JOURNAL OF NATURAL PRODUCTS

Volume 68, Number 8 August 2005

Full Papers

Pentasaccharide Glycosides from the Roots of *Ipomoea murucoides*

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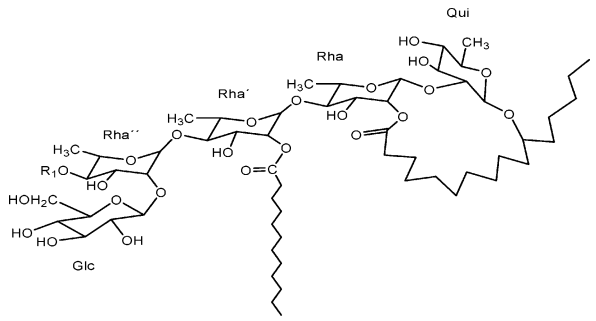
Received March 4, 2005

Five pentasaccharide glycosides, murucins 1−5 (**1**−**5**), were isolated from the roots of the arboreal species *Ipomoea murucoides*, and their structures were elucidated by spectroscopic and chemical methods. Compounds **1**−**5** were evaluated for cytotoxicity against a small panel of cancer cell lines.

Ipomoea murucoides Roem. et Schult. (Convolvulaceae) is a tree with a white bark and white flowers that grows in southern and central Mexico. This species is known in several states of Mexico as "cazahuate". Some communities use the smoke from the burned tree against mosquitos. Aqueous infusions of the leaves, bark, and flowers are used as an anti-inflammatory and against scorpion bites.[1] Initial studies on the seeds and leaves of *I. murucoides* led to reports of a mannagalactonan and fatty acids.[2,3] Perez Amador et al.[4,5] detected resin glycosides in the seeds and leaves but without divulging any chemical structures. In our continuing investigation on secondary metabolites with biological activity from *Ipomoea* species, we have studied the resin glycosidic content of the roots of *I. murucoides*. We report herein on the isolation and characterization of five pentasaccharides of jalapinolic acid, murucins 1−5 (**1**−**5**), from *I. murucoides*.



	R_1
1	Acetyl
2	Propanoyl
3	*n*-Butanoyl
4	2-Methylbutanoyl
5	3-Hydroxy-2-methylbutanoyl

Results and Discussion

The roots of *I. murucoides* were dried, pulverized, and macerated in dichloromethane, and the extract was frac-

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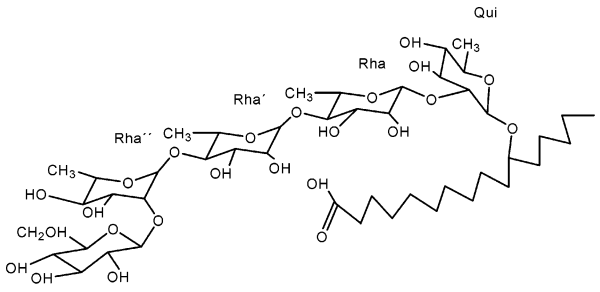
10.1021/np050075m CCC: $30.25
Published on Web 07/19/2005

tionated by column chromatography on silica gel, leading to the separation of two chromatographic fractions: a brown solid and a resinous material. The crude resin exhibited cytotoxic activity for a cancer line (OVCAR-5: ED_{50} 4.0 μg/mL) and was subjected to preparative HPLC in the reversed-phase mode, with compounds **1**−**5** being isolated by repetitive chromatography.

The resinous material was hydrolyzed in an aqueous/ethanolic acid medium, producing an organic fraction together with a water-soluble mixture of carbohydrates. Analysis of the organic fraction by GC-MS permitted the identification of acetic, propanoic, butanoic, 2-methylbutanoic, 3-hydroxy-2-methylbutanoic, dodecanoic, and 11-hydroxyhexadecanoic ethyl ester units by comparison with the mass spectra and retention times of the ethyl esters of authentic samples. HPLC and GC-MS analysis of the carbohydrates present in the aqueous phase allowed the identification of quinovose, rhamnose, and glucose.



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Basic hydrolysis of the resinous chromatographic fraction produced an organic acid fraction and a water-soluble glycosidic acid derivative (**6**). Murucinic acid (**6**) gave a quasi-molecular ion at *m/z* 1041 $[M + Na]^+$ in the positive-ion FABMS. An accurate mass measurement of the deprotonated glycosidic acid $[M - H]^-$ in the negative-ion HRESIMS gave a *m/z* value of 1017.5163, consistent with a molecular formula of $C_{46}H_{82}O_{24}$. Negative-ion FABMS showed a deprotonated molecular ion at *m/z* 1017 $[M - H]^-$ and other significant peaks at *m/z* 855 [*m/z* 1017 − 162 $(C_6H_{10}O_5)]^-$, 709 [*m/z* 855 − 146 $(C_6H_{10}O_4)]^-$, 563 [*m/z* 709 − 146 $(C_6H_{10}O_4)]^-$, 417 [*m/z* 563 − 146 $(C_6H_{10}O_4)]^-$, and 271 [*m/z* 417 − 146 $(C_6H_{10}O_4)]^-$. The sugar units in the ^{1}H NMR spectrum of **6** showed four doublet methyl signals of 6-deoxyhexose units, a methyl triplet signal at 0.95 ppm, and a triplet signal for the methylene protons at C-2 (2.34 ppm) of the aglycon, suggesting its acyclic structure.[10] The ^{13}C NMR spectrum of **6** showed five anomeric signals, indicating that part of the molecule is a pentasaccharide. The HMQC spectrum of **6** indicated that the anomeric carbons at 103.2, 103.1, 102.8, 102.0, and 101.0 ppm were correlated with the anomeric protons at 4.38 (d, J = 7.7 Hz), 5.05 (d, J = 7.6 Hz), 5.35 (d, J = 1.4 Hz), 5.25 (d, J = 1.3 Hz), and 5.45 (d, J = 1.5 Hz) ppm. A combination of one- and two-dimensional ^{1}H NMR techniques allowed all protons to be sequentially assigned within each saccharide system, leading to the identification of one glucopyranosyl, one quinovopyranosyl, and three rhamnopyranosyl units as the monosaccharides present in **6**. The anomeric configurations for the sugar moieties were assigned as β for glucopyranosyl, β for quinovopyranosyl, and α for rhamnopyranosyl, from their coupling constants of 7.7, 7.6, and 1.4 Hz, respectively. The connectivities between sugar moieties were determined from the following HMBC correlations: C-2 (79.3 ppm) of quinovose with H-1 (5.25 ppm) of rhamnose and H-1 (4.38 ppm) of quinovose; C-4 (82.4 ppm) of rhamose with H-1 (5.45 ppm) of rhamnose′; C-4 (81.3 ppm) of rhamnose′ with H-1 (5.35 ppm) of rhamnose″; and C-2 (70.9 ppm) of rhamnose″ with H-1 (5.05 ppm) of glucose. The position of the jalapinolic acid unit was determined by the correlation between jalapinolic acid H-11 (3.72 ppm) and quinovose H-1 (4.38 ppm) in the ROESY spectrum. Accordingly, the structure of murucinic acid (**6**) was assigned as (11*S*)-hydroxyhexadecanoic acid 11-*O*-β-D-quinovopyranosyl-(2→1)-*O*-α-L-rhamnopyranosyl-(4→1)-*O*-α-L-rhamnopyranosyl-(4→1)-*O*-α-L-rhamnopyranosyl-(2→1)-*O*-β-D-glucopyranoside.

Murucin 1 (**1**) gave a quasi-molecular ion at *m/z* 1247 $[M + Na]^+$ in the positive-ion FABMS. An accurate mass measurement of the deprotonated glycosidic acid $[M - H]^-$ in the negative-ion HRESIMS gave an *m/z* value of 1223.6862, consistent with a molecular formula of $C_{60}H_{104}O_{25}$. The negative-ion FABMS showed fragment peaks at *m/z* 1223 $[M - H]^-$, 1041 [*m/z* 1223 − 182 $(C_{12}H_{22}O)]^-$, 999 [*m/z* 1041 − 42 $(C_2H_2O)]^-$, 837 [*m/z* 999 − 162 $(C_6H_{10}O_5)]^-$, 691 [*m/z* 837 − 146 $(C_6H_{10}O_4)]^-$, 545 [*m/z* 691 − 146 $(C_6H_{10}O_4)]^-$, 417 [*m/z* 545 − 128 $(C_6H_8O_3)]^-$, and 271 [*m/z* 417 − 146 $(C_6H_{10}O_4)]^-$, suggesting that part of the molecule was a pentasaccharide. Basic hydrolysis of **1** afforded murucinic (**6**), dodecanoic, and acetic acids. The ^{13}C NMR spectrum of **1** showed three carbonyl signals and five anomeric signals. In turn, the ^{1}H NMR spectrum of **1** showed four doublet methyl signals of four 6-deoxyhexose units, a methyl singlet signal at 2.08 ppm of an acetyl group, and two signals at 2.31 (1H, ddd) and 2.55 (1H, ddd) ppm of the nonequivalent protons of a methylene group at C-2 in the aglycon moiety, suggesting a macrocyclic lactone-type structure. Also observed were a methyl triplet signal at 1.22 ppm and a triplet-like signal for a methylene group at C-2 (2.47 ppm) of a dodecanoyl group. The position of the jalapinolic acid moiety in the oligosaccharide was determined by the correlation between jalapinolic acid H-11 (3.62 ppm) and quinovose H-1 (4.32 ppm), in a T-ROESY[6] NMR spectrum. The HMBC spectrum of **1** permitted the esterification sites to be established through the connectivities between the carbonyls and the ^{1}H NMR signals of the monosaccharides: ^{13}C═O (172.5 ppm) of the acetyl group correlated with H-4 (4.92 ppm) of rhamnose″; the ^{13}C═O (175.1 ppm) of the *n*-dodecanoyl showed a correlation with H-2 (5.51 ppm) of rhamnose′ and H-2 (2.37 ppm) of *n*-dodecanoyl; and the ^{13}C═O (175.0 ppm) of the 11-hydroxyhexadecanoyl correlated with H-2 (5.10 ppm) of rhamnose and H-2 (2.28, 2.55 ppm) of 11-hydroxyhexadecanoyl.

The molecular formula of compound **2** ($C_{61}H_{106}O_{25}$) was determined from the negative-ion HRESIMS. The positive-ion FABMS of **2** gave a quasi-molecular ion at *m/z* 1261 $[M + Na]^+$. The negative-ion FABMS showed fragment peaks at *m/z* 1237 $[M - H]^-$, 1055 [*m/z* 1237 − 182 $(C_{12}H_{22}O)]^-$, 999 [*m/z* 1055 − 56 $(C_3H_4O)]^-$, 837, 691, 545, 417, and 271. Basic hydrolysis of **2** afforded murucinic (**6**), dodecanoic, and propanoic acids. The ^{13}C NMR spectrum of **2** was very similar to that of **1**. The ^{1}H NMR spectrum of **2** showed a methyl triplet signal at 1.22 ppm and an overlapped signal at 2.47 ppm of the methylene of a propanoyl group. The HMBC spectrum of **2** permitted the unambiguous assignments of the esterified positions of the oligosaccharide core for **2**. Thus, a propanoyl substituent was attached at C-2 of rhamnose″, a *n*-dodecanoyl residue was located at C-2 of rhamnose′, and the jalapinolic acid unit was esterified at C-2 of rhamnose.

The positive-ion FABMS of compound **3** gave a quasi-molecular ion at *m/z* 1275 $[M + Na]^+$. The negative-ion HRESIMS of murucin 3 (**3**) gave a *m/z* value of 1251.7177

$[M - H]^-$, consistent with a molecular formula of $C_{62}H_{108}O_{25}$. The negative-ion FABMS showed fragment peaks at *m/z* 1251 $[M - H]^-$, 1069 [*m/z* 1251 − 182 $(C_{12}H_{22}O)]^-$, 999 [*m/z* 1069 − 70 $(C_4H_6O)]^-$, 837, 691, 545, 417, and 271. Basic hydrolysis of **3** afforded murucinic (**6**), dodecanoic, and butanoic acids. The ^{13}C NMR spectrum of **3** was almost identical to that of **2**. The ^1H NMR spectrum of **3** showed a methyl triplet signal at 1.08 ppm and an overlapped signal at 2.49 ppm of the methylene group at C-2 of a butanoyl group. According to long-range correlations in the HMBC spectrum, a *n*-butanoyl was attached at C-2 of rhamnose″, a *n*-dodecanoyl residue was located at C-2 of rhamnose′, and the jalapinolic acid unit was esterified at C-2 of rhamnose.

The negative-ion HRESIMS of compound **4** gave a *m/z* value of 1265.7375 $[M - H]^-$, consistent with a molecular formula of $C_{60}H_{104}O_{25}$. The positive-ion FABMS gave a quasi-molecular ion at *m/z* 1289 $[M + Na]^+$. The negative-ion FABMS showed fragment peaks at *m/z* 1265 $[M - H]^-$, 1083 [*m/z* 1265 − 182 $(C_{12}H_{22}O)]^-$, 999 [*m/z* 1083 − 84 $(C_5H_8O)]^-$, 837, 691, 545, 417, and 271. Basic hydrolysis of **4** afforded murucinic (**6**), dodecanoic, and 2-methylbutanoic acids. The ^{13}C NMR spectrum of **4** was almost identical to that of **3**. The ^1H NMR spectrum of **4** showed a methyl triplet signal at 1.10 ppm and a signal at 2.50 (1H, tq) ppm of the methine unit of a methylbutanoyl group. The esterified positions of the oligosaccharide core were determined by the HMBC spectrum. Accordingly a 2-methylbutanoyl ester group was attached at C-2 of rhamnose″, a *n*-dodecanoyl residue was located at C-2 of rhamnose′, and the jalapinolic acid unit was esterified at C-2 of rhamnose.

The negative-ion HRESIMS of compound **5** gave a *m/z* value of 1281.7280 $[M - H]^-$, consistent with a molecular formula of $C_{60}H_{104}O_{25}$. Murucin 5 (**5**) gave a quasi-molecular ion at *m/z* 1305 $[M + Na]^+$ in the positive-ion FABMS. The negative-ion FABMS showed fragment peaks at *m/z* 1281 $[M - H]^-$, 1099 [*m/z* 1281−182 $(C_{12}H_{22}O)]^-$, 999 [*m/z* 1099 − 100 $(C_5H_8O_2)]^-$, 837, 691, 545, 417, and 271. Basic hydrolysis of **5** afforded murucinic (**6**), dodecanoic, and 3-hydroxy-2-methylbutaoic acids. The ^{13}C NMR spectrum of **5** was almost identical to that of **4**. The ^1H NMR spectrum of **5** showed overlapped signals at 3.90 (H-3) and 2.48 ppm (H-2) of the 3-hydroxy-2-methylbutanoyl group. The HMBC spectrum of **5** was used to determine that this 3-hydroxy-2-methylbutanoyl unit is attached at C-2 of rhamnose″, while a *n*-dodecanoyl residue was located at C-2 of rhamnose′, and the jalapinolic acid unit was esterified at C-2 of rhamnose.

Compounds **1**−**5** were subjected to a cytotoxicity evaluation using colon carcinoma (HCT-15), cervical carcinoma (UISO-SQC-1), and ovarian carcinoma (OVCAR-5) cells. Compounds **2**−**5** were inactive[7] against all three of these cell lines (ED_{50} >20.0 μg/mL). Compound **1** exhibited marginal activity against OVCAR cells (ED_{50} 5.0 μg/mL) but was inactive against HCT-15 and UISO-SQC-1 cells.

Noda et al. reported the chemical structure of stoloniferins, pentasaccharide macrolactones of jalapinolic acid.[8] The latter compounds differ from the murucins 1−5 (**1**−**5**) in that they have a branched oligosaccharide core and a fucose unit instead of a quinovose residue.[8] Murucins 1−5 (**1**−**5**) are the first oligosaccharides isolated and characterized from an arboreal *Ipomoea* species to date.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured using a JASCO DIP 360 digital polarimeter. IR spectra were recorded using a Bruker model v22 spectrometer. NMR spectra were obtained on a Varian Unity 400 spectrometer equipped with a 5 mm inverse detection pulse field gradient probe at 25 °C using standard Varian software. T-ROESY spectra were obtained on a Varian Unity 500 spectrometer. Proton and carbon chemical shifts were referenced to internal tetramethylsilane (TMS), with 20 mg of each oligosaccharide being dissolved in ca. 0.75 mL of methyl alcohol-d_4. Electrospray-ionization mass spectra were recorded on a Micromass model QTOF 2 spectrometer. Positive- and negative-ion FABMS were recorded on a JEOL MStation JMS700 mass spectrometer using *m*-nitrobenzyl alcohol as matrix. The GC-MS system consisted of a HP 6890 gas chromatograph and a HP 5970 mass selective detector in the electron-ionization mode. Silica gel (70−230 mesh, Merck, Darmstadt, Germany) was used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck, Darmstadt, Germany) plates, and spots were visualized by spraying the plates with 10% H_2SO_4 solution followed by heating. HPLC was performed using a system comprised of an Varian 9010 ternary pump, a Varian variable-wavelength UV−vis 9050 detector, and a Rheodyne injector.

Plant Material. Samples of *I. murucoides* were collected in Ixpantepec, in the state of Oaxaca, Mexico, in December 1996. Botanical classification was carried out by Biol. Francisco Ramos, Instituto de Biología, UNAM, and a voucher specimen (No. 896095) is deposited at the National Herbarium in Mexico City.

Table 1. ^1H and ^{13}C NMR Data for Murucinic Acid (6) (D_2O, δ ppm, *J* in Hz)

position	δ_H	δ_C
Qui[a]		
1	4.38 d (7.7)	103.2
2	3.60*[b]	79.3
3	3.63*	78.7
4	3.00 dd (9.0, 6.5)	77.1
5	3.30 dd (6.5, 9.0)	72.9
6	1.30 d (6.5)	18.1
Rha		
1	5.25 d (1.3)	102.0
2	3.95 dd (3.3, 1.3)	71.9
3	4.06 dd (9.1, 3.3)	72.0
4	3.58*	82.4
5	4.22 dd (6.2, 9.2)	67.9
6	1.25 d (6.2)	19.0
Rha′		
1	5.45 d (1.5)	101.0
2	4.05 dd (3.5, 1.5)	69.9
3	4.20 dd (9.0, 3.5)	71.4
4	3.75 dd (8.9, 9.0)	81.3
5	4.30 dd (6.0, 8.9)	68.1
6	1.05 d (6.0)	18.6
Rha″		
1	5.35 d (1.4)	102.8
2	4.15 dd (3.4, 1.4)	70.9
3	4.30 dd (8.9, 3.4)	71.8
4	3.65*	82.0
5	4.42 dd (6.1, 9.0)	68.9
6	1.15 d (6.1)	18.9
Glc		
1	5.05 d (7.6)	103.1
2	3.45 dd (9.1, 7.6)	78.0
3	3.65*	79.4
4	3.10 dd (9.0, 9.0)	72.8
5	3.25 ddd (2.4, 4.9, 9.0)	77.9
6	3.60*	63.9
6′	3.90 dd (2.4, 12.1)	
Jal		
1		179.0
2	2.34 t (6.7)	36.8
11	3.72*	83.3
16	0.95 t (7.1)	14.7

[a] Abbreviations: Qui = quinovopyranosyl, Glc = glucopyranosyl, Rha = rhamnopyranosyl, and Jal = 11-hydroxyhexadecanoyl. [b] Chemical shifts marked with an asterisk (*) indicate overlapped signals.

Table 2. ^{1}H NMR Data for Compounds **1**−**5** (CD_3OD, δ ppm, J in Hz)

position	1	2	3	4	5
Qui[a]					
1	4.31 d (7.4)	4.33 d (7.5)	4.31 d (7.7)	4.32 d (7.8)	4.34 d (7.9)
2	3.43 dd (9.1, 7.4)	3.44 dd (9.0, 7.5)	3.43 dd (8.9, 7.7)	3.43 dd (9.0, 7.8)	3.42 dd (9.1, 7.9)
3	3.50 dd (9.0, 9.1)	3.52 dd (8.9, 9.0)	3.53 dd (9.0, 8.9)	3.52 dd (9.0, 9.0)	3.52 dd (8.9, 9.1)
4	3.58*[b]	3.61*	3.62*	3.62*	3.63*
5	3.60*	3.60*	3.61*	3.60*	3.61*
6	1.23 d (7.0)	1.21 d (7.2)	1.22 d (7.0)	1.21 d (7.0)	1.22 d (7.1)
Rha 1	5.04 d (1.7)	5.02 d (1.8)	5.03 (1.7)	5.02 d (1.7)	5.03 d (1.8)
2	5.10 dd (3.6, 1.7)	5.16 dd (3.7, 1.8)	5.13 dd (3.6, 1.7)	5.16 dd (3.8, 1.7)	5.16 dd (3.7, 1.8)
3	4.22 dd (9.3, 3.6)	4.20 dd (9.1,3.7)	4.21 dd (9.2, 3.6)	4.22 dd (9.3, 3.8)	4.21 dd (9.2, 3.7)
4	3.46 dd (9.0, 9.3)	3.44 dd (9.1, 9.1)	3.47 dd (9.0, 9.2)	3.45 dd (9.1, 9.3)	3.46 dd (9.2, 9.2)
5	3.86*	3.87*	3.88*	3.89*	3.87*
6	1.28 d (6.6)	1.27 d (6.5)	1.28 d (6.5)	1.29 d (6.6)	1.28 d (6.5)
Rha′ 1	5.08 d (1.5)	5.09 d (1.5)	5.09 d (1.5)	5.09 d (1.5)	5.10 d (1.5)
2	5.52 dd (3.5, 1.5)	5.54 dd (3.4, 1.5)	5.54 dd (3.5, 1.5)	5.54 dd (3.4, 1.5)	5.57 dd (3.5, 1.5)
3	4.06 dd (9.0, 3.5)	4.10 dd (9.1, 3.4)	4.08 dd (9.0, 3.5)	4.07 dd (9.0, 3.4)	4.07 dd (9.0, 3.5)
4	3.66*	3.67*	3.66*	3.67*	3.67*
5	3.86*	3.86*	3.86*	3.87*	3.86*
6	1.30 d (6.5)	1.32 d (6.3)	1.30 d (6.3)	1.29 d (6.4)	1.29 d (6.4)
Rha″ 1	5.27 d (1.7)	5.25 d (1.6)	5.25 d (1.7)	5.25 d (1.7)	5.26 d (1.7)
2	4.06 dd (3.5, 1.7)	4.05 dd (3.5, 1.6)	4.05 dd (3.5, 1.7)	4.03 dd (3.6, 1.7)	4.05 dd (3.5, 1.7)
3	3.74 dd (9.0, 3.5)	3.75 dd (8.9, 3.5)	3.74 dd (9.0, 3.5)	3.75 dd (9.0, 3.6)	3.74 dd (9.0, 3.5)
4	4.93 dd (9.4, 9.0)	4.97 dd (9.2, 9.0)	4.95 dd (9.3, 9.0)	4.95 dd (9.3, 9.0)	4.95 dd (9.2, 9.0)
5	3.84*	3.83*	3.83*	3.83*	3.83*
6	1.14 d (6.0)	1.12 d (6.1)	1.12 d (6.0)	1.12 d (6.0)	1.12 d (6.0)
Glc					
1	4.43 d (7.1)	4.42 d (7.0)	4.42 d (7.1)	4.43 d (7.0)	4.43 d (7.0)
2	3.18 dd (9.1, 7.1)	3.17 d (9.0, 7.0)	3.18 d (9.0, 7.1)	3.17 d (9.0, 7.0)	3.16 d (9.1, 7.0)
3	3.31*	3.31*	3.31*	3.31*	3.31*
4	3.19 dd (9.0, 9.1)	3.20 dd (9.1, 9.0)	3.19 dd (9.0, 9.0)	3.20 dd (9.0, 9.1)	3.20 dd (9.0, 9.1)
5	3.29*	3.29*	3.30*	3.30*	3.30*
6	3.58*	3.59*	3.60*	3.60*	3.60*
6′	3.86*	3.87*	3.87*	3.86*	3.87*
Jal					
2a	2.28 ddd (14.0, 7.0, 3.5)	2.30 ddd (15.0, 7.5, 3.4)	2.29 ddd (15.0, 7.5, 3.4)	2.30 ddd (14.5, 7.0, 3.5)	2.30 ddd (14.0, 7.0, 3.5)
2b	2.55 ddd (14.0, 7.0, 3.5)	2.56 ddd (15.0, 7.5, 3.4)	2.55 ddd (15.0, 7.5, 3.4)	2.56 ddd (14.5, 7.0, 3.5)	2.57 ddd (14.0, 7.0, 3.5)
11	3.59*	3.60*	3.60*	3.65*	3.60*
16	0.89 t (7.0)	0.88 t (7.0)	0.89 t (7.0)	0.90 t (7.0)	0.89 t (7.0)
Dodeca					
2	2.37 t (6.5)	2.38 t (6.7)	2.37 t (6.6)	2.36 t (6.7)	2.37 t (6.7)
12	1.22 t (7.0)	1.23 t (7.1)	1.22 t (7.0)	1.23 t (7.1)	1.22 t (7.1)
Ac					
2	2.08 s				
Propa					
2		2.47 q(7.0)			
3		1.22 t (7.0)			
Buta					
2			2.49 t (7.0)		
4			1.08 t (7.5)		
Mba					
2				2.40 m	
4				1.10 t (7.0)	
Nla					
2					2.48 m
3					3.90*

[a] Abbreviations: Qui = quinovopyranosyl, Glc = glucopyranosyl, Rha = rhamnopyranosyl, Dodeca = *n*-dodecanoyl, Ac = acetyl, Propa = propanoyl, Buta = butanoyl, Mba = 2-methylbutanoyl, Nla = 3-hydroxy-2-methylbutanoyl, and Jal = 11-hydroxyhexadecanoyl. [b] Chemical shifts marked with an asterisk (*) indicate overlapped signals.

Extraction and Isolation. Dried and ground roots (825 g) were defatted with hexane at room temperature. The residual material was extracted exhaustively in CH_2Cl_2 to give, after removal of the solvent, a brown solid material (50 g). The brown solid showed two spots by TLC on silica gel eluted with $CHCl_3$−CH_3OH (9:1) (R_f 0.74 and 0.45) and was subjected to gravity column chromatography over silica gel (500 g) using a gradient of CH_3OH in $CHCl_3$, leading to two fractions. Purification of the resinous fraction was carried out by preparative HPLC using a MCH-10 column (10 mm i.d. × 300 mm, 5 μm, Varian), eluting with a mixture of CH_3CN−H_2O (7:3), at a flow rate 1 mL/min at 25 °C, and detection with UV at 215 nm. Compounds **1** (100 mg, t_R 9.4 min), **2** (50 mg, t_R 12.12 min), **3** (100 mg, t_R 14.0 min), **4** (78 mg, t_R 14.2 min), and **5** (45 mg, t_R 16.79 min) were collected and reinjected until pure.

Murucin 1 (1): amorphous white powder; mp 141−143 °C; $[\alpha]^{25}_D$ −19.9° (*c* 4.3 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 3; positive-ion FABMS *m/z* 1247 $[M + Na]^+$; negative-ion FABMS *m/z* 1223 $[M - H]^-$, 1041 $[M - H - C_{12}H_{22}O]^-$, 999 $[104 - C_2H_2O]^-$, 837, 691, 545, 417, and 271; HRESIMS *m/z* 1223.6862 $[M - H]^-$ (calcd for $C_{60}H_{104}O_{25}$, requires 1224.6867).

Murucin 2 (2): amorphous white powder; mp 142−144 °C; $[\alpha]^{25}_D$ −22.0° (*c* 2.0 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR,

Table 3. ^{13}C NMR Data for Compounds **1**−**5** (125 MHz, CD_3OD)

carbon	1	2	3	4	5
Qui 1	105.4	105.4	105.3	105.4	105.4
2	80.1	80.0	80.0	80.1	80.1
3	73.8	73.7	73.8	73.7	73.7
4	71.4	71.3	71.4	71.4	71.4
5	73.3	73.3	73.3	73.2	73.3
6	16.9	16.8	16.8	16.8	16.8
Rha 1	98.9	99.0	98.9	98.8	98.9
2	74.1	74.1	74.1	74.1	74.1
3	70.1	70.1	70.2	70.1	70.1
4	82.0	82.0	82.0	82.0	81.9
5	69.2	69.3	69.2	69.2	69.2
6	19.2	19.3	19.3	19.3	19.3
Rha′ 1	100.2	100.2	100.2	100.2	100.2
2	73.7	73.8	73.7	73.7	73.7
3	80.8	80.8	80.8	80.8	80.9
4	79.1	79.1	79.1	79.1	79.1
5	69.1	69.0	69.0	69.0	69.0
6	18.8	18.8	18.8	18.8	18.8
Rha″ 1	103.2	103.3	103.2	103.3	103.2
2	72.2	72.2	72.2	72.2	72.2
3	70.3	70.3	70.3	70.3	70.3
4	75.5	75.4	75.4	75.5	75.5
5	68.6	68.6	68.6	68.6	68.6
6	17.8	17.8	17.9	17.8	17.8
Glc					
1	105.5	105.5	105.5	105.5	105.5
2	75.3	75.3	75.3	75.3	75.2
3	78.3	78.3	78.3	78.2	78.3
4	71.6	71.5	71.5	71.5	71.5
5	78.1	78.2	78.1	78.1	78.1
6	63.1	63.1	63.2	63.2	63.2
Jal 1	175.0	175.0	174.9	175.0	175.0
2	34.8	34.8	34.8	34.8	34.8
11	84.0	84.0	84.0	84.0	84.0
16	14.4	14.4	14.0	14.0	14.0
Dodeca					
1	175.1	175.1	175.1	175.1	175.1
2	35.1	35.0	35.0	35.0	35.0
12	23.1	23.0	23.0	23.0	23.0
Ac					
1	172.5				
2	41.1				
Propa					
1		178.0			
2		35.1			
3		19.9			
Buta					
1			178.0		
2			42.0		
3			28.1		
4			19.4		
Mba					
1				178.0	
2				41.0	
3				28.9	
4				19.2	
Nla					
1					178.0
2					48.5
3					69.8
4					20.1

see Tables 2 and 3; positive-ion FABMS *m/z* 1261 $[M + Na]^+$; negative-ion FABMS *m/z* 1237 $[M - H]^-$, 1055 $[M - H - C_{12}H_{22}O]^-$, 999 $[1055 - C_3H_4O]^-$, 837, 691, 545, 417, and 271; HRESIMS *m/z* 1237.7021 $[M - H]^-$ (calcd for $C_{61}H_{106}O_{25}$, requires 1238.7023).

Murucin 3 (3): amorphous white powder; mp 142−144 °C; $[\alpha]^{25}_D$ −21.0° (*c* 1.9 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; positive-ion FABMS *m/z* 1275 $[M + Na]^+$; negative-ion FABMS *m/z* 1251 $[M - H]^-$, 1069 $[M - H - C_{12}H_{22}O]^-$, 999 $[1069 - C_4H_6O]^-$, 837, 691, 545, 417, and 271; HRESIMS *m/z* 1251.7177 $[M - H]^-$ (calcd for $C_{62}H_{108}O_{25}$, requires 1252.7180).

Murucin 4 (4): amorphous white powder; mp 142−144 °C; $[\alpha]^{25}_D$ −20.0° (*c* 2.0 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; positive-ion FABMS *m/z* 1289 $[M + Na]^+$; negative-ion FABMS *m/z* 1265 $[M - H]^-$, 1083 $[M - H - C_{12}H_{22}O]^-$, 999 $[1083 - C_5H_8O]^-$, 837, 691, 545, 417, and 271; HRESIMS *m/z* 1265.7335 $[M - H]^-$ (calcd for $C_{60}H_{104}O_{25}$, requires 1266.7336).

Murucin 5 (5): amorphous white powder; mp 143−145 °C; $[\alpha]^{25}_D$ −19.6° (*c* 2.0 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; positive-ion FABMS *m/z* 1305 $[M + Na]^+$; negative-ion FABMS *m/z* 1281 $[M - H]^-$, 1099 $[M - H - C_{12}H_{22}O]^-$, 999 $[1099 - C_5H_8O_2]^-$, 837, 691, 545, 417, and 271; HRESIMS *m/z* 1281.7280 $[M - H]^-$ (calcd for $C_{60}H_{104}O_{25}$, requires 1282.7284).

Murucinic Acid (6): amorphous white powder; mp 161−164 °C; $[\alpha]^{25}_D$ −39.9° (*c* 2.1 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1730 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; positive-ion FABMS *m/z* 1041 $[M + Na]^+$; negative-ion FABMS *m/z* 1017 $[M - H]^-$, 855 $[M - H - C_6H_{10}O_5]^-$, 709 $[855 - C_6H_{10}O_4]^-$, 563 $[709 - C_6H_{10}O_4]^-$, 417 $[563 - C_6H_{10}O_4]^-$, and 271 $[417 - C_6H_{10}O_4]^-$; HRESIMS *m/z* 1017.5163 $[M - H]^-$ (calcd for $C_{46}H_{82}O_{24}$, requires 1018.5168).

Acid Hydrolysis of the Resinous Chromatographic Fraction. The resinous chromatographic fraction (50 mg) was refluxed with 1.0 N HCl (10 mL of water−ethanol) for 1.0 h. The reaction mixture was taken to pH 5 with NaOH solution, and the solution was extracted with CH_2Cl_2 and analyzed by GC-MS (25 m × 0.2 mm HP-5 column: He, 1 mL/min; 40 °C, 2 min, 40−250 °C, Δ 15 °C/min, 250 °C 10 min; split 1:40), giving the following acid derivatives: ethyl acetate (t_R 3.0 min) *m/z* $[M]^+$ 88 (7), $[M - C_2H_3]^+$ 61 (14), $[M - OC_2H_5]^+$ 43 (100), 29 (24); ethyl propanoate (t_R 3.5 min) *m/z* $[M]^+$ 102 (15), $[M - CH_3]^+$ 87 (4), $[M - OC_2H_5]^+$ 75 (12), 57 (60), 29 (100); ethyl butyrate (t_R 4.0 min) *m/z* $[M]^+$ 116 (5), $[M - CH_3]^+$ 101 (10), $[M - C_2H_4]^+$ 88 (55), $[M - OC_2H_5]^+$ 71 (100), 43 (70), 29 (60); ethyl 2-methylbutyrate (t_R 6.6 min) *m/z* $[M]^+$ 130 (0.5), $[M - C_2H_4]^+$ 102 (34), $[M - OC_2H_5]^+$ 85 (26), 57 (80), 29 (100); ethyl 3-hydroxy-2-methylbutyrate (t_R 8.65 min) *m/z* $[M]^+$ 132 (5.0), $[M - OC_2H_5]^+$ 87 (20), 73 (100), 45 (40); ethyl dodecanoate (t_R 15.0 min) *m/z* $[CH_3 - (CH_2)_{10}CO_2C_2H_5]^+$ 228 (10), $[CH_3 - (CH_2)_{10}CO]^+$ 183 (10), $[CH_3 - CH_2 - CO_2C_2H_4]^+$ 101 (45), $[CH_3 - CH_2 - CO_2CH_3]^+$ 88 (100), 73 (15), 43 (18); and ethyl 11-hydroxyhexadecanoate (t_R 19.57 min) *m/z* $[CH_3 - (CH_2)_4 - CH - (OH) - (CH_2)_9CO_2C_2H_5]^+$ 300 (1), $[CH_3 - (CH_2)_4 - CH - (OH) - (CH_2)_9CO]^+$ 255 (3.0), $[(CH_2)_9CO_2C_2H_5]^+$ 199 (50), $[CH_3 - (CH_2)_4 - CH - OH]^+$ 101 (60), 83 (45), 57 (100), 73 (20), 45 (40).

Carbohydrate Analysis. The aqueous phase of the acid hydrolysis reaction was neutralized with Na_2CO_3 solution and lyophilized to give a colorless powder. The residue was dissolved in 1.0 mL of dry pyridine and treated with 1 mL of hexamethyldisilazane and 0.5 mL of chlorotrimethylsilane at 60 °C for 50 min. GC-MS analysis (25 m × 0.2 mm HP-5 column; He, 1 mL/min; 40 °C 2 min; 40−250 °C, Δ 15 °C/min, 250 °C 10 min; split 1:40) gave three peaks, t_R 14.1, 18.2, and 22.2 min, which coeluted with TMSi ethers of standard α-L-rhamnose, 6-deoxy-β-D-glucose (quinovose), and β-D-glucose, respectively. An aliquot of the hydrolysis mixture was subjected to HPLC [Alltech Nucleosil 100 NH_2, 5 μm, 250 × 4.6 mm, 1 mL/min; 80% MeCN−H_2O] and a refractive index detector (Agilent 1100): α-L-rhamnose (3 mg t_R 7.9 min), D-quinovose (1 mg, t_R 8.9 min), D-glucose (1 mg, t_R 15.2 min). These sugars were isolated and their absolute configurations were determined by their optical rotations: glucose, $[\alpha]^{25}_D$ +105° (*c* 0.9 CH_3OH), rhamnose, $[\alpha]^{25}_D$ −6.0° (*c* 2.3 CH_3OH), and quinovose, $[\alpha]^{25}_D$ +64.9° (*c* 0.8 CH_3OH).

Alkaline Hydrolysis of the Resinous Chromatographic Fraction. The resinous chromatographic fraction (250 mg) was refluxed in 0.1 N NaOH (10 mL) for 60 min. The reaction mixture was acidified to pH 5 and extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The aqueous layer was lyophilized, the residue was dissolved with methanol, and a white solid (glycosidic acid) was obtained after removal of solvent. The glycosidic acid (**6**) was characterized by NMR and mass spectrometry. The glycosidic acid was

refluxed in 1.0 N HCl (5 mL of water−ethanol) for 1.0 h. The reaction mixture was taken to pH 5 with NaOH solution, and the solution extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The observed optical rotation ($[\alpha]^{25}_D$ +0.42°) was closely comparable to that previously reported[9] for the *S* enantiomer of the ethyl ester of jalapinolic acid ($[\alpha]^{25}_D$ +0.45°).

Cytotoxicity Assay. The HCT-15, UISO, and OVCAR-5 cell lines were maintained in RPMI culture medium with 10% fetal bovine serum (FBS), and all cell lines were cultured at 37 °C in an atmosphere of 5% CO_2 in air (100% humidity). The cells at a log phase of their growth were treated in triplicate at various concentrations of the compounds (1.0−100 μg/mL) and incubated for 72 h at 37 °C in a humidified atmosphere of 5% CO_2. The cell concentration was determined by protein analysis. Results were expressed as the dose that inhibits 50% control growth after the incubation period (ED_{50}). The values were estimated from a semilog plot of the drug concentration (μg/mL) against the percent of viable cells. The HCT-15, UISO, and OVCAR-5 cell lines were donated to CIBIS, IMSS by the Natural Products Research National Cancer Institute, Frederick, MD.

Acknowledgment. Financial support from the Natural Sciences and Engineering Research Council of Canada to W.F.R. and from CONACYT to R.E.H. is gratefully acknowledged. We thank V. Labastida and M. Medina (CIQ, UAEM) for recording the FABMS.

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NP050075M