecanoyl residue on both units A and B (δ_C 173.4) was located at C-2 of the third saccharide unit (Rha′, δ_H 4–99). The methylbutanoyl residue (δ_C 166.9, unit A; δ_C 166.4, unit B) was located as the acylating group at positions C-4 (δ_H 5.86, unit A; and δ_H 5.93, unit B) of the terminal rhamnose unit (Rha″).

Common features in both ^1H and ^{13}C NMR spectra for the new compounds **2**–**4** are included in Table 2. The diagnostic resonances observed in the downfield region δ 4.8–6.4 were assigned to the anomeric protons because of their multiplicity as doublets. In the ^{13}C NMR spectra, the anomeric signals in the δ 98–105 region directly indicated the number of monosaccharide units forming the oligosaccharide core for each compound. Five diagnostic carbon signals in the anomeric region were observed and correlated with the same number of proton resonances for **2**–**4**. Expansion of the COSY spectrum for the anomeric region showed that compounds **2** and **4** presented two sites of esterification, whereas compound **3** displayed three positions. For compound **2**, the carbonyl signal centered at δ 172.6 was assigned to the lactone group because of its observed ^2J coupling with the C-2 diastereotopic methylene protons of the aglycone unit (δ_H 2.27 and 2.41), and the lactonization was corroborated at position C-2 of the second monosaccharide unit (Rha) by the observed ^3J coupling between the carbonyl group and the corresponding methyne proton (δ_H 6.36) on the saccharide core.

For compounds **3** and **4**, the carbonyl signal centered at δ 174.7 and 174.9, respectively, was assigned to the lactone group, and its place of esterification was corroborated at C-3 of the second monosaccharide unit (Rha) by the observed ^3J coupling with H-3 (δ_H 5.65) on the saccharide core. For all compounds (**2**–**4**), the dodecanoyl group (δ_{C-1} 172.1–173.5) was located at C-2 of the second rhamnose (Rha′, δ_H 5.85–6.39). For compound **3**, the 2-methylbutanoyl group (δ_{C-1} 176.6) was located at C-3 of the terminal rhamnose (Rha″, δ_H 5.76).

Structural Diversity of Sweet Potato Resin Glycosides. Forty-five intact glycolipids have been isolated from the sweet potato.[4] These compounds illustrate how nature creates structural diversity by using simple metabolic building blocks. Sugar units found in these oligosaccharide cores are D-glucose, D-fucose, and L-rhamnose. *O*-Glycosidic linkage is the only type forming connections between the monosaccharide residues as well as with the aglycone, 11(*S*)-hydroxyhexadecanoic acid. Five heteropolysaccharides have been characterized as the constitutive glycosidic acids: operculinic acids A,[9,12] C,[12] and E[9] as well as simonic acids A[9,16] and B.[8,9,11,12] The structural complexity arises from the variable linkage positions formed with the following highly conserved disaccharide subunits: L-Rha-(1→2)-D-Fuc, L-Rha-(1→4)-L-Rha, and D-Glc-(1→2)-D-Fuc. This chemical diversity is further increased by the diverging possibilities of cyclization of the glycosidic acid cores into corresponding macrolactones, mainly at C-2 and C-3 of the second saccharide unit. In addition, the multiple variations caused by acylation with fatty acids of different lengths considerably increase their structural variety.

The most complex resin glycoside structures that have been isolated are the ester-type dimers of acylated tetrasaccharides[13] and pentasaccharides[11] found in the Mexican white-skinned staple-type cultivar. Actually, a large number of resin glycoside congeners can occur in the different varieties of sweet potato. They are not very easy to isolate and purify for they are always present as complex mixtures of homologues having the same polar oligosaccharide moiety but with alkyl substituents differing in chain length. Homogeneity can be obtained for minor isolated compounds through the application of improved recycling HPLC techniques that provide maximal resolution in a short-term analysis involving the use of small particle sizes and modified stationary phases. The methodologies used for the isolation and structural characterization of **1**–**4** could be applicable for the purification and characterization of polar bioactive resin glycoside oligomers from the morning-glory family or lipooligosaccharides with large molecular weights from other plant sources.

■ ASSOCIATED CONTENT

Supporting Information

Spectra (negative FAB/MS, negative ESI/MS, MALDI-TOF/MS, ^{1}H and ^{13}C NMR, COSY, TOCSY, HMQC, HMBC) of batatin VII (**1**) and batatinosides VII–IX (**2**–**4**); observed correlations in the HMBC experiment for **1**; structures for sweet potato resin glycosides. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

Fuc, fucose; Glc, glucose; Rha, rhamnose

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