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Batatinosides II−VI, Acylated Lipooligosaccharides from the Resin Glycosides of Sweet Potato

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Sweet potato (*Ipomoea batatas*) belongs to the Convolvulaceae (morning glory family) and is native to Mexico and Central America. Its edible tuberous roots have been much appreciated since pre-Hispanic times in Mesoamerica and now play an important role as a basic diet staple and a medicinal plant worldwide. The hexane-soluble extract from roots, through preparative-scale recycling HPLC, yielded five new lipophilic oligosaccharides of jalapinolic acid, batatinosides II−VI (**1**−**5**), as well as the known pescapreins I (**6**) and VII (**7**) and murucoidin I (**8**), which are part of the purgative resin glycoside mixture. NMR spectroscopy and FAB mass spectrometry were used to characterize their structures. Compounds **1** and **2** are tetraglycosidic lactones of operculinic acid C. The pentasaccharide structures for compounds **3** and **4** were confirmed to be macrolactones of simonic acid B, and that characterized for **5** was derived from operculinic acid A. The lactonization site of the aglycone was placed at C-3 of the second saccharide unit in all compounds except **4**, where it was placed at C-2. All compounds contain an esterifying residue that is composed of a long-chain fatty acid, *n*-decanoic acid (capric) or *n*-dodecanoic acid (lauric). In compound **3**, an additional short-chain fatty acid, (2*S*)-methylbutyric acid, was also identified.

KEYWORDS: *Ipomoea batatas*; sweet potato; resin glycoside; oligosaccharide; batatinoside

INTRODUCTION

The sweet potato, *Ipomoea batatas* (L.) Lam., is a perennial morning-glory vine that has been cultivated for over 5000 years for its edible tubers in Mexico, Central and lowland South America, and the West Indies. Recent molecular studies have demonstrated that *Ipomoea trifida* is the wild progenitor of *I. batatas* (*1*), and on the basis of variation of a nuclear-encoded β-amylase gene sequences, it has been shown that *I. trifida*, *Ipomoea tabascana* (native to southern Mexico), and *I. batatas* form a monophyletic group (*2*). Consequently, the geographic center of origin of *I. batatas* is said to be between Yucatan in southern Mexico and the Orinoco river of northern Venezuela, where *I. trifida*, the wild progenitor of *I. batatas*, is native (*2*). Today, sweet potato is cultivated around the world, especially in developing countries, because, among other factors, it is easy to propagate, tolerates low temperatures, and requires low level inputs of water and fertilizer (*3*). The People's Republic of China, Indonesia, and Japan lead in the production of sweet potatoes, and this tuber is a common vegetable throughout the Orient.

In Mexico, the sweet potatoes or "camotes", as they are called today, are eaten in various salty and sweet dishes and form an important part of the Mexican diet. The camote is planted in two cycles: spring−summer and fall−winter (*4*). The varieties planted in Mexico include those with the following pulp colors: white, yellow, orange, red, and purple. Nineteen of the 32 Mexican states produce a total of about 61098 tons per year (*4*). In central Mexico, the white camote is preferred, although the purple and yellow roots are also consumed. In the street markets, the white and yellow forms are the most commonly sold. The most frequent color forms available in the supermarkets are the yellow and white ones, which can be found throughout the year. The cooked camote in brown sugar syrup is eaten with milk for breakfast or dinner.

The yellow sweet potato with a pumpkin-colored fleshy tuber has a greater content of β-carotene than carrot and is equivalent to about 12800 IU per 100 g of pulp, providing an adult with a recommended daily dose of vitamin A by eating three to six slices of the root (*5*). Consequently, it could be considered a nutraceutical for vitamin A deficiency.

Our previous research has been focused on the resin glycosides of the morning-glory family (*6*), the chemical diversity of which holds considerable potential for therapeutical applications (*7, 8*). Prior to this investigation, the simonin series, one tetrasaccharide and four pentasaccharides of jalapinolic acid, was first reported as a result of the chemical analysis of the resin glycosides from the roots of a Brazilian cultivar (cv. Simon) of *I. batatas* grown in Japan (*9*). An investigation

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conducted in China using roots collected in Hunan Province, where this species is used in traditional medicine to eliminate secretion in abnormal quantity of blood or other body fluids (apocenosis effect), was also reported. In that study seven new pentasaccharides, the batatoside series, were elucidated (*10*). The present investigation was undertaken to identify lipophilic oligosaccharides in the roots of cultivated white-fleshed and white-skinned staple-type varieties of sweet potato.

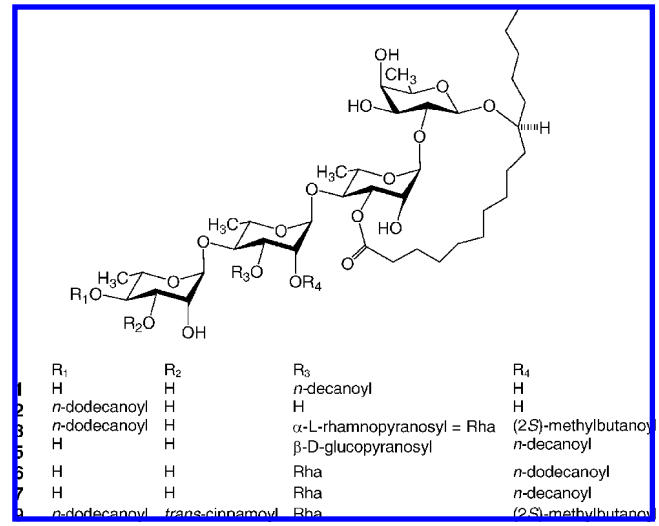
MATERIALS AND METHODS

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. ^{1}H (500 MHz) and ^{13}C (125.7 MHz) NMR experiments were conducted on a Bruker DMX-500 instrument. The NMR techniques were performed according to previously described methodology (*11*). The instrumentation used for HPLC analysis consisted of a Waters (Millipore Corp., Waters Chromatography Division, Milford, MA) 600 E multisolvent delivery system equipped with a refractive index detector (Waters 410). Negative-ion low and high-resolution FAB-MS were recorded using a matrix of triethanolamine on a JEOL SX-102A spectrometer. GC-MS was performed on a Hewlett-Packard 5890-II instrument coupled to a JEOL SX-102A spectrometer. GC conditions: 30 m × 0.25 mm, film thickness = 0.25 μm, 5% phenyl-methylpolysiloxane HP-5MS column (Agilent Technologies, Santa Clara, CA); He, linear velocity = 30 cm/s; 50 °C isothermal for 3 min, linear gradient to 300 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.

Plant Material. The roots of *I. batatas* were collected on plantations in Salvatierra, Guanajuato, Mexico, in 1999. The plant material was identified by Dr. Robert Bye. A voucher specimen (Robert Bye FB 1314) was deposited in the Ethnobotanical Collection of the National Herbarium (MEXUE), Instituto de Biología, UNAM.

Extraction and Isolation. The powdered dry roots (2.6 kg) were extracted by maceration at room temperature with hexane to give, after removal of the solvent, a dark orange syrup (13.1 g). The crude extract (13.1 g) was subjected to column chromatography over silica gel (150 g) using gradients of CH_2Cl_2 in hexane (1:1 and 1:0), Me_2CO in CH_2Cl_2 (1:9 and 3:7), and MeOH in $Me_2CO-CH_2Cl_2$ (0.5:2.5:7, 1:2:7, and 2:1:7). A total of 95 fractions (200 mL each) were collected, examined by TLC, and combined in seven fractions containing resin glycoside mixtures. Fraction V (eluates 82−84; MeOH/Me_2CO/CH_2Cl_2, 1:2:7; 180 mg) and fraction VI (eluates 85−87; MeOH/Me_2CO/CH_2Cl_2, 2:1:7; 590 mg) were partially purified by passage through activated charcoal to eliminate pigmented residues.

Recycling HPLC Separation. The instrumentation used for HPLC analysis consisted of a Waters 600E multisolvent delivery system equipped with a refractive index detector (Waters 410). Control of the equipment, data acquisition, processing, and management of chromatographic information were performed by the Millennium 2000 software program (Waters). The crude fractions V and VI were independently subjected to preparative reversed-phase HPLC on a 300 mm × 19 mm i.d., 7 μm, Symmetry C_{18} column (Waters). The elution was isocratic with CH_3CN/MeOH (9:1) using a flow rate of 9 mL/min and a sample injection of 500 μL (30 mg/mL). Fraction V eluates with t_R of 8.5 min (peak IA, 20.3 mg), 9.6 min (peak IIA, 15.8 mg), and 15.5 min (peak IIIA, 34.8 mg) and fraction VI eluates with t_R of 6.3 min (peak IB, 13.2 mg), 7.6 min (peak IIB, 30.5 mg), 12.8 min (peak IIIB, 27 mg), and 29.21 min (peak IVB, 58.7 mg) were collected by the technique of heart cutting and independently reinjected in the apparatus operated in the recycle mode (*12*) to achieve total homogeneity after 10−20 consecutive cycles employing the same isocratic elution. These techniques afforded pure compound **1** (4.1 mg) from peak IA, **2** (3.3 mg) from peak IIA, **3** (10.1 mg) from peak IIIA, **4** (22.0 mg) from peak IVB, **5** (3.0 mg) from peak IB, pescaprein I (**6**, 13.0 mg) from peak IIB, pescaprein VII (**7**, 16.0 mg) from peak IVB, and murucoidin I (**8**, 13.1 mg) from peak IIIB (**Figures 1** and **2**).



	R_1	R_2	R_3	R_4
1	H	H	*n*-decanoyl	H
2	*n*-dodecanoyl	H	H	H
3	*n*-dodecanoyl	H	α-L-rhamnopyranosyl = Rha	(2*S*)-methylbutanoyl
5	H	H	β-D-glucopyranosyl	*n*-decanoyl
6	H	H	Rha	*n*-dodecanoyl
7	H	H	Rha	*n*-decanoyl
9	*n*-dodecanoyl	*trans*-cinnamoyl	Rha	(2*S*)-methylbutanoyl

Figure 1. Structures of compounds **1**−**3**, **5**−**7**, and **9** isolated from *Ipomoea batatas* with oligosaccharide core lactonization at C-3 of the second sugar moiety.

Batatinoside II (1): white powder; mp 107−110 °C; $[\alpha]_D$ −89.2 (*c* 0.13 MeOH); ^{1}H and ^{13}C NMR, see **Tables 1** and **2**; negative FABMS, *m/z* 991 $[M - H]^-$, 837 $[M - H - C_{10}H_{18}O]^-$, 691, 545, 417; HRMS-FAB (*m/z*) $[M - H]^-$ calcd for $C_{50}H_{87}O_{19}$, 991.5841; found 991.5826.

Batatinoside III (2): white powder; mp 88−90 °C; $[\alpha]_D$ −23 (*c* 0.1 MeOH); ^{1}H and ^{13}C NMR, see **Tables 1** and **2**; negative FABMS, *m/z* 1019 $[M - H]^-$, 837 $[M - H - C_{12}H_{22}O]^-$, 691, 545, 417; HRMS-FAB (*m/z*) $[M - H]^-$ calcd for $C_{52}H_{91}O_{19}$, 1019.6155; found 1019.6178.

Batatinoside IV (3): white powder; mp 123−125 °C; $[\alpha]_D$ −51.8 (*c* 0.1 MeOH); ^{1}H and ^{13}C NMR, see **Tables 1** and **2**; negative FABMS, *m/z* 1249 $[M - H]^-$, 1067 $[M - H - C_{12}H_{22}O]^-$, 921 $[1067 - C_6H_{10}O_4]^-$, 545, 417; HRMS-FAB (*m/z*) $[M - H]^-$ calcd for $C_{63}H_{109}O_{24}$, 1249.7309; found 1249.7282.

Batatinoside V (4): white powder; mp 136−138 °C; $[\alpha]_D$ −13.6 (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR, see **Tables 1** and **2**; negative FABMS, *m/z* 1137 $[M - H]^-$, 983 $[M - H - C_{10}H_{18}O]^-$, 837 $[983 - C_6H_{10}O_4]^-$, 545, 417, 271; HRMS-FAB (*m/z*) $[M - H]^-$ calcd for $C_{56}H_{97}O_{23}$, 1137.6420; found 1137.6421.

Batatinoside VI (5): white powder; mp 142 °C; $[\alpha]_D$ −58 (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR, see **Tables 1** and **2**; negative FABMS, *m/z* 1153 $[M - H]^-$, 999 $[M - H - C_{10}H_{18}O]^-$, 837 $[999 - C_6H_{10}O_5]^-$, 545, 417, 271; HRMS-FAB (*m/z*) $[M - H]^-$ calcd for $C_{56}H_{97}O_{24}$, 1153.6369; found 1153.6370.

Pescaprein I (6): white powder; mp 131−133 °C; $[\alpha]_D$ −65 (*c* 0.1, MeOH); HRMS-FAB (*m/z*) $[M - H]^-$ calcd for $C_{58}H_{101}O_{23}$, 1165.6733; found 1165.6730; identified by comparison of NMR data with published values (*13*).

Pescaprein VII (7): white powder; mp 135 °C; $[\alpha]_D$ −72 (*c* 0.1, MeOH); HRMS-FAB (*m/z*) $[M - H]^-$ calcd for $C_{56}H_{97}O_{23}$, 1137.6420; found 1137.6424; identified by comparison of NMR data with published values (*14*).

Murucoidin I (8): white powder; mp 154−156 °C; $[\alpha]_D$ −46 (*c* 0.1, MeOH); HRMS-FAB (*m/z*) $[M - H]^-$ calcd for $C_{51}H_{87}O_{23}$, 1067.5638; found 1067.5640; identified by comparison of NMR data with published values (*15*).

Alkaline Hydrolysis of Resin Glycoside Mixture. A combined solution of the crude fractions V and VI (100 mg) in 5% KOH−H_2O (5 mL) was heated 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 was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was directly analyzed by GC-MS (*16*), and four peaks were detected: 2-methylbutanoic acid (t_R 7.2 min) [*m/z* $[M]^+$ 102 (3), 87 (33), 74 (100), 57 (50), 41 (28), 39 (8)]; cinnamic acid (t_R 16.5 min) [*m/z* $[M]^+$ 148 (100), 147 (96), 131 (25), 103(40), 102 (20), 77 (25), 74 (8), 51 (20), 50 (8), 39 (5), 38 (4)]; *n*-decanoic acid (t_R 14.6 min) [*m/z* $[M]^+$ 172 (2), 155 (3), 143 (12), 129 (62), 115

Table 1. ^{1}H NMR Data of Batatinosides II−VI (**1**−**5**)[a]

proton[b]	1	2	3	4	5
Fuc-1	4.78 d (8.0)	4.75 d (8.0)	4.82 d (7.5)	4.73 d (8.0)	4.80 d (8.0)
Fuc-2	4.56 dd (8.0, 9.0)	4.52 dd (8.0, 9.5)	4.53 dd (7.5, 8.5)	4.16 dd (8.0, 9.0)	4.49 dd (8.0, 9.7)
Fuc-3	4.21 dd (2.0, 9.0)	4.15 dd (9.5, 2.5)	4.20 dd (3.0, 8.5)	4.07 dd (9.0, 3.5)	4.18 dd (9.7, 3.5)
Fuc-4	3.93 d (2.0)	3.91 br s	3.93 dd (1.0, 3.0)	3.99 d (3.5)	3.91 d (3.5)
Fuc-5	3.83 q (6.5)	3.80 qd (1.5, 6.0)	3.82 qd (1.0, 6.3)	3.77 q (6.5)	3.80 q (6.5)
Fuc-6	1.53 d (6.5)	1.51 d (6.0)	1.52 d (6.3)	1.51 d (6.5)	1.51 d (6.0)
Rha-1	6.41 d (2.0)	6.35 d (2.0)	6.35 d (1.5)	5.48 d (1.5)	6.31 d (1.5)
Rha-2	5.27 dd (2.0, 3.0)	5.24 dd (2.0, 3.0)	5.31 br s	5.94 dd (1.5, 3.0)	5.21 dd (1.5, 3.5)
Rha-3	5.68 dd (3.0, 10.0)	5.57 dd (3.0, 10.0)	5.62 dd (2.8, 10.0)	5.01 dd (3.0, 9.0)	5.63 dd (3.5, 9.5)
Rha-4	4.73 dd (10.0, 10.0)	4.62 dd (10.0, 10.0)	4.68 dd (10.0, 10.0)	4.20 dd (9.0, 9.9)	4.64 dd (9.5, 9.5)
Rha-5	5.09 dq (6.0, 10.0)	5.00 dq (6.5, 10.0)	5.01 dq (6.5, 10.0)	4.43 dq (6.1, 9.9)	4.97 dq (6.0, 9.5)
Rha-6	1.56 d (6.0)	1.60 d (6.5)	1.57 d (6.5)	1.58 d (6.1)	1.55 d (6.0)
Rha′-1	5.92 d (1.5)	5.78 d (1.0)	5.64 d (1.5)	6.13 d (2.0)	5.61 d (2.0)
Rha′-2	4.73 br s	4.92 br s	5.79 dd (1.5, 3.0)	6.01 dd (2.0, 3.0)	5.97 dd (2.0, 3.0)
Rha′-3	5.72 dd (3.0, 9.5)	4.43 dd (2.5, 9.5)	4.53 dd (3.0, 9.3)	4.58 dd (3.0, 9.0)	4.61 dd (3.0, 9.0)
Rha′-4	4.62 dd (9.5, 9.5)	4.37 dd (9.5, 9.5)	4.28 dd (9.3, 9.3)	4.30*	4.33*
Rha′-5	4.36 dq (6.0, 9.5)	4.34 dq (5.5, 9.5)	4.33 dq (6.5, 9.3)	4.30*	4.32*
Rha′-6	1.56 d (6.0)	1.60 d (5.5)	1.60 d (6.5)	1.62 d (6.0)	1.58 d (6.0)
Rha″-1	5.74 d (1.5)	6.21 d (1.5)	5.88 d (1.0)	5.94 br s	6.21 d (1.5)
Rha″-2	4.53 br s	4.82 br s	4.68 dd (1.0, 3.0)	4.72 dd (1.5, 3.5)	4.88 dd (1.5, 3.0)
Rha″-3	4.38 dd (3.0, 9.3)	4.51 dd (3.5, 9.5)	4.46 dd (3.0, 9.5)	4.43 dd (3.5, 9.0)	4.41 dd (3.0, 9.0)
Rha″-4	4.29 dd (9.3, 9.3)	5.81 dd (9.5, 9.5)	5.84 dd (9.5, 9.5)	4.31 dd (9.0, 9.0)	4.23 dd (9.0, 9.0)
Rha″-5	4.26 dq (6.0, 9.3)	4.37 dq (9.5, 6.5)	4.36 dq (6.5, 9.5)	4.36 dq (6.0, 9.0)	4.33*
Rha″-6	1.61 d (6.0)	1.37 d (6.5)	1.43 d (6.5)	1.66 d (6.0)	1.64 d (6.0)
Rha‴-1			5.60 d (1.0)	5.62 d (1.0)	
Rha‴-2			4.81 dd (1.0, 3.5)	4.87 br s	
Rha‴-3			4.44 dd (3.5, 9.3)	4.50 dd (3.0, 9.0)	
Rha‴-4			4.23 dd (9.3. 9.3)	4.29 dd (9.0, 9.0)	
Rha‴-5			4.29 dq (5.5, 9.3)	4.26 dq (9.0, 7.0)	
Rha‴-6			1.72 d (5.5)	1.60 d (6.0)	
Glc-1					5.08 d (8.0)
Glc-2					3.94 dd (8.0, 9.0)
Glc-3					4.11*
Glc-4					4.13*
Glc-5					3.86 m
Glc-6					4.36 dd (7.0, 12.0)
					4.46 dd (3.0, 12.0)
Jal-2	2.13 ddd (2.5, 7.0, 14.0)	2.11 ddd (3.0, 7.0, 14.0)	2.24 ddd (2.0, 6.5, 15.0)	2.23 ddd (2.0, 6.0, 13.0)	2.26 ddd (2.0, 7.0, 14.5)
	2.26 ddd (3.5, 7.5, 13.5)	2.26*	2.78 ddd (2.5, 6.5, 14.5)	2.40 ddd (3.5, 8.5, 13.0)	2.90 ddd (9.0, 12.5, 14.5)
Jal-11	3.89 m	3.89 m	3.88 m	3.86 m	3.86 m
Jal-16	0.97 t (7.0)	1.00 t (7.0)	0.94 t (7.0)	0.86 t (7.0)	0.85 t (7.0)
Mba-2			2.39 tq (7.0, 7.5)		
2-Me			1.14 d (7.0)		
3-Me			0.89 t (7.0)		
Deca-2	2.27 ddd (2.5, 7.5, 16.0)			2.33 t (8.0)	2.33 t (8.0)
	2.43 ddd (3.5, 7.5, 16.0)				
Deca-10	0.86 t (7.0)			0.88 t (6.5)	0.92 t (7.0)
Dodeca-2		2.26*	2.43 ddd (3.0, 7.3, 15.5)		
		2.44 ddd (6.0, 7.0, 16.0)	2.46 ddd (3.0, 8.0, 15.5)		
Dodeca-12		0.87 t (7.0)	0.87 t (7.0)		

[a] Data recorded in C_5D_5N (500 MHz). Chemical shifts (δ) are in ppm relative to TMS. The spin coupling (J) is given in parentheses (hertz). Chemical shifts marked with an asterisk indicate overlapped signals. Spin-coupled patterns are designed as follows: s, singlet; d, doublet; t, triplet; m, multiplet; q, quartet; sept, septet; br s, broad signal. All assignments are based on ^{1}H−^{1}H COSY and TOCSY experiments. [b] Abbreviations: Fuc, fucose; Rha, rhamnose; Jal, 11-hydroxyhexadecanoyl; Mba, 2-methylbutanoyl; Deca, decanoyl; Dodeca, dodecanoyl.

(15), 112 (12), 87 (20), 73 (100), 60 (90), 57 (40), 55 (45), 43 (30), 41 (35), 39 (6)]; and *n*-dodecanoic acid (t_R 17.8 min) [*m*/*z* [M]$^+$ 200 (15), 183 (2), 171 (18), 157 (40), 143 (20), 129 (48), 115 (20), 101 (15), 85 (33), 73 (100), 60 (80), 57 (30), 55 (47), 43 (30)]. The residue (50 mg) extracted from the aqueous phase was subjected to preparative HPLC on a 300 mm × 7.8 mm i.d., 10 μm, μBondapak NH_2 column (Waters). 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 (35 mg/mL). This procedure yielded three glycosidic acids: operculinic acid C (7.3 mg, t_R 6.70 min) (*17*), simonic acid B (28.5 mg, t_R 12.17 min) (*9*), and operculinic acid A (3.40 mg, t_R 14.30 min) (*17*), which were identified by comparison of their physical constants and NMR data with published values. Saponification of compounds **3** and **8** afforded (*S*)-(+)-α-methylbutyric acid: $[\alpha]_D$ +10 (*c* 1.0, $CHCl_3$).

Sugar Analysis. A solution of glycosidic acids (20 mg) obtained from the saponification of the crude resin glycoside mixture in 4 N HCl (10 mL) was heated at 90 °C for 2 h. The reaction was diluted with H_2O (5 mL) and extracted with Et_2O (30 mL). The aqueous phase was neutralized with 1 N KOH, extracted with *n*-BuOH (30 mL), and concentrated to give a colorless solid. The residue was dissolved in CH_3CN/H_2O (1:1) and directly analyzed by HPLC using a 300 mm × 3.9 mm i.d., 10 μm, μBondapak NH_2 standard column for carbohydrate analysis (Waters), an isocratic elution of CH_3CN/H_2O (85:15), a flow rate of 1 mL/min, and a sample injection of 20 μL (5 mg/mL). Coelution experiments with standard carbohydrate samples allowed the identification of rhamnose (t_R 6.3 min), fucose (t_R 8.0 min), and glucose (t_R 10.5 min). Each of these eluates was individually collected, concentrated, and dissolved in H_2O. Optical activity was recorded after the solutions had been stirred for 2 h at room temperature: L-rhamnose $[\alpha]_{598}$ +8, $[\alpha]_{578}$ +8, $[\alpha]_{546}$ +9, $[\alpha]_{436}$ +14, $[\alpha]_{365}$ +20 (*c* 0.1, H_2O); D-fucose $[\alpha]_{598}$ +81, $[\alpha]_{578}$ +82, $[\alpha]_{546}$ +95, $[\alpha]_{436}$ +154, $[\alpha]_{365}$ +235 (*c* 0.1, H_2O); D-glucose $[\alpha]_{598}$ +50, $[\alpha]_{578}$ +51, $[\alpha]_{546}$ +56, $[\alpha]_{436}$ +98, $[\alpha]_{365}$ +151 (*c* 0.1, H_2O).

Table 2. ^{13}C NMR Data of Compounds **1**−**5** (125 MHz)[a]

carbon[b]	1	2	3	4	5
Fuc-1	101.7	101.7	101.6	104.3	101.5
Fuc-2	73.0	72.9	73.4	80.2	73.6
Fuc-3	76.9	76.8	76.6	73.3	76.5
Fuc-4	73.6	73.5	73.6	73.0	74.0
Fuc-5	71.2	71.2	71.2	70.8	71.2
Fuc-6	17.3	17.2	17.2	17.4	17.2
Rha-1	100.3	100.3	100.3	98.8	100.1
Rha-2	69.7	69.6	69.8	73.9	65.0
Rha-3	79.1	78.6	78.1	69.8	77.9
Rha-4	77.2	77.6	76.6	80.1	76.2
Rha-5	67.4	67.7	67.9	68.6	68.0
Rha-6	19.4	19.4	19.2	19.5	19.2
Rha′-1	102.3	103.4	98.4	99.2	99.2
Rha′-2	72.6	72.4	72.7	73.2	72.6
Rha′-3	75.9	73.1	79.6	80.1	80.3
Rha′-4	77.4	80.4	79.8	79.0	77.9
Rha′-5	69.1	68.6	68.5	68.6	68.3
Rha′-6	18.5	19.1	18.7	18.7	18.7
Rha″-1	103.5	103.3	103.8	103.6	103.4
Rha″-	70.4	72.9	72.6	72.6	72.4
Rha″-3	72.6	70.2	70.2	72.8	72.8
Rha″-4	73.6	75.5	75.0	73.6	74.0
Rha″-5	70.7	67.8	68.2	70.8	70.8
Rha″-6	18.3	18.0	17.9	18.4	18.5
Rha‴-1			104.6	104.7	
Rha‴-2			72.6	72.5	
Rha‴-3			72.5	72.6	
Rha‴-4			73.6	70.7	
Rha‴-5			70.5	73.7	
Rha‴-6			18.8	18.6	
Glc-1					104.9
Glc-2					75.2
Glc-3					78.1
Glc-4					70.7
Glc-5					77.9
Glc-6					62.5
Jal-1	174.4	174.5	174.7	173.1	174.9
Jal-2	34.7	34.6	33.8	34.3	34.2
Jal-11	79.8	79.8	79.4	82.3	79.6
Jal-16	14.6	14.5	14.4	14.3	14.3
Mba-1			175.4		
Mba-2			41.4		
2-Me			16.9		
3-Me			11.8		
Deca-1	172.7			172.5	173.5
Deca-2	34.4			34.5	34.5
Deca-10	14.3			14.3	14.5
Dodeca-1		173.4	173.5		
Dodeca-2		34.7	32.1		
Dodeca-12		14.3	14.3		

[a] Data recorded in C_5D_5N. Chemical shifts (δ) are in ppm relative to TMS. All assignments are based on HMQC and HMBC experiments. [b] Abbreviations: Fuc, fucose; Rha, rhamnose; Glc, glucose; Jal, 11-hydroxyhexadecanoyl; Mba, 2-methylbutanoyl; Deca, decanoyl; Dodeca, dodecanoyl.

Identification of the Aglycone. The organic layer residue (Et_2O-soluble fraction) obtained during the acid-catalyzed hydrolysis of the crude resin glycoside mixture was treated with CH_2N_2 and directly analyzed by normal phase HPLC on a 250 mm × 21.2 mm i.d., 10 μm silica gel column (Teledyne ISCO, Lincoln, NE), using an isocratic elution of *n*-hexane/$CHCl_3$/Me_2CO (6:3:1) and a flow rate of 6 mL/min to give 5.3 mg of methyl (11*S*)-hydroxyhexadecanoate (jalapinolic acid methyl ester) (*16*): t_R 16.8 min; mp 43−44 °C; $[\alpha]_D$ +7.0 (*c* 1, $CHCl_3$); ^{13}C NMR δ 174.4, 72.0, 51.4, 37.5, 37.4, 34.1, 31.9, 29.6, 29.5, 29.4, 29.2, 29.1, 25.6, 25.3, 24.9, 22.6, 14.1.

RESULTS AND DISCUSSION

Characterization of the Oligosaccharide Core of Batatinosides. The present investigation describes the isolation of five lipooligosaccharides, to which the names batatinosides II−VI (**1**−**5**) have been given, from the hexane-soluble resin glycosides of sweet potato. The lipophilic crude extract obtained from the dried roots was fractionated by column chromatography on silica gel. The two major fractions containing resin glycosides were further separated by using preparative-scale recycling reversed-phase HPLC (*12*). This methodological approach led to the isolation of the new glycolipids **1**−**5** and the known pescapreins I (**6**) and VII (**7**) and murucoidin I (**8**) (**Figures 1** and **2**).

A small portion of each crude resin glycoside fractions was saponified to liberate an H_2O-soluble mixture of oligosaccharides of jalapinolic acid. Three glycosidic acids were isolated: the major product was characterized as a branched pentasaccharide and identified as 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, which has been previously identified in the resin glycosides of *I. batatas* (*9, 10*), *Ipomoea pes-caprae* (*13, 14*), *Ipomoea murucoides* (*15*), and *Ipomoea stolonifera* (*18, 19*). Batatins I and II are two ester-type dimers of this pentasaccharide previously isolated from the hexane-soluble fraction of sweet potato (*20*). The second and third glycosidic acids represented minor constituents and were characterized as the linear tetrasaccharide operculinic acid C [(11*S*)-hydroxyhexadecanoate 11-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)−*β*-D-fucopyranoside] and the branched 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], both previously isolated from *Ipomoea operculata* (*17*). Evidence for the absolute stereochemistry of the sugars and the configuration of the anomeric linkages as well as the sequence of glycosidation was published when these oligosaccharide cores were first elucidated (*9, 17*). HPLC analysis of the acid hydrolysis-liberated monosaccharides led to the identification of rhamnose, fucose, and glucose by coelution experiments with standard commercial samples. The three monosaccharides were in their naturally occurring form in agreement with their optical activity measurements, that is, L-series for rhamnose and D-series for fucose and glucose (*21*). The liberated organic acids were identified as *trans*-cinnamic, 2-methylpropanoic, 2-methylbutanoic, *n*-decanoic, and *n*-dodecanoic acids by GC-MS comparison of their spectra and retention times with those of authentic samples (*16, 21*). The aglycone was identical, in all chromatographic and spectrometric aspects, to an authentic sample of jalapinolic acid (*16*).

Interpretation of FAB Mass Spectra of Batatinosides. Negative-ion FABMS of compounds **1**−**5** were obtained and provided intense pseudomolecular $[M - H]^-$ ions for the analysis of this type of glycolipids (*6, 11*). Batatinoside II (**1**) gave a $[M - H]^-$ at *m/z* 991 ($C_{50}H_{87}O_{19}$); the observed difference of 154 mass units between this ion and the fragment at *m/z* 837 $[M - H - C_{10}H_{18}O]^-$ corroborated the presence of decanoic acid as the acylating residue of the oligosaccharide core. Similarly, the observed difference of 182 mass units between the pseudomolecular ion $[M - H]^-$ at *m/z* 1019 ($C_{52}H_{91}O_{19}$) and *m/z* 837 ion $[M - H - C_{12}H_{22}O]^-$ indicated the presence of a dodecanoyl residue in glycolipid **2**. In compound **4**, a pseudomolecular ion $[M - H]^-$ was detected at *m/z* 1137, indicating a molecular formula of $C_{56}H_{98}O_{23}$ for this batatinoside. For compound **5**, the ion $[M - H]^-$ was detected at *m/z* 1153 ($C_{56}H_{97}O_{23}$). Glycolipids **4** and **5** showed the same initial loss, affording peaks at *m/z* 983 $[1137 - C_{10}H_{18}O]^-$ in compound **4** and at *m/z* 999 $[1153 - C_{10}H_{18}O]^-$ in compound **5**. This 154 mass difference corroborated by its initial loss the

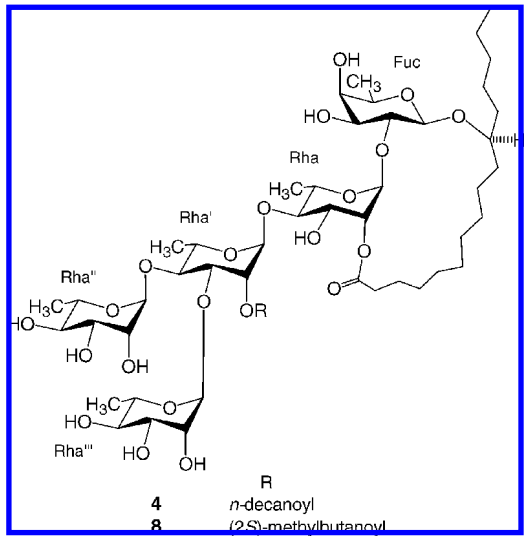


Figure 2. Structures of compounds **4** and **8** isolated from *Ipomoea batatas* with oligosaccharide core lactonization at C-2 of the second sugar moiety.

presence of decanoic acid as the acylating residue in both compounds. Batatinoside IV (**3**) produced a $[M - H]^-$ ion at *m/z* 1249 ($C_{63}H_{109}O_{24}$), and the loss of one of the esterifying groups afforded a peak at *m/z* 1165 representing $[M - H - C_5H_8O]^-$; this 84 mass unit loss corroborated the presence of 2-methylbutanoic acid in addition to the peak $[M - H - C_{12}H_{22}O]^-$ at *m/z* 1067. All spectra displayed the common fragments produced by glycosidic cleavage of each sugar moiety at *m/z* 691 [1067 − 2 × 146 ($C_6H_{10}O_4$) − $C_5H_8O]^-$, 545 [691 − 146 ($C_6H_{10}O_4$)]$^-$, which indicated that the lactonization was located at the first rhamnose unit (Rha), 417 [545 + H_2O − 146 ($C_6H_{10}O_4$)]$^-$, and 271 − [417 − 146 ($C_6H_{10}O_4$)]$^-$, as previously reported (*8, 13, 14*).

Structure Elucidation of Batatinosides by NMR. Common features in both ^{1}H and ^{13}C NMR spectra of the new compounds **1**−**5** are noted in **Tables 1** and **2**. The diagnostic resonances observed in the downfield region δ 4.80−6.40 (**Table 1**) were assigned to the anomeric protons because of their multiplicity as doublets. In the ^{13}C NMR spectra (**Table 2**), the anomeric signals at δ 98−105 directly indicated the number of monosaccharide units forming the oligosaccharide core for each compound. The observed coupling constant values for the anomeric resonances are distinctive and could be used as "reporter signals" (*22*) for each monosaccharide type: 1.0−3.0 Hz for rhamnose, 7.0−8.0 Hz for fucose, and 8.0−9.0 Hz for glucose (**Table 1**). All ^{1}H NMR spectra showed a group of paramagnetically shifted nonanomeric protons reflecting the presence of esterification sites that could be either H-2 of the second rhamnose unit (Rha′) or H-4 of the third rhamnose unit (Rha″), suggesting esterification at these positions (**Table 1**).

The two multiplets centered at δ 2.1−2.8 correspond to the nonequivalent diastereotopic protons of the methylene C-2 of the aglycone (jalapinolic acid) and provide evidence for the macrocyclic lactone-type structure of compounds **1**−**5** (*6*). The lactonization could be placed at C-3 of the second saccharide (Rha) in compounds **1**−**3** and **5** (**Figure 1**) by the observed $^{2,3}J_{CH}$ correlations through HMBC experiments for C-1 (δ 174) of the aglycon with Jal H-2 and Rha H-3 (ca. δ 5.60). In compound **4** (**Figure 2**), the lactonization could be placed at C-2 of the second saccharide (Rha) by the observed $^3J_{CH}$ correlation.

Diagnostic resonances for each of the esterifying residues were observed in the ^{1}H NMR spectra (**Table 1**): a triplet of quartets (δ_H 2.39) generated by H-2 of the 2(*S*)-methylbutanoyl group for batatinoside IV (**3**); for compound **4**, the C-2 methylene protons of the fatty acid residue (*n*-decanoyl ester) generated a triplet-like signal (J = 7.0 Hz), and in compounds **1**−**3** and **5**, the signals for the C-2 methylene group α to the carbonyl group in the decanoyl and dodecanoyl residues were observed as a pair of nonequivalent signals (δ_H 2.27−2.43) like a doublet of doublet of doublets (J = 14, 10, and 7 Hz), suggesting some conformational restrictions for these fatty acid residues and resulting in a set of two diastereotopic anisochronous protons as previously reported for some members of the pescaprein series (*14*). In all cases it was possible by HMBC analysis (*6, 22*) to establish the links between a specific carbonyl ester group with their corresponding pyranose ring proton at the site of esterification ($^3J_{CH}$). For example, the location of the *n*-dodecanoyl residue at C-4 of Rha″ was verified by the observed $^3J_{CH}$ coupling between the carbonyl resonance at δ 173.5 with the signal at δ5.84. Therefore, the remaining esterified position at C-2 of the second rhamnose unit (Rha′, δ_C 72.7) represented the location of the additional ester linkage for the methylbutanoyl group as confirmed by the $^3J_{CH}$ coupling between the carbonyl resonance at δ 175.4 and the signal at δ_H 5.79. Batatinoside IV (**3**) is closely related to batatinoside I (**9**), which represents the pentasaccharide macrocyclic monomeric moiety of the ester-type dimers batatins I and II (*20*).

Mexican Perspective for the Use of Sweet Potato. Despite being a pre-Hispanic center of origin and diversity of sweet potato and having ample Spanish Colonial documentation of its consumption for three centuries, today Mexico is not a major producer of *I. batatas*. Of the more than 129 million tons of sweet potato grown in the world in 2004, China ranked number one with the production of over 105 million tons. Mexico, on the other hand, harvested only 61098 tons on 2908 ha, with the states of Guanajuato and Michoacan yielding half of the crop (*4*). Between 1990 and 2004, the production augmented nationwide with notable increases of 400% in Guanajuato and of 100% in Aguascalientes, Mexico, Nayarit, Yucatan, Jalisco, and Chihuahua (*4*). In Mexico, sweet potato has served as a supplementary human food, in contrast to China, where half is used as livestock food. Given the existing diversity of colors and flavors of the Mexican sweet potato, which has been rediscovered in contemporary Mexican cuisine, this crop has a promising future not only as a basic foodstuff but as a major culinary ingredient in specialty foods.

Prior to consumption, the camotes are roasted or boiled. The ingestion of raw roots can produce flatulence, diarrhea, or even a drastic purgation due, in part, to their high content of resin glycosides (up to 5%, e.g., the yield of the hexane-soluble resin glycoside mixture for the analyzed material in the present study was 0.25% in dry weight), which are similar structurally to those present in the Mexican jalap roots, a pre-Hispanic medicinal plant complex also from the morning-glory family still considered to be a useful laxative (*16*). Cooking procedures as decoction or roasting denature these purgative principles, making the camote edible.

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